

**Raman Spectroscopic Characterisation and
Chemometric Analysis of Facial Cosmetics as
Associative Trace Evidence**



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**This thesis is presented for the degree of
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Declaration

I declare that the work presented here is the result of my own research, and where applicable, I have clearly acknowledged published work. This work has not been submitted for a qualification at any other institution or for any other degree.

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Abstract

Decorative cosmetics such as facial foundations or finishing powders are widely used and easily transferred upon physical contact. As such, they may be used as associative trace evidence to link people to each other or to places in criminal investigations. To maximise their probative value, it is important to understand the variability amongst representative market products and the degree of sample discrimination that can be achieved. Additionally, it is required that analysis techniques be non-destructive, readily available and relatively inexpensive. Raman spectroscopy is a powerful tool for probing the chemistry of facial cosmetics. As well as fitting the criteria above, it offers the capability of studying a wide range of sample types with minimal prior preparation. The information derived from Raman spectra can help analysts to understand and visualise spectral variability, potentially enabling discrimination between samples.

This thesis presents the novel application of Raman microspectroscopy to the analysis of 297 facial cosmetic samples with subsequent chemometric methods for objective spectral interpretation. The analysis of 177 newly purchased cosmetic products revealed the most important chemical components for sample discrimination and highlighted the issue of spectral heterogeneity, leading to the separation of 126 spectrally homogeneous from 44 spectrally heterogeneous samples. Microscopic examination of samples allowed for the assessment of visual homogeneity but revealed that this was not a reliable indicator of spectral homogeneity. Subjective assessment of Raman spectra did not always correlate with the principal component analysis (PCA) models as the dimensionality reduction technique applied different variable weightings. Assessment of PCA loadings showed the primary distinction between samples to be their titanium dioxide polymorph, followed by iron oxide and lecithin content.

The effects of ageing were more noticeable visually among the water-based products yet nearly undetectable via Raman spectra. Drastic colour and texture changes were evident after 15 months of passive ageing, as were the detachment of borosilicate glass pigments and silica microspheres from their respective matrices, which may have implications for casework. The spectral changes were more pronounced amongst the aged samples that contained a higher organic composition, indicated by the loss of organic components or their degradation to other species. Furthermore, inter-batch comparisons of some products showed a change of titanium dioxide polymorph used (from anatase to rutile) showing a lack of formulation consistency.

Donations of 120 used samples, old discarded products and expired shop testers allowed for the assessment of a set of “*real world*” samples. Raman analysis of these donated samples and comparisons with their newly purchased counterparts revealed an increase in baseline fluorescence, increased spectral heterogeneity, and extra peaks in the spectral mid-range.

These spectral alterations suggest chemical changes associated with ageing, and/or the contamination of these samples, most likely from a biological source.

This study describes the first microscopic and Raman spectroscopic characterisation of facial cosmetics within a forensic context, further enhanced by the addition of multivariate data analysis methods, which addresses the need for objective, unbiased interpretation of spectral data. These cosmetic traces may be exploited in criminal investigations in questioned (Q) versus known (K) comparisons, or sample eliminations, or to provide investigative leads. The discovery of a counterfeit product demonstrated the utility of these analyses not just in a crime scene investigation context but also for the verification of genuine consumer goods. The assessment of cosmetic traces on a purely visual basis is not recommended, owing to subjective descriptions and the difficulties associated with analysing interference pigments; the addition of Raman spectroscopy offers much needed enhanced discrimination potential. Raman microscopy is well suited for the analysis of microtraces such as those that might be encountered in casework, and involves less sample preparation than many other analytical techniques such as surface enhanced Raman spectroscopy (SERS).

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Abbreviations

AAS	Atomic absorption spectroscopy
ATR	Attenuated total reflectance
BB	Beauty balm (cream)
CARS	Coherent anti-Stokes Raman spectroscopy
CC	Colour correcting (cream)
CCD	Charge-coupled device
CSRS	Coherent Stokes Raman spectroscopy
DA	Discriminant analysis
DNA	Deoxyribonucleic acid
DP	Discriminatory power
EDX	Energy dispersive X-ray
FTIR	Fourier transform infrared
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionisation detector
GC/MS	Gas chromatography/mass spectrometry
HCA	Hierarchical cluster analysis
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
ICS	Intensity correction system
IPA	Isopropyl alcohol
IR	Infrared
kNN	k-nearest neighbours
LDA	Linear discriminant analysis
MDS	Multidimensional scaling
MEKC	Micellar electrokinetic capillary chromatography

MEEKC	Microemulsion electrokinetic capillary chromatography
MS	Mass spectrometry
MSP	Microspectrophotometry
MVA	Multivariate data analysis
NA	Numerical aperture
NAS	National Academy of Sciences
ND	Neutral density
NIPALS	Non-linear iterative partial least squares
NIR	Near infrared
OMC	Octyl methoxycinnamate
PBT	Polybutylene terephthalate
PC	Principal component
PCA	Principal component analysis
PCAST	President's Council of Advisors on Science and Technology
PDMS	Polydimethylsiloxane
PE	Polyethylene
PEG	Polyethylene glycol
PET	Polyethylene terephthalate
PLS-DA	Partial least squares-discriminant analysis
PLSR	Partial least squares regression
PMMA	Polymethyl methacrylate
PPG	Polypropylene glycol
PS-MS	Paper spray mass spectrometry
PVP	Polyvinylpyrrolidone
Py-GC/MS	Pyrolysis-gas chromatography/mass spectrometry
Q vs. K	Questioned versus known

QDA	Quadratic discriminant analysis
RRS	Resonance Raman spectroscopy
RTD	Real time display
SEM	Scanning electron microscopy
SEM-EDX	Scanning electron microscopy-energy dispersive X-ray spectroscopy
SERDS	Shifted excitation Raman difference spectroscopy
SERRS	Surface enhanced resonance Raman spectroscopy
SERS	Surface enhanced Raman spectroscopy
SIMCA	Soft independent modelling of class analogies
SLS	Sodium laureth sulphate
SNV	Standard normal variate
SPF	Sun protection factor
SRS	Stimulated Raman spectroscopy
SVD	Singular value decomposition
SVM	Support vector machines
SWGDOC	Scientific Working Group for Document Examination
SWGMA	Scientific Working Group for Materials Analysis
TERS	Tip-enhanced Raman spectroscopy
ToF-SIMS	Time of flight secondary ion mass spectrometry
TLC	Thin layer chromatography
UV	Ultraviolet
XRD	X-ray diffraction
XRF	X-ray fluorescence

Chapter One: Introduction

1.1 Introduction

Trace evidence provides a means for forensic scientists to study past activities such as an individual's whereabouts, actions or interactions, within the criminal justice system. Physical traces are vestiges of the past that can aid in piecing together the circumstances of a crime via their detection, recognition, recovery, examination and interpretation. This is performed in a case-based and research-oriented way to enable the formation of logical hypotheses and explanations that may be presented in court¹.

It is usually accepted that trace evidence is a very small amount of a substance of interest that remains after an occurrence or action by an agent². Many forms of trace are universally accepted by the criminal justice system as tried-and-tested methods of proving a link between suspect and scene, or suspect and victim, such as DNA or fibre evidence³⁻⁵; while others are in their infancy and are the subject of academic research and peer review.

Associative traces are physical remnants that help to prove a link between people, places, and/or objects and may aid in crime reconstruction. They can be generated from different sources and are found in varied forms and locations. A fundamental principle of associative trace is that it is bidirectional; the suspect will not only leave traces of their presence but also take traces from the scene away on their person. Some examples of commonly encountered traces in forensic casework include paint flakes, hairs, glass fragments, adhesive tapes, and fibres^{6,7}. Trace evidence also includes soil, explosives, drugs, gunshot residue, and arson accelerants⁸.

Best forensic practice stipulates that DNA should not be the sole form of evidence utilised in casework; indeed, the courts increasingly require complementary evidence to avoid further miscarriages of justice⁹. The inclusion of trace evidence in a case adds further probative weight to key issues, rather like adding extra pieces to a jigsaw in order to see the full picture. It is proposed in this thesis that cosmetic traces have the potential to be a valuable form of associative trace evidence in forensic casework.

1.2 Cosmetics

Cosmetics are defined as *“any substance or mixture intended to be placed in contact with the various external parts of the human body (...) with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and /or protecting them or keeping them in good condition”*¹⁰⁻¹². It can therefore be seen that the term cosmetics covers an extraordinarily wide array of products, many of which are commonly used

every day, such as toothpaste, handwash, and deodorant. Perhaps the most frequently associated type of cosmetic is makeup; these are formally known as decorative cosmetics and account for around 30% of the global billion-dollar industry¹³. Decorative cosmetics encompass a broad range of products intended to beautify the wearer's appearance or to conceal real or perceived skin imperfections such as blemishes, scars, hyperpigmentation, or tattoos.

Cosmetic products are used by a wide cross-section of society for skin care and beautification. As a result, the global cosmetic market was worth an estimated USD\$676 billion in 2023 and continues to grow¹⁴. The use of cosmetics is independent of age, gender, ethnicity, and socio-economic status; thus, it is not unreasonable to expect that cosmetic traces of some description may be encountered during criminal investigations.

1.2.1 Cosmetic use throughout history

The use of cosmetics dates back around 12,000 years, to the ancient Sumerian and Egyptian civilisations¹⁵. Then, cosmetics were used primarily to indicate membership of a tribe or to protect against the elements rather than to beautify. People were innovative and resourceful, using ingredients such as clay, crushed gemstones, paprika, turmeric, seaweed and cochineal (carmine). Ancient Egyptians lined their eyes with kohl (otherwise known as kajal or Surma) not only to accentuate the eyes, but also to protect the skin against the burning effects of the sun¹⁶. Some also believed wearing kohl would ward off evil spirits.

From the bronze age right through to the Edwardian era, cosmetic use fluctuated in terms of popularity and social acceptance¹⁷. During the Tudor reign, lead-based white pigments, often containing arsenic, were used to give the fashionably pale complexions of the time¹⁸, while cheeks were stained with rouge made from mercuric oxide. Nowadays, non-toxic alternative white pigments such as oxides of titanium and zinc are used.

At the beginning of the 20th century, the creation of the cosmetic powerhouses: Max Factor, Elizabeth Arden, L'Oreal, and Nivea, amongst others, resulted in a vast array of new and exciting products¹⁷. The cosmetics market has seen sustained growth since, with Europe and the US being the largest cosmetics markets in the world¹⁹.

1.2.2 Cosmetic types and compositions

Cosmetics are formally divided into seven categories: sun care, oral care, skin care, hair care, body care, perfumes, and decorative cosmetics¹¹. Examples of products within these seven cosmetic categories are given in Figure 1.1.

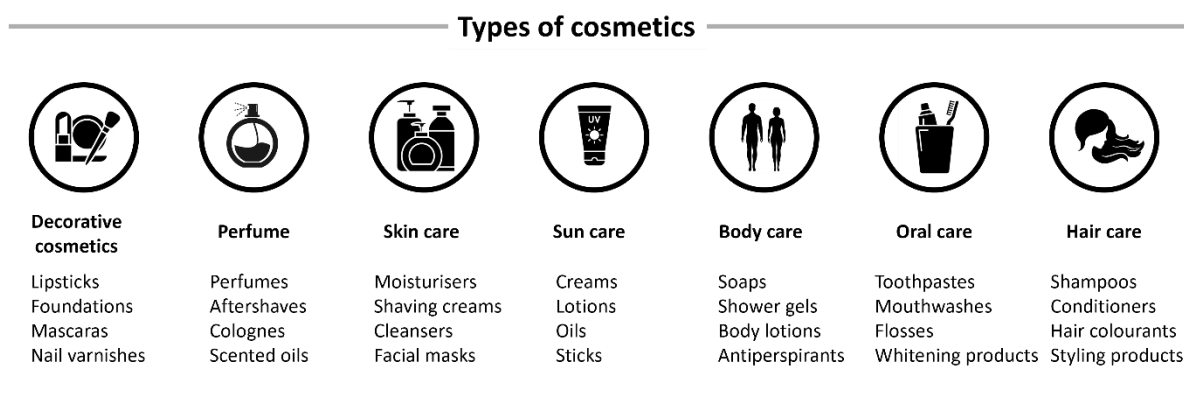


Figure 1.1: Schematic representation of the primary categories of cosmetics with example products, replicated from Bruce *et al.*²⁰.

Decorative cosmetics include products such as foundation, powder, blush, lipstick and lip gloss, lip and eyeliner, mascara, eyeshadow, and nail polish²¹. They tend to be mass-produced with the ingredients subject to highly regulated laws and restrictions, to ensure consumer safety^{12,22}. Alternatively, they can comprise local or homemade items; some consumers may even choose to use common household cooking ingredients such as cocoa powder in place of manufactured blusher or bronzer¹⁷. If we consider which sort of cosmetic products are likely to become trace evidence within a forensic context, it is reasonable to assume that decorative cosmetics would be the most relevant. Therefore, these form the focus of the following discussion.

The compositions of the different types of cosmetics vary but some components are commonly found across most products. Examples of such components include colourants (as pigments or dyes), opacifying agents, and moisture absorbents²¹. The most common pigments are titanium dioxide, zinc oxide, muscovite mica (hydrated potassium aluminium silicate), bismuth oxychloride, and iron oxides; common opacifying agents are kaolin (hydrated aluminium silicate) and calcium carbonate; while talc (hydrated magnesium silicate) and boron nitride act as moisture/oil absorbents^{23–29}.

Products that require structure such as lipsticks, eyeliners, and mascaras contain a proportion of animal or plant-based waxes such as beeswax, carnauba wax, or candelilla wax, and petroleum-based waxes such as microcrystalline wax, ozokerite, or paraffin wax²¹. Eye and lipliners contain polyethylene glycol (PEG) or polypropylene glycol (PPG) as humectants (moisture-retaining compounds)³⁰ and emulsifiers (oil and water phase binding agents)³⁰, while mascaras tend to contain polyvinylpyrrolidone (PVP) as a film former. Some mascaras also contain tiny fibres made of nylon or rayon for a thickening effect²¹.

Lip balms contain occlusive agents that prevent water evaporation, such as petroleum, and often UV absorbers such as oxybenzone. Eyeshadows may have ingredients added for marketing appeal such as proteins or vitamins but these rarely contribute to tangible skin effects. Nail polishes are very similar to paints in that they mostly consist of film formers (as nitrocellulose), resins, plasticisers and solvents.

Other ingredients used across decorative cosmetic ranges include solvents such as isopropyl alcohol (IPA); surfactants such as sodium laureth sulfate (SLS); thickeners such as magnesium aluminium silicate, and fragrances. Plant-derived butters like cocoa and shea act as emollients (moisturising agents) and texture modifiers, while smectite clays, for instance bentonite and hectorite, function as viscosity adjusters³⁰. Many products contain pH buffers to counteract the effect of skin oils, sweat or tears; and antioxidants such as tocopherol (vitamin E) to stop rancidity of unsaturated oils.

Components systematically observed in many of the decorative cosmetics are parabens and isothiazolinones, which are used as preservatives to avoid the development of microbes in the cosmetic. However, both preservatives are known to be skin irritants, and parabens specifically are suspected to act as endocrine (or hormone) disruptor chemicals³⁰ and, therefore, their use is strictly limited in decorative cosmetics. None of the research discussed in Section 1.4 detected preservatives and this is likely due to a concentration below the instrumental detection limit. Nonetheless, for decorative cosmetics it is the whole product composition that is of forensic interest, as the various proportions of the components in the mixture confer a specific chemical pattern.

1.2.2.1 Facial cosmetics

Facial cosmetics specifically describe products that enhance or correct the complexion. They are decorative cosmetics that are designed to be used over large areas of the face or neck, as opposed to eye or lip products that are “*point*” style products intended to be used on those areas only. Facial cosmetics encompass products such as foundations, concealers, tinted moisturisers, beauty balm (BB) and colour correcting (CC) creams, finishing powders, bronzers, highlighters, and primers. They are either translucent/transparent, skin-coloured or, in the case of bronzers and highlighters, slightly darker than or lighter than skin tone respectively.

Primers are intended to be the first makeup product applied after skin care and provide a smooth base for facial cosmetics thereafter. They serve different functions depending on the skin concerns of the consumer: some absorb excess oil, helping makeup to stay in place longer, while others contain emollients to reduce the appearance of dry patches.

Foundations are the workhorse of facial cosmetics. They are used as the underpinning “*canvas*” onto which all other decorative cosmetics may be applied, hence the use of the word to describe these products. Foundations function to correct skin tone, cover blemishes, blur imperfections, and give an even appearance to the skin³⁰. They come in a variety of different formulations: liquids, gels, mousses, creams, pressed powders and loose powders; and a variety of different coverage types: sheer, light, medium or full. Various finishes are available depending on the desired skin effect: natural, matte (for oily complexions or stage makeup), or dewy (for sallow or dry complexions). Recent innovations in foundations have seen them become hybrid products of makeup and skincare, an example of which is serum foundation.

Typically, foundations are water-based and contain a large proportion of either linear siloxanes such as dimethicone (polydimethylsiloxane, PDMS) or cyclic siloxanes such as cyclopentasiloxane (D5) and cyclohexasiloxane (D6)³¹. Many of the other components discussed in the previous section are also found, but not repeated here. Remarkably, the huge variety of shades and tones available on the market are created using only differing ratios of the white pigment, titanium dioxide, and the three iron oxides: red, yellow, and black³². Occasionally, other inorganic pigments such as zinc oxide and ultramarines may be added to fine tune the shades³³.

Perhaps the most versatile of facial cosmetics is concealer. These are similar to foundations but contain a higher proportion of pigment in order to cover blemishes, under-eye dark circles and hyperpigmentation, or to highlight and contour facial features. Some concealers use green, purple, or orange pigments to cancel out unwanted redness, sallowness or acne scarring, respectively. They tend to have a thicker consistency than foundation so they may be layered on top for extra coverage³⁴. As with foundations, concealers may be obtained across all the different formulations, coverage types and finishes already described. However, they exhibit a greater variety of application types, dependent on their desired use, for example: “*doe-foot*” applicators for precise product placement; sponge applicators for dabbing product onto the skin; integral roller ball applicators for under-eye dark circles; or stick-based products (in the style of a traditional lipstick) for applying directly onto the skin. These are all in addition to the standard application methods of using various brushes or simply the fingertips³⁵. Some consumers who require full coverage may choose to swap foundation for concealer and use this all over the face. This is a consideration explored later in this Chapter. The immense variety of differing compositions leads to a highly complex chemical mixture that may be exploited for discrimination between products in forensic investigations.

The main function of a tinted moisturiser is an emollient; however, the tint they provide, categorises them as decorative cosmetics rather than skin care. BB creams contain more

pigment than tinted moisturisers and an array of other active ingredients such as sunscreens, vitamins and antioxidants. CC creams are improved versions of BB creams that offer a lighter consistency but higher pigment content due to the built-in colour correcting concealers³⁶. Examples of commonly used ingredients include hyaluronic acid, glycerin, squalane (rarely squalene due to its instability), aloe vera, and niacinamide (vitamin B3). Formulations that boast added SPF tend to contain a combination of physical sunscreens such as titanium dioxide and zinc oxide, alongside chemical sunscreens such as octyl methoxycinnamate (OMC) and oxybenzone³⁰.

The purpose of finishing powders is to set the makeup underneath it, reduce shine and reduce the appearance of pores to give an “*airbrushed*” effect. Some finishing powders are tinted to give extra coverage over foundation, but all finishing powders will be either the pressed “*cake*” formulation or a loose powder³⁷. Typical ingredients for pressed powders include capric/caprylic triglycerides and magnesium, aluminium, zinc, or calcium stearates as the binding agents and magnesium myristate as an anticaking agent³⁸. Ingredients common to both powdered formulations comprise: talc, nylon-12, tin oxide, kaolin, lauroyl lysine, and corn or rice starches. The addition of microspheres to a formulation aids smooth application and fills wrinkles, while the light scattering properties provide a soft-focus effect. Microspheres are composed of silica, glass, or polymers, such as polyethylene (PE) and polymethyl methacrylate (PMMA). Microspheres may also be used to encapsulate and deliver active ingredients³⁹.

Bronzers are used to contour facial features or give a suntanned complexion, whereas highlighters enhance the high points of the face and give a radiant complexion. Both products are routinely used in tandem to accentuate the contours of the face. They come in a variety of different formulations similarly to foundations and comprise similar ingredients. Bronzers, being darker in colour, tend to rely heavily upon iron oxide pigments, whereas highlighters contain large amounts of pearlescent and/or iridescent components. These may derive from metal oxide coated mica, bismuth oxychloride, guanine (fish scales), nacre (mother or pearl), or borosilicate glass interference pigments. It should be noted that naturally occurring mica (muscovite, sericite or biotite) is distinct from synthetic mica (synthetic fluorophlogopite); the latter being safer for use owing to traces of lead in natural sources, making its use less controversial⁴⁰.

It is hypothesised that owing to their rich chemical diversity and prevalence in society, that decorative cosmetics (facial cosmetics in particular) would be an excellent form of associative trace in criminal investigations as explored in the next section.

1.3 Cosmetics as associative trace

Cosmetic traces may be used as associative evidence in casework, where their purpose is to establish the *corpus delicti*⁴¹. Traditionally, the analysis of cosmetics has been based either within the pharmaceutical or archaeological contexts, however, cosmetic products are also of significant interest in forensic casework^{42–46}. They have the potential to add valuable evidence to the most serious of criminal cases, such as murder, rape, assault, child abuse, trafficking and kidnapping^{47,48}. Cosmetic traces would aid the understanding and reconstruction of the crime activity, by providing a link between suspect and victim, or suspect and scene. This would be particularly beneficial in cases where there is a lack of standard physical evidence, the type of cosmetic residue is uncommon, or cosmetics have been layered and therefore provide unique combinations.

The prevalence and use of cosmetics have increased year on year¹⁹ and their traces may be left behind on many objects, such as cigarette butts, drinking glasses or clothing⁴⁹. Many of these products are easily transferred from one surface or one person to another, making them useful in linking individuals or locations involved in criminal activity. Makeup products of any single type can be purchased in a wide array of formulations, leading to the possibility of source discrimination. Furthermore, consumer trends reveal that facial makeup (such as those described in Section 1.2.2.1) is typically applied in a layered sequence, with the resulting product mixture likely to give distinctive residues. As such, these cosmetics have significant probative potential as transfer evidence.

There are a huge variety of potentially discriminating cosmetic features: the scope of different brands, series, formulations, and shades coupled with geographical location manufacture, inter/intra-batch variations, and variability in particle sizes/morphologies/layers/coatings. When considered alongside differing ages of used products, differing application methods, sources of contamination, and differing modes of trace deposition, this leads to a hypothetically unique trace.

The incidence of foundation use (and similar complexion enhancing products) is increasing at a rapid rate, meaning the likelihood of encountering such a trace at a crime scene is also increasing. When the forces involved during violent contact are considered, it is even more likely that cosmetic traces will be transferred between victim and perpetrator. It is becoming more socially accepted for men to use complexion products, i.e. for concealing blemishes, scars, hyperpigmentation and even tattoos and therefore these traces are no longer restricted to the assumption that the transfer will have occurred due to female suspects/victims. However, it must always be borne in mind that Locard's Exchange Principle dictates that trace transfer is a two-way event¹. Additionally, it is worth mentioning that foundation is typically

applied to the entire face and often neck or chest. Some consumers also apply complexion products to areas of exposed skin such as the arms or legs using a technique familiarly known as “*body painting*”. This implies a large surface area will be covered by the cosmetic and therefore the likelihood of accidental transfer is also increased. Cosmetic traces can also be expected to persist well on fabrics such as garments, bedding or upholstery. Anyone who has ever attempted to remove makeup stains from clothing can attest to its persistence.

Glitter particles are made from tiny pieces of plastic and/or aluminium and may be added to cosmetic preparations for special effect purposes. Blackledge⁵⁰ believes glitter evidence to fulfil all the properties of the ideal trace and many of those properties can also be applied to cosmetic residues, as explained below:

- Glitter particles are nearly invisible, meaning that suspects may not notice them on their skin/clothing or in their surroundings and therefore not actively destroy them. Depending on the size of the trace this could also apply to cosmetic traces.
- There is a high probability of transfer and retention as the particles are so small. This could also apply to certain cosmetics, particularly those containing shimmer particles (interference pigments).
- They are individualistic as there are thousands of potential sizes, shapes, colours, thicknesses, densities, materials, layers or coating variations. Variety within a class type and the benefit of small sub-classes is important in terms of associative evidence value. Theoretically there is even more potential variation among cosmetic samples as the complex mixtures can be blended with varying proportions of raw ingredients, of which there are many.
- Glitter is quickly collected and processed as it retains its solid form and is relatively easy to identify; given the right lighting, it reflects just as a mirror would. The collection of cosmetic traces may not be as straightforward as they are likely to be present as a stain rather than a solid to collect. If present as a trace on a suspect’s skin the collection complexity is increased. That being said, if the stain is present on a garment, confocal Raman spectroscopy allows precise focussing on the sample, reducing background interference.
- Tiny glitter traces can be analysed, particularly when using confocal Raman spectroscopy. The same applies to cosmetic samples.
- Results can be databased to create a library of known samples against which, searches of crime scene samples can be made. The spectra generated by the Raman analysis of cosmetic samples could also be databased but due to constant formulation changes and the release of new products, it would be almost impossible to ascertain whether or not every sample was accounted for.

- Glitter will survive in the environment as it is made from cut out pieces of foil and/or plastic. Cosmetic traces may not be as hardy but can still be expected to persist on fabrics.

Some glitter producing companies only manufacture the glitter rolls but do not cut it into the desired shapes; other companies buy the film and process it into glitter. The same principle applies to cosmetics. It is assumed the majority of cosmetic manufacturers formulate their products using purchased raw materials rather than making everything from scratch. This introduces another layer of variability and complexity as the raw material sources may vary between factories of a given manufacturer.

Nonetheless, the analysis and interpretation of cosmetic traces in casework is not without its limitations. Cosmetics may be altered during wear such that a deposited trace may not accurately represent its true original composition. Factors such as the evaporation of volatile organic components, absorption of water-soluble components into the skin, addition of sebum, eccrine secretions or skin cells to the cosmetic, the application and mixing of other cosmetics, contamination from external sources and UV or heat degradation during wear could all impact upon the deposited trace composition. Furthermore, the trace may alter in composition depending on the time since deposition, UV or heat degradation *in situ*, contamination from the local environment or interactions with the surface on which it is found. There is also the possibility that the item exhibiting the deposited trace is laundered, partially removing it and potentially contaminating with detergents or optical brighteners. Thus, it is crucial to investigate and model these potential alterations to original cosmetics in order to best interpret cosmetic traces encountered in casework.

1.3.1 Forensic case studies

There are very few cases that describe the use of cosmetic trace throughout history, it is suggested that this is due to the lack of underpinning research in this area rather the criminal justice system's acceptance of its value. The first case of cosmetics being used as trace evidence was in 1912 and describes the strangulation and murder of Marie Latelle, in which Edmond Locard recorded a "*pink dust*" found underneath her partner's (the suspect) fingernails. These were chemically identified by Locard as being a specific cosmetic preparation matching Marie's custom-made face powder and, thus, provided the important physical link between the suspect and victim⁵¹. Despite this early demonstration of cosmetic trace value, this type of evidence remains under-researched and under-utilised.

There have been instances where glitter traces have been used as associative evidence in criminal trials and these have contributed towards successful convictions. Examples include

glitter particles found on the clothing of women who had been sexually assaulted; glitter particles found in two suspects' cars following a murder and a double murder; glitter found in bedsheets in the case of a child homicide; and glitter present in the design on a T-shirt which aided in the conviction of a knife attacker^{50,52}.

Various makeup deposits identified on a deployed airbag post fatal motor vehicle collision in 1999 were analysed to ascertain the driver that caused the deaths of four of the five passengers⁴¹. Smears of two differently coloured deposits – pink and black – were observed on the airbag and analysed using a combination of visual examination, orientation measurements, Pyrolysis-gas chromatography (Py-GC), Fourier transform infrared (FTIR), scanning electron microscopy–energy dispersive X-ray spectroscopy (SEM-EDX) and X-ray diffraction (XRD). The black deposits contained iron oxide, a hydrocarbon and acrylic components and it was assumed that these had originated from mascara. The pink deposits contained talc and kaolin and possible identification of eyeshadow and/or face powder were proposed. This cosmetic evidence was used alongside DNA evidence to prove the identity of the driver⁴¹.

Lipstick has also been used as physical evidence, where it was used by the suspect to scrawl a message onto a mirror at a murder scene^{53,54} and as associative evidence found underneath the fingernails of a suspect in a murder case^{55–57}. Lip and lipstick impressions have also been used as biometric identifiers⁵⁸. Facial cosmetics have been used to conceal victims' injuries⁵⁹, to disguise suspects during various bank robberies^{60–62}, and have been recovered from vehicles following hit-and-run road collisions with pedestrians⁶⁰.

1.3.2 Current practice

Forensic research into the evidential value of cosmetic residues began in the 1980s^{60,63–67}. Some of this research was case-based⁶⁰ and was able to guide future directions, for example by illustrating likely target substrates. Despite all the sample discrimination models proposed, very few recent applications to casework are reported in the literature. The primary aim of these models is to provide information about the sample itself, i.e., the extraction and analysis of the sample, without further consideration to the interpretation of the results. Assessing a sample at the source of the evidence is a valuable endeavour. However, comparing it to material seized from a suspect or a crime scene, and thus determining if the trace is linked to the criminal activity itself, would be far more beneficial to forensic practice and the criminal justice system.

Recently, cosmetics have become increasingly present as new types of evidence recovered in forensic casework. Although they are mass-produced products, the diversity of their

chemical compositions may be exploited for the forensic identification of their source material and, consequently, aid in reconstructing the case scenario. Identification of the compounds likely to be detected in casework is a key starting point. With a high variability demonstrated among different sample types, most research has based sample discrimination purely on visual assessment of the presence or absence of specific chemical patterns⁴⁹.

Given that cosmetic trace evidence is a relatively new research area, the current focus seems to be the discrimination of standard samples and furthering our understanding of how those samples compare to one another. Notwithstanding this and bearing in mind the nature of evidence transfers during violent exchange, it is highly unlikely that any cosmetic sample retrieved from a scene will reliably compare with its original product. This is especially true if the original product's formulation were a liquid or gel as opposed to a loose powder⁶⁸. Ergo, by changing the substrate or performing ageing experiments (or a combination of both parameters, as it may be that products age dependent upon their supporting material), additional variables are studied and are likely to impact upon the initial models, while being more representative of casework samples.

Cosmetic evidence interpretation is principally considered as a source enquiry. That is to say, the forensic scientist wishes to know whether a trace detected on a substrate can be linked to either a reference sample from a database (investigative purpose) or to some material seized by/from a suspect (evaluative purpose). Comparison of samples at a source level is essential and these studies are conducted in a logical fashion, starting with the samples in their most basic format and limiting the number of variables, so that models can be created.

However, the investigation of cosmetic trace evidence also requires comprehension of which cosmetic smears may be present on textiles for legitimate reasons (background considerations), how cosmetics transfer to various substrates, and their persistence on those substrates until collection. This framework is derived from the basic principles of forensic trace evidence and is used to understand the properties of the sample, how it got there, and how it has evolved over time, until detected and collected by crime scene investigators. It is worth mentioning at this point that the likelihood of incidentally finding decorative cosmetic traces in the environment is low, and thus the discovery of these traces indicates a high probability that transfer has occurred⁶⁹.

According to crime scene investigators, decorative cosmetic traces are most commonly encountered on garments and bedding^{70,71}, thus indicating a need to investigate substrate interference. Recent studies have targeted substrates including cotton fabric, tissues, A4 paper sheets, skin, cigarette butts, drinking glasses and plastics, as the main substrate likely to be recovered from casework samples. Numerous personal communications suggest that,

in the UK at least, crime scene examiners, police officers and forensic scientists have little or no experience of dealing with cosmetic trace evidence^{70–74}. Private communications also suggested that some laboratories actively seek to exclude the contribution of cosmetic traces from their investigations. Curiously, according to several reporting scientists, cosmetics analysis is performed in forensic laboratories, albeit on a minor scale, but it seems these analyses are not shared with the wider forensic community^{75,76}.

A current challenge facing the use of cosmetic evidence in forensic casework is the collection of that evidence. It has been discovered that crime scene investigators – at both Kent Police and within the Metropolitan Police – do not routinely collect cosmetic evidence at scenes, irrespective of the type or substrate (e.g. smears on textiles or walls, or source material from victims or suspects), even if those traces are immediately apparent, unless there is a particular indication to do so^{72,73}. Furthermore, no post-mortem cosmetic samples are taken from the cadaver (other than routine DNA swabs), either as a reference for further investigations, or as a contact-and-trace specimen⁷⁰. This is a missed opportunity to add potentially valuable evidence to a case and demonstrates the urgent need for scientific validation of these types of trace. Ultimately, evidence that is not collected at the scene, is evidence that is lost.

The implementation of cosmetic trace evidence into criminal cases would be hugely advantageous and the initial research in this area (as is discussed in Section 1.4) is encouraging. In addition to further research, what also needs to change is acceptance of this type of evidence and their perception of its value. Every piece of evidence from a scene has the potential to provide the “*missing piece of the jigsaw*” and cosmetics are no exception.

1.4 Forensic cosmetic research

Following an extensive review of the relevant literature, general themes have emerged regarding the forensic analysis of cosmetic samples. The various approaches fall under one of the following four areas:

- Studying the composition of the samples, listing sample components present, or patterns to look for during an investigation.
- Discrimination of samples using market surveys, or differentiation capability among seemingly indistinguishable samples.
- Identification of chemical markers on various substrates, casework simulations, and investigations into matrix effects.
- Interpretation of evidence, alterations over time, background interference, and transfer and persistence considerations.

All of these approaches are essential to forensic investigation; the aim of which is to determine if a trace is present (detection), if it is possible to link this trace to a sample class (classification), and how to interpret this result with regards to the context of the case, in particular if persistence needs to be considered (interpretation). A schematic representation of the number of publications involving forensic analysis of cosmetic products is given in Figure 1.2, categorised by those examining population variability, contact transfer, and/or persistence.

								
Population	46	10	12	13	10	5	8	4
Transfer	15	6	4	5	5	1	2	4
Persistence	8	1	1	2	3	0	0	3

Figure 1.2: Schematic representation of the number of publications involving forensic analysis of cosmetics focused on examining population variability; contact transfer; and/or persistence. Categories from left to right: Lip cosmetics, face cosmetics, eye cosmetics, nail cosmetics, skin care products, hair care products, glitters, and perfumes. Replicated from Bruce et al.²⁰

Significant challenges arise for forensic scientists when faced with new types of evidence, especially with regards to the identification, discrimination and attribution of a source in casework. This is when the value of chemometric analysis can be realised. Chemometrics (discussed further in Chapter 2) is used complementarily to visual observations of the data, and at different stages over the course of the research. An important focus is the discrimination of pure samples purchased from different markets (“*market surveys*”), in order to build models dedicated to source identification and interpretation. The most common algorithms used in cosmetic product discrimination are hierarchical cluster analysis (HCA) and principal component analysis (PCA), coupled with classification algorithms such as linear discriminant analysis (LDA), support vector machines (SVM), quadratic discriminant analysis (QDA) or k-nearest neighbours (kNN); the choice of technique is determined according to highest discrimination level and most accurate classification rate.

As previously mentioned, the discrimination between decorative cosmetic samples is dependent upon which compounds are detected; accordingly, it is dependent on the analytical method used. However, some sample types preclude the use of certain techniques, for example, the incompatibility of liquid samples with photoacoustic spectroscopy⁷⁷. Consequently, the sample itself may dictate the analytical method used. Spectroscopic techniques are the most commonly employed, with FTIR being the selected method in approximately half of the published papers. Separation techniques and mass spectrometric methods are rarely used and this is likely due to their destructive nature. The next sections will present the analytical and discrimination results obtained for each technique category.

1.4.1 Forensic analysis of cosmetics using separation techniques

One of the most appealing separation methods in forensic science generally, is gas chromatography (GC), yet, this technique has drawbacks when applied to the analysis of some decorative cosmetics⁷⁸. Coupled to flame ionisation detection (FID), GC has been used for the analysis of facial foundations²⁴; its limitation for identifying this type of sample was revealed, with half of the samples providing no chemical profile. The discrimination power (DP) – the probability of discriminating between two samples selected at random from the population of interest⁷⁹ – was calculated at 82%, which is significantly lower than the results obtained via attenuated total reflectance-Fourier transform infrared (ATR-FTIR) and SEM-EDX²⁴. Furthermore, when coupled with both techniques, GC-FID only added an advantage of 0.2% to the discrimination of the samples²⁴. This is due to the incompatibility of GC-FID with inorganic compounds and also indicates a lack of volatile components within these samples.

Gas chromatography mass spectrometry (GC/MS) was more successful when applied to the discrimination of 38 lipsticks⁸⁰. Based on a set of samples previously analysed using ATR-FTIR⁸¹, the DP of GC/MS (0.998) was superlative to that obtained with ATR-FTIR (0.933). Visual discrimination of the GC/MS data created six sample groupings, which were then confirmed using multidimensional scaling (MDS). Although correlations were observed between samples of the same manufacturer and series, no conclusive trends were identified. Focusing on enhancing the quality of the discrimination of the samples, the same set of 38 lipsticks was analysed using micellar electrokinetic capillary chromatography (MEKC)⁸². No definitive trends could be observed among manufacturer, series, or index number and, furthermore, misclassifications were evident for samples originating from the same manufacturer and series. It should be noted though, that this method permitted the discrimination of one pair of samples that could not be achieved using GC/MS⁸². An alternative version of capillary electrophoresis, microemulsion electrokinetic capillary chromatography (MEEKC), was employed for the analysis of red lipsticks. Regrettably, the sample set only

comprised six lipsticks and analyses were performed via visual interpretation of the electropherograms, rather than based on chemometric/statistical techniques. The focus of the research was instead directed towards optimisation of the technique as a tool for the forensic analysis of lipstick traces⁸³.

The lack of research using chromatographic techniques for decorative cosmetics analysis suggests that there are significant limitations when applied in a forensic context. It is not only the destructivity of the technique that must be considered, but also the nature and the chemistry of the different cosmetics that render them unsuitable for GC analysis, e.g., the lack of volatile components. Py-GC/MS is a variant of GC/MS specifically designed for the analysis of involatile samples and has been applied to the investigation of lipglosses⁸⁴. The authors reported that Py-GC/MS allowed the successful visual discrimination of 15 of the 21 samples based on variations in styrene and cyclohexane concentrations. The other samples could not be clearly visually distinguished; however, a subsequent blind validation procedure correctly attributed all randomly selected samples to their respective sources, revealing the potential of Py-GC/MS for sample analysis and discrimination in forensic casework⁸⁴.

Despite garments and bedding being the most commonly encountered substrates for decorative cosmetic residues⁸⁵, fingerprints and lip prints contaminated with cosmetics may also be found in casework^{86–88}. To this extent, a time of flight-secondary ion mass spectrometry (ToF-SIMS) method was developed for the discrimination of lipsticks, various cosmetic creams, foundations and nail polishes that had been applied to a volunteer's hands and lips⁸⁹. When coupled to PCA, the type of product could be identified from the deposited trace, and, moreover, be linked to its source. The persistence of moisturising products on skin has also been investigated using GC/MS⁹⁰.

In an interesting case, the comparison of seized counterfeit perfumes with their genuine counterparts was examined using paper spray mass spectrometry (PS-MS)⁹¹. Trends in the data were observed using PCA, the genuine perfumes having more negative values along PC2. The classification methods, partial least squares-discriminant analysis (PLS-DA) and soft independent modelling of class analogies (SIMCA) were then employed to create models of the data; PLS-DA proved to be the superlative technique with no misclassifications observed. The authors note that, contrary to what might be predicted, the most abundant ions were not the most discriminating variables, and, furthermore, proposed which ions might be the best diagnostic predictors of counterfeit perfumes⁹¹. Perfume variability, their transfer onto substrates of interest, and their persistence thereon, have also been analysed using various GC/MS techniques by Gherghel *et al.*^{92–95}.

Thin layer chromatography (TLC) has been used to profile a wide variety of cosmetic products such as lipstick, lip gloss, hairspray, and moisturising cream. Whilst TLC has been used as a rapid and effective analytical technique in forensic laboratories, it yields limited information about a sample and is generally inferior to the other separation techniques herewith^{63,65,96–104}.

1.4.2 Forensic analysis of cosmetics using elemental techniques

Elemental analysis of decorative cosmetics has been performed using SEM-EDX^{24,105,106}, atomic absorption spectroscopy (AAS)^{29,107–109}, X-Ray fluorescence (XRF)^{27,28,110–113} and inductively coupled plasma mass spectrometry (ICP-MS)¹¹⁴ or coupled to optical emission spectrometry (ICP-OES)¹¹⁵, for the detection of metals.

Two of the AAS studies investigated eyeshadows containing heavy metals^{108,109}. A number of different metals were detected in the composition of the samples; the discrimination between those samples was defined by variations in their relative concentrations. Eyeshadows of a golden colour, for example, contain more cadmium than blue-colored eyeshadows^{108,109}.

Nail polishes have been investigated using portable XRF^{110–112}. The discrimination of 42 nail polishes from five different brands was based on the elements: phosphorus, sulfur, potassium, calcium, titanium and iron, with calcium and titanium content being the main discriminators. Using PCA, five groupings were successfully created and these correlated directly back to the sample brand. A combination of PCA and PLS-DA was used to successfully identify sulfur content within a sample set of 100 nail polishes¹¹¹. XRF has also been used for the discrimination of 39 traditional and mineral foundation samples²⁸. Applying HCA and then PCA, the majority of the mineral-based foundations were separated from the traditional formulations, and correctly clustered into their respective manufacturer groupings. The main indicators of a traditional foundation were the magnesium and calcium concentrations, presumably due to the presence of talc and chalk in the formulations. Differentiation within the mineral-based foundations was achieved by looking at the pigmentation profiles of titanium, iron and zinc, the addition of bismuth, and aluminium-containing clays.

Another set of 53 foundation samples, were subjected to a full sequence analysis of FTIR, SEM-EDX and GC-FID²⁴. Similar elemental profiles were observed across all samples; however, the relative intensities of those elements were highly variable and provided a discrimination potential of 93.8%. Comparatively, SEM-EDX proved inferior to FTIR, but superior to GC-FID. When coupled to FTIR, SEM-EDX analysis elevated the discriminating power to 99.5%, compared to 98.3% for FTIR analysis alone.

SEM-EDX has been employed for the discrimination of 36 glitter and 40 shimmer particles¹⁰⁵. The distinction between the two cosmetic sample types is that glitter comprises man-made

multilayered foil and/or plastic pieces, cut into regular shapes such as hexagons, whereas shimmer is a generic term that encompasses naturally occurring mineral flakes (or their synthetic analogues), such as mica, which are randomly shaped and sized. HCA and PCA yielded similar groupings for the glitter and shimmer samples; the common elements found to generate the discrimination being iron, aluminium, sulfur, silicon, and titanium. Among the results obtained, the ability to discriminate between natural and synthetic versions of mica was demonstrated. Nevertheless, observations of the clusters revealed that samples of different colours were clustered together. This highlights one of the limitations of exclusively using elemental analysis, and the necessity of combining instrumental techniques with the operator's visual assessment for the accurate discrimination of samples.

1.4.3 Forensic analysis of cosmetics using vibrational spectroscopies

Many of the aforementioned techniques are limited by their destructive nature and/or the requirement for specialist instrumentation and user knowledge that may not be readily available in all forensic science laboratories. For that reason, there has been significant interest in vibrational spectroscopy modalities such as ATR-FTIR and Raman spectroscopy as powerful, non-destructive (samples can be analysed *in situ*) and accessible methods for the analysis of cosmetic traces. They require little to no sample preparation, thereby minimising the risk of sample alteration or contamination. Indeed, Raman spectroscopy was found to be effective at analysing samples through plastic evidence bags, further mitigating any sample interference or degradation as well as reducing any risk to the operator¹¹⁶. These techniques provide complementary information on the rotational and vibrational modes present in a material with minimal sample preparation and are suited to samples in both solid and liquid forms.

The largest category of decorative cosmetics investigated using spectroscopic techniques is lipsticks, representing over half of the literature focused on these types of samples. Analysis is performed more frequently with ATR-FTIR than with Raman spectroscopy, but the reason for this preference is unclear. The populations of samples are fairly similar: the use of around 40-50 samples is reported when running ATR-FTIR analysis^{81,100,117–125}, whereas between 50-80 samples are used for Raman analysis^{116,126–128}. The sample pools are relatively different, with products being sourced all over the world, nevertheless, the conclusions are all congruent as are discussed herewith.

1.4.3.1 Forensic analysis of cosmetics using infrared spectroscopy

With regards to ATR-FTIR analysis, lipstick samples are found to primarily cluster in accordance with their colour¹¹⁷. The most effective use of PCA is to firstly separate the

samples into groups based on visual observation of their colour^{117,119}. Wax and liquid lipsticks generate different chemical patterns based on their silicone and organic content, leading to an additional level of discrimination¹¹⁷. The variability between samples collected from the same manufacturer and series (or range) is found to be less than between samples originating from the same manufacturer but from a different series⁸¹. All the studies report the use of PCA and LDA for the discrimination and classification of lipsticks, and within these datasets, all the lipsticks are differentiated based on either the brands or the brands and series. Correct classification rates are found to vary depending on the colour of the sample, suggesting that the homogeneity and composition of the samples are important factors^{47,81,117,119,120,129}.

Red and nude shaded lipsticks (including wax, liquid and gloss formulations) were studied using ATR-FTIR by Wong *et al.*¹¹⁷. The authors noted distinct changes in the spectra of liquid lipsticks in the ten minutes immediately following application, presumably due to drying. They noted the significance for sampling within a forensic context, as traces recovered at a scene are likely to be days to weeks old rather than freshly deposited. PCA determined that although many products exhibited similar formulations, certain products had distinct chemical components; LDA models produced classification accuracies between 93 and 100% depending on colour. These models were validated using a test set deliberately selected to reflect a range of similar and dissimilar samples, retaining a 93% classification for red-shaded lipsticks but a reduced classification (when compared to the original LDA classification) of 73% for nude-shaded samples¹¹⁷.

Gladysz *et al.*^{81,130} have performed multiple studies using ATR-FTIR and other techniques for the analysis of lipsticks. Initially, they studied 38 red wax-based lipsticks across several brands, using ATR-FTIR coupled to PCA and cluster analysis. They demonstrated that whilst lipsticks could be grouped, they showed no clear trends by shade or manufacturer, similar to previous Raman studies^{126,128}. They later studied seven red lipsticks by ATR-FTIR alongside GC-MS and capillary electrophoresis¹²¹. All traces were analysed by smearing lipstick onto substrates such as paper, cotton and glass and storing under different conditions. Analysis was then performed at varying time intervals over the course of 12 months. Additional variables such as transfer by different individuals, the length of time lipstick had been worn prior to deposition, and the impact of smoking or beverage consumption on the resultant transfer were investigated in an attempt to model likely crime scene trace. They saw significant spectral changes amongst products with oily or liquid formulations whilst “*drier*” samples remained unchanged. Additionally, they reported that light appeared to accelerate changes in chemical composition, independent of substrate.

Sharma *et al.* studied 25 non-branded, unregulated marketplace lipsticks which were “kissed” onto paper substrates⁴⁷. They reported 100% discrimination between spectra using factor analysis and 99% using HCA/k means clustering techniques using an average spectrum generated from five replicates for each sample. Given many of the samples are closely arranged in the reported scores plot, this may suggest that use of the original spectra could have resulted in some sample overlap lowering the discrimination rate. It is also worth mentioning that due to the unregulated nature of these samples, there is less control of ingredients and/or manufacturing processes; this may result in the high sample differentiation seen.

Foundations have been studied far less than lipsticks, with only three papers detailing the use of vibrational spectroscopic methods. For example, 31 cosmetic foundation creams from 23 brands were investigated using ATR-FTIR, with samples deposited onto wet and dry cotton substrates¹³¹. Visual comparison of the spectra resulted in a discrimination power of 98%, however, once PCA was applied to the data the discriminating power rose to 99.3%, and increased further to 100% upon combination with LDA, albeit by using averaged spectra.

Similarly, liquid foundations across three different brands on four different fabrics (including cotton blend and acrylic) have been analysed using near infrared (NIR) spectroscopy by Skobeeva *et al.*²³. They chose to interrogate their NIR spectra using PCA-LDA and noted that the lighter shaded foundations yielded a more accurate classification rate (96.7%) than those of the darker shaded foundations (90%). The proposal was that this was due to the optical effects of the white titanium dioxide pigments and black iron oxide pigments respectively. Despite optical effects arising from the darker pigments, resulting in almost featureless spectra, and standard normal variate (SNV) pre-treatment surprisingly failing to improve models, the authors still reported 93% discrimination rates from PCA-LDA using a test set. It appears that this was largely based on differences in background position rather than resolvable spectral features. Whilst NIR appeared to be a promising technique, the authors concluded that the use of ATR-FTIR would likely result in higher classification rates.

Gordon and Coulson investigated and compared the effectiveness of FTIR, GC-FID and SEM-EDX for the forensic analysis of 53 foundation samples²⁴. These samples represented a cross spread of the New Zealand cosmetic foundation market and were analysed straight from the source as well as deposited onto cotton substrates. FTIR was found to be the best single technique with 98.3% discriminating power, although challenges were encountered when analysing samples containing talc as this dominated the spectrum. Interestingly, there was no apparent trend between pairs of indistinguishable products and the manufacturer or formulation, consistent with what has been reported for lipstick samples^{126,128}. A small number

of foundations were also unable to be distinguished from the cotton substrate due to absorption of the product into the fabric on application.

Eyeliners and mascara traces have also been studied by ATR-FTIR on paper²⁵. PCA and PLS-DA were performed on data acquired from 102 cosmetic samples (62 eyeliners from 37 brands and 40 mascaras from 24 brands), developing a classifier capable of distinguishing between the two categories with 95% accuracy. However, individual product identification was not examined. A subset of samples was also analysed on cotton fabric and wet versus dry tissue paper. Minor contributions from the substrate were observed, which could be adequately removed through background subtraction, although it is noted that this resulted in the loss of some minor peaks associated with the cosmetic, which may need to be considered when taking discrimination into account.

The forensic analysis of nail varnishes has received moderate attention, with four studies using ATR-FTIR^{132–135} and one using FTIR microspectroscopy²⁶.

Mixing decorative cosmetic types likely to be encountered together in casework, into a sample dataset, yields extremely useful information. Work by Chopi *et al.* demonstrate this by analysing a selection of eyeliners and eyeshadows¹³⁶. PCA of the eyeliners initially showed good separation of the samples, but when triplicates of some of the eyeliners were plotted, they were not in close enough proximity to one another, to permit interpretation. The PCA scores plot for the eyeshadows showed clustering for the majority of the samples but also some overlapping results.

ATR-FTIR has been used to investigate 36 cosmetic glitters from a single manufacturer covering a wide colour palette¹³⁷. Najjar *et al.*, demonstrate that glitter classes are separated based on their acrylate, polyethylene terephthalate (PET) and polybutylene terephthalate (PBT) composition. HCA analysis separated ATR-FTIR spectral data into clusters. Subsequent PCA suggested that the data could be classified into 6 groupings. Validation of this model was investigated using LDA, kNN and SVM methodologies. LDA resulted in 99.2% classification rate, with 11 misclassifications due to the spectral similarities between some of the groupings. In contrast, both kNN and SVM gave higher classification rates of 100% and 99.3% respectively, with the latter only misclassifying one pair. However, it is not clear from these data if the groupings relate to any specific colours and thus dyes¹³⁷.

Vernoud *et al.*¹³⁸ studied four layered glitter samples from a single manufacturer using synchrotron FTIR microscopy. The use of synchrotron facilities provides data with increased resolution and intensity, enabling cross-sectional studies to be performed at the micro-level. Within a single cross-section, seven spectrally distinct layers were able to be readily resolved. Layer thicknesses and composition then allowed for samples to be discriminated. However,

from a forensic perspective, access to synchrotron facilities can be limited and expensive, restricting their applicability to casework, except perhaps in high-profile cases.

Another illustration of spectroscopic cosmetic analysis can be seen by Ricci *et al.* who used ATR-FTIR imaging coupled to PCA, to detect traces of face cream, foundation, body butter, and body lotion in deposited fingerprints on nonporous surfaces¹³⁹. The contaminated fingerprints were recovered via gelatine lifters; the resultant spectra demonstrated that, despite the traces being invisible to the naked eye, they were successfully identified with this technique. Furthermore, fingerprints contaminated with traces of lip gloss were deposited upon a polycotton shirt (porous surface) and subjected to ATR-FTIR analysis. Reconstruction of the fingerprint pattern was not possible, nonetheless, the lip gloss traces were successfully located on the garment.

Vermilion, in the form of Sindoor, has also been investigated as a type of cosmetic evidence¹⁴⁰. Sindoor is used as a cosmetic powder in some religious and cultural practices. Chophi *et al.* analysed 42 vermilion samples using ATR-FTIR and performed PCA-LDA on the data set, achieving a 95% calibration accuracy¹⁴¹. However, this level of accuracy could only be reported for PCA-LDA models which were generated using a truncated spectral range. When the model was generated using the entire spectral range, the accuracy fell to 78%. This indicates that the initial models were based on highly variable spectral regions. This opens debate around sampling ranges and the consideration of data which should be incorporated into models to enable forensically valid conclusions to be reached.

The preceding discussion has focused on the detection and classification of decorative cosmetics for forensic purposes. However, in the literature, the analysis of other cosmetics can be found^{125,142}. For instance, the discrimination of facial moisturising creams was investigated using ATR-FTIR coupled to chemometrics¹⁴³. ATR-FTIR was used to analyse a set of 57 cosmetic samples comprising herbal and non-herbal creams; the PCA and LDA tools highlighted two clear groups of samples based on the sample type. Moreover, the ATR-FTIR discrimination of 109 sunscreens¹⁴⁴ revealed that separation of the PCA classes was found to be primarily due to the presence/absence of solvents, which are likely to evaporate and secondly, based on the mineral composition (talc and mica) and paraffin concentrations. Nevertheless, the solvent evaporation poses some ambiguity as it is unknown whether or not the solvent had already started to evaporate between the time of deposition and the subsequent analysis. This may render discrimination based on solvent concentration unreliable.

1.4.3.2 Forensic analysis of cosmetics using Raman spectroscopy

Gardner *et al.* reported the Raman spectroscopic analysis of 80 red, pink, brown and purple-brown lipsticks, from 23 different manufacturers, across 40 different product lines¹²⁷. They utilised laser wavelengths of both 532 nm and 780 nm, noting the latter was more effective for minimising the background of the fluorescent samples¹²⁷. Yet, when the researchers analysed minimally pigmented lip cosmetics (such as lip glosses or lip balms) they found the products yielded very similar spectral profiles, inhibiting discrimination within brands¹⁴⁵.

Similarly, López-López *et al.* used confocal Raman microscopy to study 49 lipsticks encompassing different brands and product lines, in different colours (red, pink, brown and purple)¹²⁸. They did not report high discriminatory rates but rather that the data could be assembled into 30 different groups, which showed no relationship between brand or colour¹²⁸. Five of the red lipsticks were further “kissed” onto 12 different substrates including fabric, glass, paper (cigarettes), metal and plastic household objects to simulate natural contact transfer. The authors found that the substrates did contribute to the spectrum, but that the cosmetic remained identifiable (by comparison with the original spectral data) within the composite spectrum. A substrate spectral subtraction was trialled, importantly noting that this approach could introduce both new and negative bands in the resultant spectrum. This finding is significant as it shows that spectral subtraction techniques may yield a spectrum that is not representative of the original sample.

Salahioğlu and Went investigated 69 brown, pink, and red lipsticks across ten different brands using Raman spectroscopy¹¹⁶. The authors found that whilst 15 of the lipsticks gave distinct spectra, the remaining 54 could only be grouped into seven families based on their peak profiles (chemical composition)¹¹⁶. These groupings did not always follow those expected for either colour or brand in line with the observations of López-López *et al.*¹²⁸. They also noted that red lipsticks were the most difficult to distinguish, with all the red lipsticks analysed falling into a single group. A caveat to this study was that a large proportion of the lipsticks analysed were also obtained via donation in the form of store testers, introducing doubt as to the origin of spectral variability (due to potential contamination) and thus, the reliability of the results.

The authors later extended this study to look at lipstick traces on forensically relevant substrates such as glass, tissues and cigarette filters, alongside ageing studies¹²⁶. They demonstrated that whilst the spectra for some lipsticks remained unchanged over long periods of time (up to two years), others saw changes related to individual spectral peaks. The authors made no assertion as to the likely origin of this phenomenon, but one can assume it may relate to either the material's consistency, the loss of water content, or chemical degradation of components. A sub-set of lipsticks were also analysed *in situ* on different substrates. The

authors found that fluorescence arising from the various substrates could largely be mitigated using different laser wavelengths, concluding that a 473 nm laser was best suited for these analyses. This contrasts with the Gardner *et al.* study, which found that a 780 nm laser was most effective¹²⁷.

Improvement of the visual discriminatory power by Raman spectroscopy has also been investigated for a small number of red wax-based lipsticks using surface enhanced resonance Raman spectroscopy (SERRS)¹⁴⁶. SERRS involved the addition of surfactants containing silver nanoparticles to a lipstick smear on either glass or cotton. The authors investigated the best surfactants for providing reduced fluorescence and high discrimination and concluded that poly(L-lysine) was the most favourable, considerably quenching the background fluorescence. However, the effectiveness of this protocol on more contemporary formulations such as liquid lipsticks, glosses or balms remains untested.

A combination of ATR-FTIR and Raman spectroscopy was used to investigate 20 pink MAC branded lipsticks by Alblooshi *et al.*¹²². These authors found that a 780 nm laser was best suited for Raman analysis of these lipsticks. However, it is not clear whether this relates to enhanced spectral information or removal of fluorescence. With the exception of eight pairings, the majority of these samples could be visually differentiated from each other. It was also reported that Raman was more effective than ATR-FTIR at differentiating between lipsticks presumably as a result of differences in inorganic components which are more readily probed using Raman spectroscopy. Nonetheless, this study only considered MAC lipsticks so the conclusions drawn are only applicable to a specific sample set and thus may not be representative of wider lipstick populations.

Zhang and co-workers performed shifted excitation Raman difference spectroscopy (SERDS) at a wavelength of 785 nm for 70 lipstick samples¹⁴⁷ deconvoluting the spectra using a back-propagated neural network. *k*-means clustering combined with PCA gave a reported 99.88% discrimination. However, only 34% of the total variance was accounted for in the first three principal components, suggesting a high level of sample variability that may pose challenges in applying this model to expanded sample sets.

The analysis of kajal samples was carried out by Asri *et al.*¹⁴⁸ using a combination of ATR-FTIR and Raman spectroscopy. Seven brands of kajal samples were applied to substrates including dry tissue, wet tissue, cotton, nylon, polyester and plastic. Interestingly, PLS-DA modelling allowed for 100% discrimination of the samples analysed via Raman, but only 86.7% for the ATR-FTIR analyses. This difference is likely due to the better suitability of Raman spectroscopy for the analysis of pigments^{149–152}.

López-López and co-workers¹⁵³ used confocal Raman spectroscopy to examine 56 regular and 21 gel-based nail polishes. In keeping with other Raman studies discussed herein, they found that the 780 nm laser was superlative to the 532 nm laser due to sample burning and fluorescence effects^{122,127}. In addition to analysing the nail polishes on aluminium foil, they also investigated nail polish transfer smears on white paper, flakes of polish, and polish removed from a volunteer's nails using acetone-soaked cotton swabs. All methods yielded spectra where the sample could be identified but substrate interference remained an issue. Most importantly, this study highlighted the problem of sample heterogeneity, with nearly 20% of the nail polishes exhibiting distinct spectral variations across replicates. Visual comparison of the Raman spectra revealed that only 64% of the samples yielded a characteristic spectrum, further highlighting the need for chemometrics when analysing complex, multivariate data.

Finally, 40 facial moisturising creams have been profiled using a combination of Raman spectroscopy with PCA and PLS-DA¹⁵⁴. Despite claiming 100% correct classification using PLS-DA, the PCA revealed that only 69% of the total variance was accounted for in the first four PCs. Furthermore, the authors used a limited Raman spectral range of 400 cm⁻¹ – 2000 cm⁻¹ and, despite the study using 40 moisturising creams this only represented eight different products, thereby increasing discrimination potential¹⁵⁴.

1.5 Motivation and aims

Facial cosmetic samples are complex mixtures of organic and inorganic components that are present in varying proportions, and thus offer a rich and plentiful source of discrimination, yet relatively few researchers have chosen to analyse facial makeup when compared to lip and eye cosmetic products. It is assumed that this apparent cosmetic selection bias relates to people's perception of product use, and therefore its value in a forensic context. Nonetheless, the discussion in Section 1.3 argues that facial makeup may be more prevalent as trace evidence, owing to the larger surface area application (and therefore higher incidence of transfer), and the increasing popularity of these kinds of cosmetics in society.

Although the focus of this research is the analysis of facial cosmetic evidence within a crime scene context, the scope of forensic science practice is broad and could be used for alternative purposes. For example, analysis of cosmetic products could also help in the detection of counterfeit products by manufacturers, with the regulation of cosmetic approved components for statutory bodies, i.e. toxicity level determination, and in consumer authentication, for example, trading standards, appropriate labelling, or misleading information.

Only five papers discovered thus far detail the forensic analysis of facial cosmetic or skin complexion products,^{23,24,28,89,131} but none of these utilise Raman spectroscopy. It is therefore

proposed that the application of Raman spectroscopy to the forensic analysis of facial cosmetic products is novel.

Initial visual discrimination between samples entails simply observing the colour of the sample. However, discriminating between cosmetic samples based on visual comparison alone is a risky approach, as the thickness of the cosmetic trace may alter its perceived colour¹⁵⁵. Furthermore, cosmetic foundation samples that may appear visually similar by eye, can look very different when viewed on a microscopic scale¹⁵⁶. Incidentally, this hypothesis is refuted by Cho *et al.* when considering nail polishes²⁶. The variation between facial cosmetic product shades is often minor and owing to the thin layer applied, this makes visual identification difficult and unreliable²⁴. This is primarily due to vision differences between individuals but even in cases where this does not apply, visual colour assessment is still subjective¹⁵⁷. Additionally, it has been experimentally demonstrated that the apparent observed colour of transparent or translucent glitter particles or interference pigments, changes depending on the orientation of the sample illumination and upon the angle of viewing, making visual discrimination highly subjective and circumstantial^{69,155,158}. Furthermore, visual assessment cannot be quantified, which poses problems for reporting and comparison. Therefore, the addition of analytical techniques capable of yielding chemical information greatly augments a visual assessment of a sample.

The published literature clearly demonstrates the power of Raman spectroscopy for the non-destructive (*in situ*), cheap and effective analysis of decorative cosmetic samples, particularly in combination with emerging methods of statistical data analysis. Furthermore, the limited information on inorganic components that ATR-FTIR affords, poses a significant problem when analysing mineral makeup that exclusively comprises metal oxides and micas, such as the BareMinerals Original Foundation range. Therefore, facial cosmetics such as those described in Section 1.2.2.1, would benefit from a Raman spectroscopic analytical approach combined with powerful MVA methods. This presents an opportunity to add to the body of knowledge and thus, forms the basis of this thesis.

Previous forensic cosmetic analyses have demonstrated that there are frequently experimental barriers or instrumental limitations. It may be the case that Raman spectroscopy is able to circumvent these issues but at the very least this technique may be used in conjunction with other methods in order to provide the maximum amount of chemical detail and the highest possible level of discrimination. Raman microspectroscopy in particular, affords the important advantage over many of the other techniques described in this Chapter, of being able to provide a colour photomicrograph of the sample area of interest. Not only does this aid in determining microscopic structure and degree of homogeneity but would also

allow a lay jury access to an image of the sample which may help them to understand and interpret the complex data.

Care needs to be taken in decisions about how to treat background-related effects in spectral data. It is not an easy task to determine if background variation is linked to sample variability, small changes in measurement conditions (e.g. focusing in Raman spectroscopy), or to matrix (substrate) effects. It is essential not to remove true chemical variability, but more importantly not to introduce variability where there is none. Background treatment has largely focused on the use of SNV treatments aimed at background smoothing and minimising the effects of background slopes²⁵. More recently, Liu *et al.* have demonstrated that machine learning and in particular, convolutional neural networks (CNN), are capable of identifying substances from noisy spectra without the need for pre-processing¹⁵⁹. Nonetheless, all types of spectral pre-processing must be carried out conservatively to avoid overfitting of the data; overzealous processing could result in spectra that bear little resemblance to their original version, invalidating variability or similarity conclusions drawn. Therefore, a variety of spectral preprocessing methods will be trialled, with a view to applying the minimum amount possible.

To overcome substrate effects, authors have looked at collecting spectral data for blank substrates and then simply subtracting this from the spectral data of interest⁴⁷. Generally, this approach has been successful, but there are instances where this either results in negative peaks or removes sample peaks of interest that happen to overlap with those of the underlying substrate¹²⁸. Few studies consider substrate variability and the effects that these may have on the resultant spectral variability. It has been noted previously that the ability to discriminate between samples is also dependent on colour of substrate and thickness of smear¹²⁷. It is clear that further research is required in this area to understand the complexities that are added through choice of substrate. With this in mind, efforts will be directed towards obtaining data across a large number of samples placed on standard microscope slides, to acquire representative spectra that illustrate the underlying chemical variability, rather than experimenting with different substrates. Attempts at modelling the impact of substrate variability are futile if the baseline data upon which models will be projected are not available.

When discussing substrates, it is also important to mention the growing use of SERRS and other enhancement methodologies such as those reported by Rodger in their analysis of lipsticks¹⁴⁶. These methodologies are typically dependent on resonance effects between substrates (or nanoparticles) and the material of interest. The resonance effects enhance spectral features at wavelengths related to the resonance. With complex mixtures, such as those expected for decorative cosmetics, understanding both the chemistry and the physics behind spectral behaviour becomes critical as variation may not be inherent to sample

differentiation but rather the underlying chemistry and physics of using such enhancement methods. Thus, the benefits and necessity of using Raman spectral enhancement methodologies need to be carefully considered with respect to their applicability to forensic casework. For this reason, standard confocal Raman spectroscopy will be applied to the differentiation of cosmetic samples.

The discrimination of samples based on the observation of an acquired chemical profile or spectrum is highly dependent on the analytical method used. Visual discrimination of chemical profiles is mostly derived from the presence and/or absence of peaks, and differences in the relative intensities of those peaks, indicating that the samples are not identical. Some chemical profile differences may appear obvious but minor differences will be affected by operator interpretation and may also be extremely time consuming. Consequently, it is necessary to employ chemometric techniques for objective and unbiased data analysis of these forms of trace evidence^{110,160}.

Many authors have reported a discriminatory power (DP) based on the visual variability of spectral information^{131,144}. However, it remains unclear in much of the literature what level of visual variability constitutes discrimination between two samples; assignments may be arbitrarily decided. Additionally, statistical calculations are hugely dependent on the number of samples being analysed, and it is well known that increasing sample numbers decreases our likelihood of discriminating all possible pairs. For this reason, we need to be careful with the weighting given to DP values. Throughout this thesis, chemical variability will be assessed using well-established chemometric techniques that will ensure objective measures of differentiation. Moreover, the largest possible number of samples will be analysed in each Chapter so that the maximum amount of variability can be assessed, rather than minimising sample numbers in favour of quoting superior statistical accuracies.

The contradicting results reported across different studies may link back to the choice and number of samples, as researchers may naturally gravitate to availability within a price bracket rather than considering samples which might represent a true market survey. In that vein, we need to be mindful of the global market and account for jurisdictional differences in cosmetic manufacture and/or regulation. For example, an ingredient that is permitted for use in cosmetic products in one country, may be prohibited in another. Furthermore, given the increase in globalisation it is also pertinent to consider that international cosmetic products may be of relevance in investigations beyond country of origin.

Insofar as is reasonably practicable, the range of cosmetic samples will be chosen from as many different manufacturers and across as many different product lines, shades, price brackets, and formulations as possible. Furthermore, as has been discussed earlier in this

Chapter, facial cosmetics are designed to be worn in a layered sequence with likely resultant mixing. Therefore, products from all seven of the product types detailed in Section 1.2.2.1 will be assessed so that a general model that takes all products into account can be constructed. Where possible, samples will be obtained from countries other than the UK to check for differences across the global market.

1.5.1 Thesis outline

This Chapter has introduced the concept of associative trace evidence and discussed how cosmetics fit this notion. The chemical compositions of decorative cosmetics specifically have been considered in the context of spectral interpretation and a detailed review of the literature has been conducted.

Chapter 2 gives an overview of Raman spectroscopy, its theory, instrumentation, limitations and methods to circumvent them, and its application to forensic trace analysis. It also gives an introduction to chemometric methods, in particular, principal component analysis (PCA), and finishes with an account of experimental parameter determination.

Chapter 3 is the first of the four results chapters and details the use of Raman microspectroscopy for the assessment of chemical variability amongst 126 newly purchased facial cosmetic products, and their subsequent PCA results.

Chapter 4 takes 44 different newly purchased facial cosmetic products of varying types and determines the level of spectral heterogeneity between them using a combination of PCA and PCA projection: a classification technique.

Chapter 5 builds upon the knowledge gained in Chapters 3 and 4 and investigates the effects of ageing on a selection of newly purchased foundation samples. Evaluation combines visual microscopic assessment with Raman spectroscopic analysis and chemometric interpretation.

Chapter 6 applies the same microscopic, spectroscopic and chemometric analyses to a substantial group of old, expired and used donated cosmetic samples, to assess the impact of advanced ageing, sample mixing, and contamination.

Chapter 7 gives overall conclusions and provides suggestions for future work.

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Chapter Two: Experimental methods

2.1 Introduction

This Chapter gives an overview of Raman spectroscopy, chemometrics, and the experimental procedures used for the characterisation of the cosmetic products investigated during this project. Any deviations from the standard procedure are discussed in the experimental sections of the relevant Chapters.

2.2 Raman spectroscopic theory

The Raman effect was discovered in 1922 by Sir C.V Raman. He reported this effect, which he termed a “*modified scattered radiation*”, in the journal *Nature*, in 1928¹. Due to the Raman effect being an inherently weak process, there was no commercial usage at that time, however, advances in lasers and sensitive detectors from the 1960s have realised its potential as an analytical technique.

When light interacts with a molecule, it can be reflected, absorbed, transmitted, or scattered. When light is scattered this involves a change in direction, and this forms the basis for Raman spectroscopy. Light photons interact with the electron cloud surrounding the molecule causing it to distort and shift to a higher energy “*virtual*” state. As this effect is unstable, the photon is quickly scattered from the molecule, allowing the molecule to relax back down to one of its “*ground*” states but this can happen in one of two ways: Rayleigh or Raman scattering (Figure 2.1).

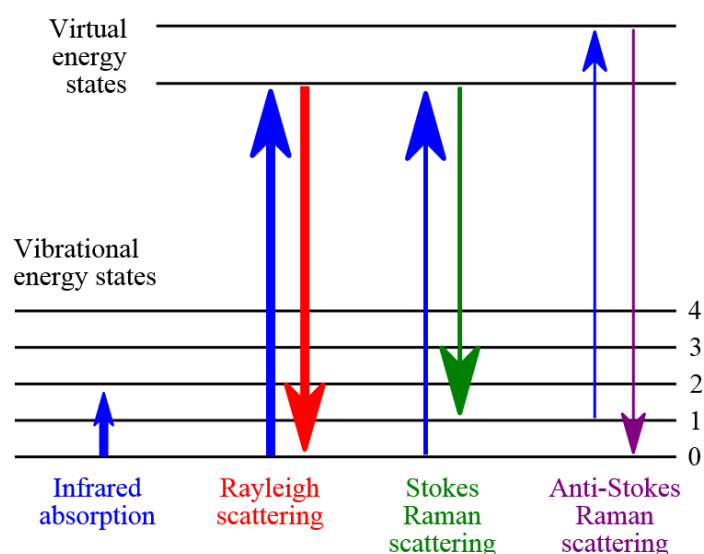


Figure 2.1: Energy level diagram showing the Rayleigh and Raman scattering processes, adapted from Unruh and Meyers².

Rayleigh scattering is an elastic process and the majority of photons are scattered in this manner. After the incoming photon has interacted with the molecule's electron cloud, it is released with no appreciable change in its energy state. Conversely, Raman scattering is an inelastic process as the photon is released from the molecular complex with a change in energy. In Stokes scattering, the incident photon polarises the electron cloud of a molecule in a low energy vibrational state and promotes it to a higher energy vibrational state. This results in the photon being scattered with less energy than the incident photon and describes the majority of Raman scattering that will occur in materials at room temperature. If the molecule is already in an excited state – as might be the case at higher temperatures – then it will transfer energy to the incident photon, such that the photon is scattered with a higher energy. This is called anti-Stokes scattering. Raman scattering is a significantly weaker process than Rayleigh scattering, with only one in approximately every ten million incident photons being Raman scattered³. Stokes scattering tends to be the preferred analytical mode as anti-Stokes is an even weaker process, but the information gained from both inelastic scattering modes is equivalent.

The difference in energy between the incident and the Raman scattered photons result in Raman shifts that are plotted as peaks on a spectrum. This means it is important to use an excitation source that has only a single frequency so this Raman shift distinction can be made accurately, hence a monochromatic laser source is used to irradiate the sample. Traditionally Raman spectrometers use visible lasers but near-infrared (NIR) and ultra-violet (UV) sources may also be utilised.

2.2.1 Raman spectral interpretation

Each spectral peak corresponds to an interaction with a molecule. The position of a peak along the x-axis (Raman shift) denotes the magnitude of the energy shift and these are given in wavenumbers with the units cm^{-1} . The intensity of each shift (peak) is measured along the y-axis in arbitrary units or counts. By convention, Stokes scattered shifts appear on the positive side of the Raman spectrum (i.e. as positive values) despite them being associated with a loss of energy from the incident photon and therefore a negative shift. Anti-Stokes shifts are plotted as negative shifts and appear on the opposite (negative) side of the spectrum.

Molecular bonds vibrate at specific, characteristic frequencies meaning that functional group assignments can be made. Lighter atoms, e.g. C–H vibrations, give rise to peaks that appear in the higher frequency end of the Raman spectrum ($\sim 3000 \text{ cm}^{-1}$), whereas heavier atoms, metal oxides, and vibrations associated with crystal lattices appear at the opposite end of the spectrum ($\sim 100\text{--}500 \text{ cm}^{-1}$). The strength of a molecular bond also has an effect on the Raman peak position. The more electron pairs two atoms share, the stronger the bond between them

and the higher the Raman shift. Ergo, a C–C bond appears in the 1200 cm^{-1} region, but a C=C bond appears around 1650 cm^{-1} , and a C≡C bond is further along in the 2200 cm^{-1} region.

The intensity of a Raman peak is related to the degree of polarisability of the molecule, i.e. how easily the electron cloud can be perturbed by an external electromagnetic field, which in this case is laser light. The oscillating electric field produced by the laser light induces a dipole moment in the molecule, in other words, it becomes polarised⁴. This induced dipole gives rise to Raman scattering and governs which molecular vibrations are Raman active, as per the basic selection rule. If there is a polarisability change during a molecular vibration, then the molecular bond is Raman active. The strongest Raman peaks are those due to symmetric vibrations, as these induce the greatest change in polarisability. This is contrary to IR spectroscopy where the strongest peaks are due to a change in dipole, meaning the asymmetric vibrations are more intense in IR spectra¹. These differing modes mean that IR and Raman spectroscopy are regarded as complementary techniques and using both to interrogate a sample often yields better information than one technique alone.

The intensity of a Raman peak is also governed by the frequency and power of the laser light. Higher frequency (i.e. shorter wavelength) lasers are powerful and give high signal intensities but also suffer from unwanted effects such as sample warming and/or photodecomposition. Examples of high energy lasers include argon ions operating at 488 or 514 nm⁵. Lower energy lasers such as He-Ne and diode operate at wavelengths around 633 nm and 785 nm and may circumvent these problems but they can also suffer from fluorescence effects, particularly when analysing biological samples¹. Even lower energy lasers that operate in the near-IR region around 1064 nm, e.g. Nd-YAG, can mitigate all these unwanted effects but due to the 4th power of frequency factor, the Raman intensity is considerably reduced and this impacts on the signal-to-noise ratio. Increasing acquisition times can mitigate this lack of intensity but obviously results in a longer analytical time, which may be problematic in industry.

2.2.2 Raman spectrometer instrumentation

Raman spectrometers come in two main types: dispersive (conventional) or Fourier-transform (FT-Raman). Dispersive Raman spectrometers have far higher sensitivity and lower detection limits than their FT counterparts. Raman spectrometers may also be coupled to a light microscope enabling analysis at the microscopic level. A schematic of a typical Raman microspectrometer setup is given in Figure 2.2.

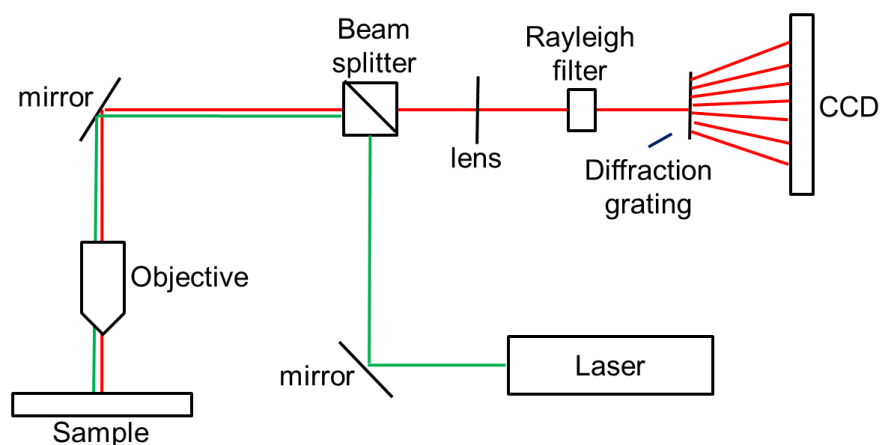


Figure 2.2: Schematic showing the basic dispersive Raman microspectrometer setup.

Laser light enters the spectrometer and is guided via a series of mirrors and conduits down through the microscope objective onto the sample of interest. The sample is mounted onto a motorised stage and the laser light is focused onto the sample's surface via a digital camera. The scattered light is collected via the same objective and guided back into the spectrometer where it passes through focusing lenses and a Rayleigh filter in order to remove the elastically scattered light but retain that which is Raman scattered. This light then passes through the entrance slit and a confocal pinhole and strikes a diffraction grating, where it is dispersed into its separate component wavelengths. From there, the light is collected by a detector; usually a charge-coupled device (CCD) and the electrical signals are converted by the operating software's algorithm into a Raman spectrum. Prior to spectrum acquisition, the spectrometer will need calibrating, usually against the silicon line at 520.6 cm^{-1} , to ensure the subsequent Raman peak positions are correctly sited.

2.2.3 Spectral fluorescence

Raman spectroscopy suffers from one main complication: fluorescence. This is an emission process that occurs when the molecule receives enough energy to be excited beyond the required virtual state and into a higher energy electronic state. Upon relaxing back down to the ground state, light of a different wavelength is emitted. Unfortunately, due to this change in wavelength, fluorescence will bypass the Rayleigh filter. Fluorescence is a far more efficient process than Raman scattering and therefore overwhelms the spectrum, masking true Raman peaks (Figure 2.3). This effect can be mitigated by using lower energy lasers, so the electrons are not promoted to the higher energy levels needed for fluorescence to occur.

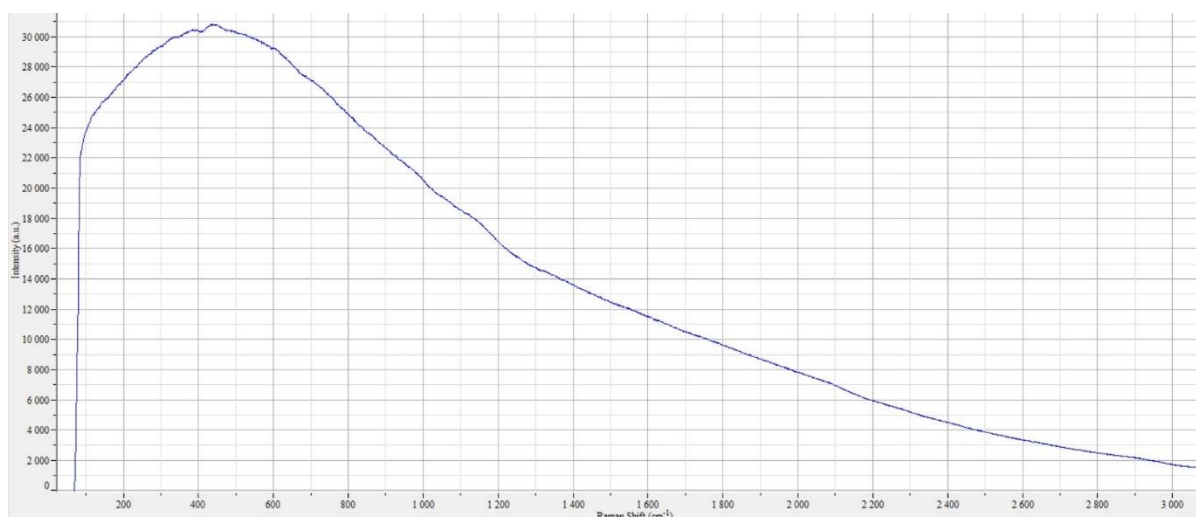


Figure 2.3: An example of a fluorescent Raman spectrum.

Fluorescence occurs as a result of impurities in the sample of interest: fluorophores or chromophores. Unfortunately, forensic evidence cannot be manipulated to remove such impurities as this would compromise the integrity of the sample. Furthermore, cosmetic trace evidence is often coloured, i.e. contains chromophores, and will therefore be prone to fluorescence. Nevertheless, there are other advanced forms of Raman that may help combat fluorescence, whilst maintaining the integrity of the sample; examples of these are resonance Raman spectroscopy (RRS) and surface enhanced Raman spectroscopy (SERS). These techniques work by increasing the number of Raman scattered photons: in RRS the laser excitation wavelength is specifically chosen to promote electronic transitions to the excited state, whereas SERS uses a metal surface to amplify the intensity of the Raman scattered light. A combination of the two techniques, surface enhanced resonance Raman spectroscopy (SERRS), can result in even better amplification of Raman signal. However, given that many forensic analyses are carried out on unknown samples, the utility of RRS may be minimal.

Other advanced techniques include: stimulated Raman spectroscopy (SRS), coherent anti-Stokes Raman spectroscopy (CARS), tip-enhanced Raman spectroscopy (TERS), coherent Stokes Raman spectroscopy (CSRS), and shifted excitation Raman difference spectroscopy (SERDS). However, it must be noted that these techniques often require specialist sample preparation, which may alter the integrity of the sample and would therefore be undesirable for forensic applications. Some of these techniques are also only applicable to certain sample types. Resultant spectra are often complex and difficult to interpret, hampering efforts at spectral library searching and matching.

A relatively easy method of reducing fluorescence is sample photobleaching. This is achieved by irradiating the sample of interest with laser light for a given amount of time prior to spectrum

acquisition, in order to cause photodecomposition of the fluorescent molecules. Nonetheless, photobleaching must be used with care to prevent sample degradation or chemical alteration.

Another simple method of removing fluorescence from a Raman spectrum is via the use of various spectral preprocessing techniques, such as linear or polynomial baseline correction. This involves either applying an automatic correction so that the algorithm decides how best to improve the spectrum, or by manual baseline correction whereby the operator decides where and by how much the spectrum should be corrected. Both methods have their drawbacks; spectral preprocessing may remove minor but important peaks, or add spurious peaks, artificially skewing the data.

2.2.4 Applicability to forensic science

Raman spectrometers are now more efficient and affordable than their earlier counterparts, making them desirable instruments in a forensic laboratory. The principal benefit of Raman spectroscopy is its non-destructive nature; in forensic science, maintaining the integrity of a sample is of paramount importance. This is not only so that other analyses can be carried out in sequence but also to safeguard the sample against any alteration that might occur during sample preparation or analysis.

Raman spectroscopy is able to identify unknown substances in solid, liquid, or gaseous form, and from microscopic amounts up to bulk samples. It determines the Raman-active molecular bonds present within a sample and gives information on the crystalline structure or polymorphic form. It is a highly sensitive technique, meaning it can detect very small changes between items of evidence, and highly selective, meaning it can recognise particular chemical species in complex mixtures such as those that might be encountered in forensic casework.

Samples may be admitted to the spectrometer with no sample pretreatment and minimal preparation. For Raman microscopy, if the sample fits on the stage and underneath the microscope objective, it can be analysed. With handheld spectrometers, analysis is via “*point-and-shoot*” style. However, neither of these techniques involve physical contact with the sample; a further benefit of Raman spectroscopy in addition to being non-destructive is that it is non-contact. This prevents sample interference and, therefore, alteration.

Raman spectroscopy is well suited to forensic analysis as spectral acquisition is rapid and the instruments can be operated by non-specialists. Furthermore, samples can be analysed through transparent and translucent polyethylene evidence bags or rigid containers by changing the focal length of the laser light. This is useful when the sample of interest is particularly hazardous or it is desirable to keep the evidence bag sealed.

As explained in Section 2.2.1, the information gained via Raman spectroscopy is complementary to IR spectroscopy, owing to their different selection rules. Therefore, the use of both techniques in tandem would result in higher sample discrimination ability. IR spectroscopy – ATR-FTIR in particular – is widely reported in forensic literature as a suitable trace evidence analytical technique and most forensic laboratories use these instruments for material identification. Whilst IR spectroscopy is able to distinguish organic molecular bonds, it struggles with inorganic functional groups as these tend to fall in the low wavenumber region (below 400 cm^{-1}) that is cut off by the spectrometer. Conversely, Raman spectroscopy is able to identify both organic and inorganic functional groups simultaneously, depending on the specification of the instrument and the Rayleigh filters, making it an ideal technique for interrogating minerals, inorganic pigments in various types of evidence, different polymorphic forms, and polymer backbone vibrations⁶. Moreover, due to these differing selection rules, water absorbs strongly in the IR but is a weak scatterer in Raman. This means that Raman spectroscopy is advantageous for aqueous samples; in a forensic context this would be beneficial for the analysis of biological fluids or material.

The variety of different lasers that can be used in Raman spectrometers not only helps combat fluorescence – as described in Section 2.2.3 – but can also prevent damage to fragile or thermally sensitive samples. Furthermore, acquisition parameters (such as exposure time or neutral density filtering) can be adjusted to suit the sample of interest and mitigate sample degradation.

With regards specifically to Raman microscopy, tiny amounts of analyte may be examined. This is particularly relevant for the analysis of trace evidence. Additionally, the high spatial resolution afforded by the microscope enables specific components within a matrix to be analysed separately; this would be beneficial for mixtures or heterogeneous samples⁷. Some Raman microscopes feature a motorised stage and a Raman mapping function, allowing visualisation of the extent of sample heterogeneity and distribution patterns. Use of a confocal microscope can help overcome substrate interference as the laser can be exclusively focused upon the sample. Depth profiling can be achieved by decreasing the size of the confocal hole aperture, again, this would be beneficial for forensic specimens such as multilayered automotive paint samples. Raman microspectrometers that possess a camera function, allow for colour photomicrograph imaging. This means the sampling area may be visualised alongside the corresponding Raman spectrum in forensic reports.

Handheld and portable Raman spectrometers do not possess the features boasted by benchtop Raman microscopes, as discussed in the previous paragraph. However, they are an invaluable tool for the *in situ* analysis of items that cannot be transported to the laboratory,

or those that would not be suitable for benchtop spectrometers, for example, assessing large works of art in forgery claims. Handheld instruments are able to rapidly assess potentially hazardous materials at scenes, such as explosives, toxic materials, and chemical warfare agents, or narcotics screening at customs, giving results in minutes via the use of built-in spectral libraries⁸. Some of these instruments can even analyse through opaque containers, protecting the operator from exposure to the harmful contents.

In a forensic context, being able to spectrally assess multiple stains at a crime scene for the presence of blood or other biological material, would be hugely cost effective and time efficient, compared to presumptive testing methods such as the Kastle-Meyer test. As discussed earlier in this Section, Raman spectroscopic analysis does not require any sample pretreatment, thus reducing the use of plastic consumables. It can therefore be regarded as a “*green*” analytical technique and offers a sustainable alternative to traditional single-use plastic generating procedures for forensic science in the future.

Finally, the data produced by Raman spectrometers is compatible with chemometric techniques such as multivariate data analysis. Considering that a typical Raman spectrum includes intensity measurements for around 3000 different wavenumber shifts, the complexity of the information is huge. Furthermore, with the ambiguity of unknown traces from crime scenes and the many ways in which traces may have been altered since deposition, the need for chemometric interpretation of instrumental data is becoming more relevant. Chemometric techniques are covered further in the next Section.

2.3 Chemometrics

The large number of variables (in the form of wavenumber shifts) and thus, the complex nature of data created by Raman spectrometers, lends itself to chemometric analysis. Mathematical algorithms can extract the maximum amount of chemical information from the spectra and identify patterns in the data, far more readily than can be achieved by eye. They also enable discrimination between samples and the identification of groupings; a particular advantage when working with large datasets that need to be compared simultaneously⁹.

Regardless of the specific technique chosen, chemometric methods reduce subjectivity in reporting and help to mitigate sources of operator bias and/or error. They also provide reproducible results; i.e. the same answer will be obtained with the same algorithm and data every time, even when carried out by a different operator. These are crucial attributes for the analysis of forensic evidence, where accurate and reliable analyses are of paramount importance. Indeed, the United States National Academy of Sciences (NAS) report in 2009, the President’s Council of Advisors on Science and Technology (PCAST) in 2016, and the

British House of Lords Science and Technology Select Committee inquiry in 2019 stipulated that more rigorous and objective protocols were required for the interpretation of forensic evidence^{10–12}. They highlighted the need to establish limits and measures of performance and identify sources of variability and bias. Chemometric techniques fulfil these requirements as they are universally accepted statistical methods used to model chemical data¹³.

Chemometric analyses are divided into those that do not require any prior knowledge of the sample population and simply perform exploratory data analysis: unsupervised (pattern recognition) techniques; and those that require sample groupings to have been ascertained or some other prior knowledge of the samples: supervised techniques¹⁴.

2.3.1 Unsupervised techniques

There are different methods of performing exploratory data analysis (EDA), such as cluster analysis (CA), multivariate curve resolution (MCR), or principal component analysis (PCA). The overarching aims of EDA include dimensionality reduction, pattern recognition, identification of sample groupings, discrimination between samples, and data mining. They seek to detect underlying trends in the data that may have otherwise gone unnoticed, such as small spectral differences that are difficult to discern visually.

EDA techniques are often the initial step for effective sample classification as they extract the important chemical information from the data, define the most discriminatory variables, give a statistical measure of how similar or dissimilar samples are, and thereby reveal sample groupings. They are far quicker than visual analyses and therefore, more efficient, in addition to being more reliable and reproducible as previously mentioned. Arguably, the most commonly used EDA and dimensionality reduction technique is PCA, and this is the technique used throughout this thesis.

2.3.1.1 Principal component analysis

PCA is a dimensionality reduction technique that seeks to uncover latent trends in the data via the creation of a new set of variables called principal components (PCs), from the original variables¹³. In the case of Raman spectra, these original variables would be the Raman shifts, or wavenumber shifts (in cm^{-1}). The PCs are linear combinations of the original variables multiplied by their respective loadings that are calculated to extract the maximum amount of variation within the data.

Each variable defines an axis; when all variables are defined this becomes a coordinate system called the variable space. Each sample is then projected into this variable space; once all data has been projected this constitutes a data swarm. In most cases this data swarm has a specific shape and a direction. Where this direction is elongated it encompasses the centre

of the data swarm: the average. This direction is then taken as the first PC (PC1) as it points along the line of maximum variation. Whatever variation has not been accounted for in the first PC (the unexplained variance) results in residuals. The projection process is then repeated in the remaining part of the variable space to find PC2, but this must be orthogonal to PC1 such that it is unrelated to the variance described by PC1. Subsequent PCs describe decreasing amounts of residual variance, until all the variance in the entire dataset has been modelled.

The PCs are then used to create a new coordinate system into which each sample is projected. The distance measure from the mean (i.e. the plot origin) along the PC axis gives each sample its score, and hence this new coordinate system is called a scores plot. Scores are dimensionless, can be any value, and may be positive or negative. A scores plot, therefore, is a map of the samples and shows how the samples relate to each other: samples with similar attributes cluster together, whereas dissimilar samples will be disparate in space. This permits the visualisation of sample groupings, discerns patterns in the data, highlights extreme or outlying samples, and assesses total variation simultaneously. It also helps to identify the level of analytical repeatability (when instrumental replicates are plotted), whether the number of replicates is sufficient, and the degree of sample homogeneity. Figure 2.4 demonstrates how a 3-dimensional scores plot can instantly reveal the chemical differences between genuine and counterfeit cosmetic products.

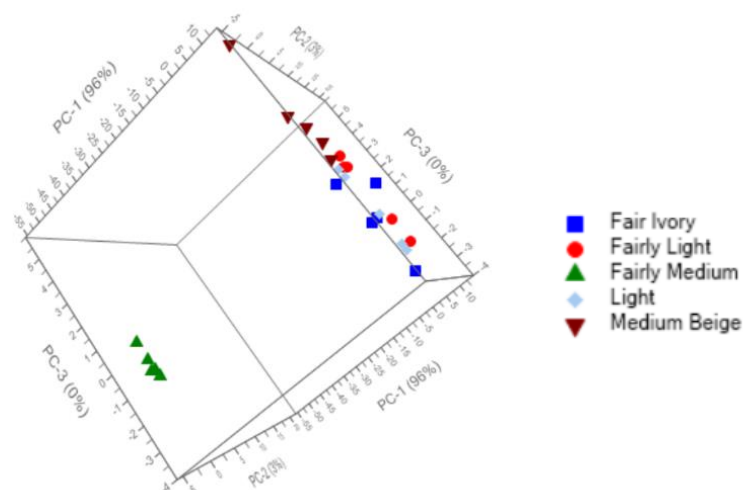


Figure 2.4: 3-dimensional PCA scores plot of five BareMinerals Original Foundation samples in varying shades, showing a clear distinction between the Raman spectra obtained for the genuine (Fair Ivory, Fairly Light, Light, and Medium Beige) and the counterfeit (Fairly Medium) products.

Inspection of the scores plots in conjunction with the loadings plots allows interpretation of the chemical variability underpinning the sample groupings. The loadings plots isolate which

variables have the greatest influence on each PC, and their respective magnitudes, therefore identifying the important components that contribute the most to sample discrimination. They are also useful to understand the correlations between the original variables and to detect the onset of noise in the PCs.

The data matrix (X) describes the relationship between the objects (the samples) and the variables and is represented by the sum of all its PCs, the so-called “*structure*”, plus the residual error (E). Each PC is the product of its scores (T) and loadings (P); thus, the original data is derived as:

$$X = TP + E$$

In theory, the number of PCs can equal the number of variables, but in reality, this is neither practical nor helpful. The aim of PCA model construction is to retain the fewest number of PCs possible to explain the majority of the variance in the data, so that the model is neither too specific, nor lacking in variability. The optimum number of PCs for a given model can be ascertained by inspecting the scree plot (also known as the explained variance or cumulative variance plot), to determine the point at which the addition of further PCs adds only minimal information to the model. This is usually taken as <1% of the total variance but should be accompanied via inspection of the scores and loadings plots, to detect the onset of noise.

PCA may be based upon either a correlation or a covariance matrix. A correlation matrix involves mean centring and scaling each sample when the variables have different measurement units, to ensure equal weighting, whereas a covariance matrix involves mean centring the data but does not apply scaling. This is appropriate when the variable metrics are comparable, as is the case with spectral data¹⁵.

2.3.2 Supervised techniques

Supervised techniques describe those that require some knowledge of sample groupings or class assignment, in order to build predictive models for unknown sample classification. There are many different methods of classification, but they all ultimately seek to determine how future samples will relate to an existing training set. A classification model needs to be robust enough to interrogate new data, but at the same time, not excessively overtrained or subjected to overfitting, so that it is unable to categorise new samples.

According to the complexity of the PCA scores in Chapter 3 of this thesis and the lack of discernible sample groupings, there was no indication to use standard classification methods such as discriminant analysis. Furthermore, given that the size of a sample set greatly influences the classification accuracy, the large datasets contained within this thesis would yield disproportionately lower percentage accuracies. It is also worth mentioning that high

classification accuracy rates are not necessarily a good indicator of a model's discrimination ability, as some classifications may arise purely by chance.

2.3.2.1 PCA projection

PCA projection is a very basic form of multivariate classification and is regarded as the PCA equivalent of prediction¹⁶. This technique may be used to see how new samples compare to an original model and is an important step in the modelling process. Inspection of the residual variance plot indicates the closeness of fit between the original model and the projected dataset, demonstrating the robustness of the original measurements and a reliable prediction certainty. It is a highly valuable method of outlier detection, as deviations from the reference samples become apparent in the combined scores plot. For all these reasons, PCA projection was deemed a suitable technique to analyse the samples investigated during this project, despite the lack of clearly defined classes.

The method works by transforming new samples using the original PCA model's loadings. The new samples are given scores that are comparable with the existing samples and can then be projected onto the original model. The positions of the projected samples in relation to the existing samples determines whether they are part of the same population or distinctly different. A Hotelling's ellipse is a confidence interval (usually set to 95%) that may be added to the PCA projection scores plot to verify whether or not the new samples lie within acceptable limits set by the operator.

2.3.3 Analytical conditions

The analytical conditions detailed in this Section were applied to all samples in every Chapter of this thesis. Any deviations from this generic account are further elaborated upon in the relevant Chapters.

Spectral data were imported into a data matrix in the Unscrambler X 11.0 software, as GRAMS files (spc format), each sample represented with five replicates. The interpolate function was used during data import as some spectra had been assigned slightly different start and finish wavenumbers by the Labspec 6 software. Row sets and column sets were defined and then category variables set up to allow visualisation of sample groupings in resultant plots. The category variables used were: brand (as designated on the product packaging), product type (foundation, concealer, tinted moisturiser, finishing powder, bronzer, highlighter, or primer), formulation (liquid, cream, mousse, pressed powder, or loose powder), and shade (light, medium, or dark).

All spectra were initially plotted to assess for quality and presence of obvious outliers. Descriptive statistics (i.e. mean, median, range, quartiles, etc.) were computed for each data

matrix and the scatter effects plot viewed. Assessment of the scatter effects determined the most appropriate method of preprocessing; in this case, SNV. Every sample was subjected to a SNV spectral transformation and the transformed data matrix was taken forward for PCA. A covariance matrix was used to construct the PCA model; as the data was spectral it only required mean-centring and did not require scaling.

The Unscrambler software gives two algorithm options for calculating PCs: singular value decomposition (SVD) and non-linear iterative partial least squares (NIPALS). According to the literature, the NIPALS algorithm is better for datasets that either have a large number of samples or a large number of variables¹⁴. It is also able to compute where there are missing values; the SVD algorithm cannot. Based on this assessment, it was decided the NIPALS algorithm would be better suited to the amount of data contained in this study.

Cross-validation is a crucial step in ensuring a model is fit for purpose. It is necessary to prevent overfitting or underfitting of the data, where the former results in a highly specific model that is incapable of dealing with new samples (the validation samples), and the latter results in a poorly performing model that fails to capture the essential patterns and trends in the data. The standard technique is leave-one-out cross-validation (LOOCV). This entails keeping some samples out and using the remaining samples used to predict the outcome for the validation samples. This process is repeated for every sample and the prediction residuals are combined. The overall predictive ability is the average correct classification rate across all iterations. The cross-validation method used during this study was the random 20 segment variant, due to the large number of samples in the data matrix.

The best estimate of future prediction error is to perform a separate test set validation. This is the most reliable and robust measure of prediction ability as it exposes the model to samples that were not used to create the model: the calibration samples. This gives a far more realistic indication of model performance than internal cross-validation, whereby the same samples are used for both the calibration and validation stages of model development. However, as the predominant aim of this thesis was to explore the chemical diversity across the facial cosmetic population, it was desirable to include as many different products as possible in the PCA model, rather than keeping some aside to be used as test set validation samples.

A Hotelling's ellipse was added to each PCA scores plot and set at a 95% confidence interval. Outliers to this ellipse were marked and investigated further in the influence plots for each PC. Assessment of the sample's residual variance (via the F-residuals) and its leverage determined whether the sample was poorly described by the model, an extreme sample (high leverage), or a true outlier that warranted removal from the model. No samples were removed from any of the models in Chapters 3 to 6, as they were found to either be exceptions to the

general rule, i.e. poorly described by the model, or extreme samples. Removal of high leverage samples from a model has a significant impact on the residual variability and is not recommended unless necessary, as it decreases the model's discrimination potential^{14–17}.

Where samples were subsequently subjected to a PCA projection, the same SNV transformation was applied to the data and the same number of PCs was used to construct the projection.

2.4 Experimental conditions

The Raman microspectrometer used for all experiments in this thesis was a Horiba LabRAM HR 800 as shown in Figure 2.5. This instrument benefits from a variety of microscope objectives for optimal sample magnification, and a motorised stage in the XYZ directions. The instrument is fitted with four different lasers – 473 nm blue, 532 nm green, 632 nm red, and 784 nm NIR – each with edge and notch filter options. An edge filter transmits Raman scattered light; this is usually Stokes scattered and rarely anti-Stokes. However, a notch filter is able to transmit both Stokes and anti-Stokes scattered light. Both filters block the majority of the laser light transmitted, allowing only a small percentage through to permit calibration. Two diffraction gratings allow differing spectral resolutions: the 600 lines/mm option gives a spectral resolution of 2 cm^{-1} but the 1800 gr/mm gives an enhanced resolution of 0.5 cm^{-1} . Light detection is via a Peltier cooled CCD operating at $-70\text{ }^{\circ}\text{C}$.

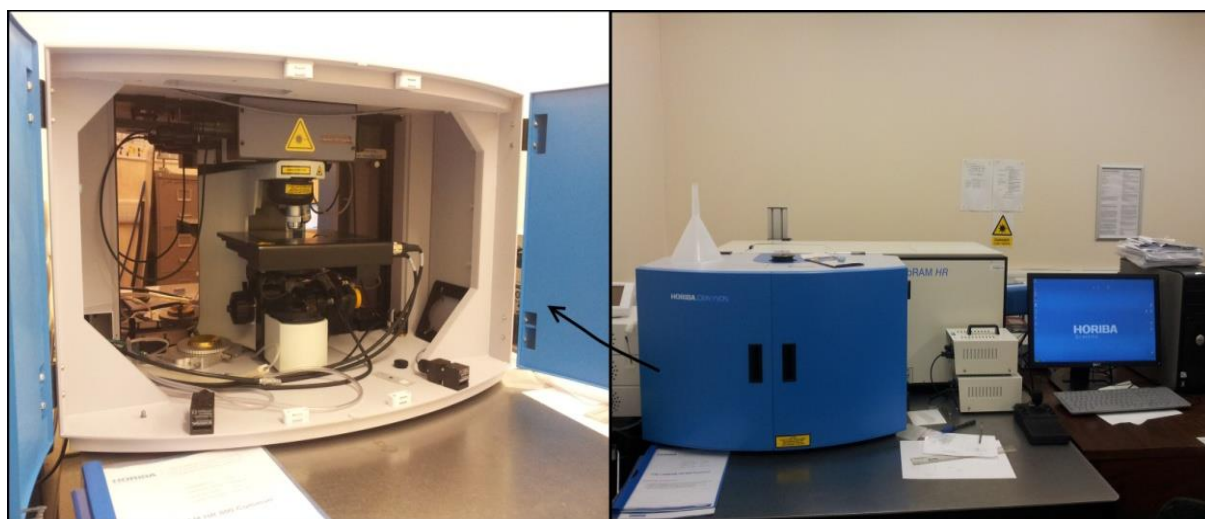


Figure 2.5: The Horiba LabRAM HR800 Raman microspectrometer used throughout this thesis.

From the outset of this project, it was decided that maintaining the spectral acquisition parameters across all experiments would be the best way of ascertaining how the samples differed from one another. Had the spectral parameters been altered per sample to achieve

superlative spectra, the origin of any differences between samples would be difficult to deduce, i.e. spectral differences could not be readily attributed to true chemical differences as they may have derived from differing measurement parameters. Therefore, initial experiments entailed trialling each of the four different lasers and then selecting different combinations of acquisition parameters to find a standard procedure that suited the majority of the samples.

2.4.1 Parameter determination

The most suitable Raman spectral acquisition parameters were assessed by analysing five foundation samples with varying conditions in turn. The first parameter to consider was the laser wavelength; it was deemed important to keep the excitation wavelength consistent as any fluorescence produced could alter spectra significantly and prohibit accurate sample comparison. Figure 2.6 shows the different spectral profiles achieved for a Max Factor foundation sample using the blue, green, red, and NIR lasers.

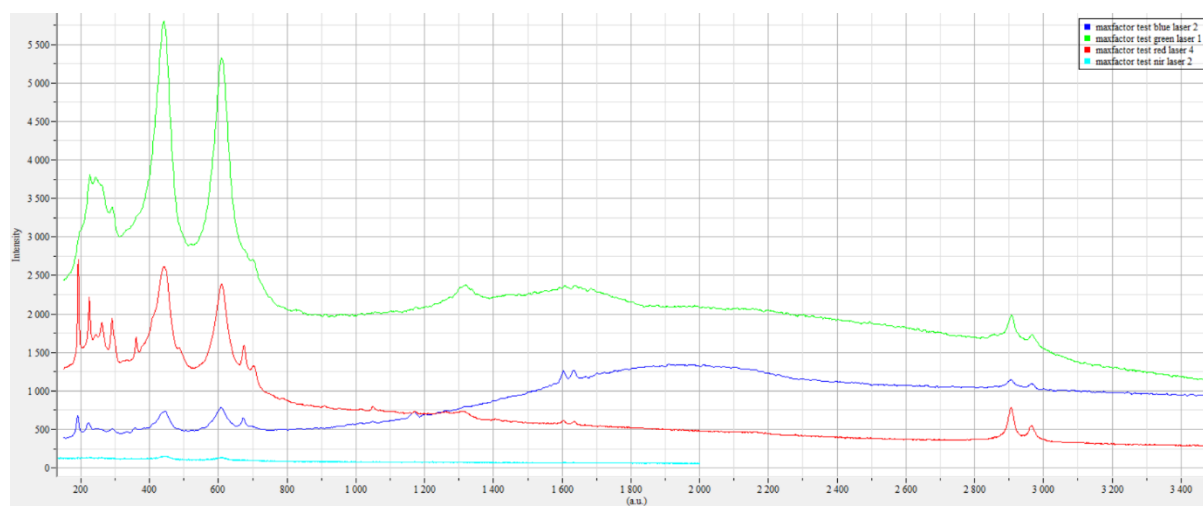


Figure 2.6: Raman spectra (unprocessed) of a Max Factor foundation sample, acquired using blue, green, red and NIR lasers, highlighting differences in spectral detail and baseline fluorescence.

Generally, the red laser outperformed the other three lasers in giving spectra with the greatest number of peaks and the least fluorescent baseline, i.e. the best signal to noise ratio. It also mitigated sample burning and/or degradation, whereas the higher-powered green laser frequently damaged the samples (even with an ND filter applied) as is indicated in Figure 2.7 by the circular area in the centre of the Bourjois foundation sample. The central black dot within the red dashed-demarcated area is indicative of the laser having burnt the sample.

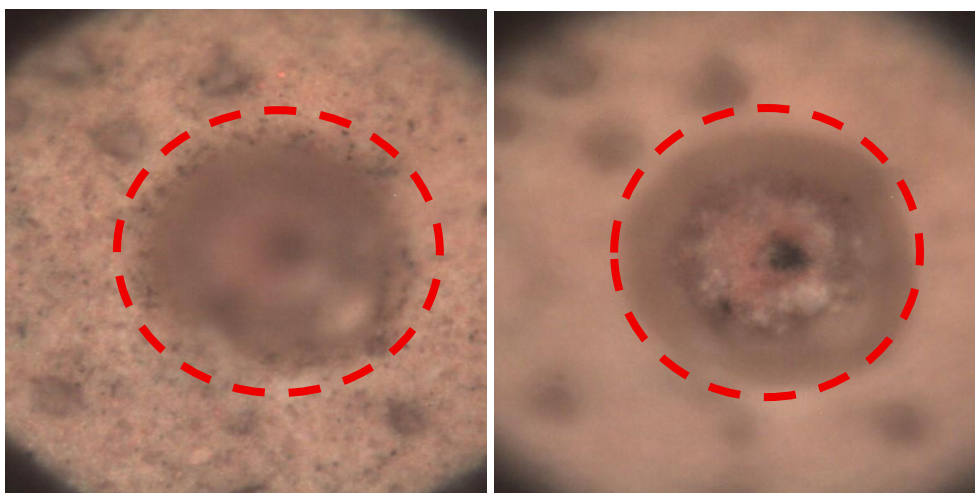


Figure 2.7: Laser damage (outlined with red dashes) seen on a Bourjois foundation sample, caused by the green laser at only 25% power, (L) focused on sample surface, (R) focused on damaged region. Sample deposited onto glass microscope slide.

Surprisingly, despite having the longest wavelength, the NIR laser also caused sample photodecomposition, even when applying various ND filters as can be seen in Figure 2.8. However, measurements of the maximum power outputs at the laser apertures of all four wavelength lasers revealed the reason for this induced sample damage: 473 nm = 500 mW, 532 nm = 542 mW, 632 nm = 30 mW, 784 nm = 150 mW. An additional consideration was the limited spectral range possible with the NIR laser as indicated in Figure 2.6 where collection of spectral data is only possible to $\sim 2000\text{ cm}^{-1}$.

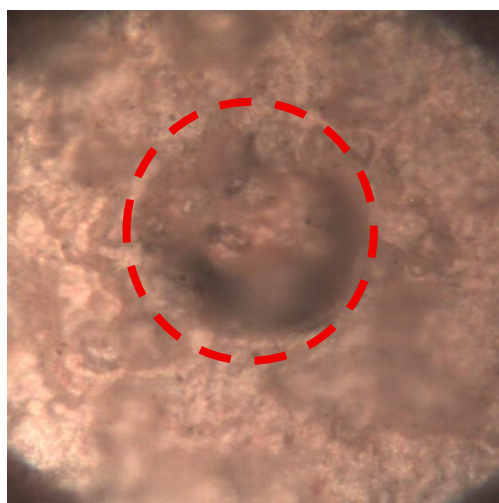


Figure 2.8: Laser damage (outlined with red dashes) seen on a Maybelline foundation sample, caused by the NIR laser at only 25% power. Sample deposited onto glass microscope slide.

Thereafter, all four lasers were assessed using both grating options available on the spectrometer: 600 and 1800 lines/mm. The higher grating value disperses the collected light

to a greater extent and is therefore able to resolve overlapping peaks, but none of the spectra obtained using the enhanced grating option gave an appreciable benefit, and the spectra took three times longer to acquire. Therefore, the 600 lines/mm option was selected for all analyses.

A balance between suitable analytical spot size and adequate magnification also needed to be achieved. It was decided that the x50 objective – giving a beam diameter of approximately 1.4 μm and a numerical aperture (NA) of 0.55 – was a good compromise. A microscope's NA indicates the range of angles over which light can be collected; it is directly related to the focal length and the size of the lens. A long focal length gives a smaller NA and therefore collects a smaller range of light. Whereas a shorter focal length gives a higher NA resulting in collection of a greater range of light and higher resolution. Therefore, the higher the magnification, the better the Raman signal, but some samples were visually heterogeneous and therefore the highest magnification (x100) might not have been suitable. In hindsight, an even lower magnification such as x10 or x15 might have been better, particularly for the very heterogeneous samples, as it would have been easier to obtain a more representative spectrum. Unfortunately, the extent of sample heterogeneity only became apparent after parameter optimisation; it transpired that all five initial samples selected were, coincidentally, spectrally homogeneous. Furthermore, some of the cosmetic samples suffered from low Raman signal intensity; an objective lens with a low NA would have collected less Raman scattered light and thus, would have only exacerbated this.

Varying acquisition times were trialled but it became apparent that longer times often resulted in sample damage, and therefore an acquisition time of two seconds was selected. Acquisition times of fewer than two seconds resulted in decreased Raman responses and proportionate decreases in the signal to noise ratios. The neutral density (ND) filters were applied in succession, and it was found that the 10% option, i.e. blocking out 90% of incident laser light, permitted acceptable signal intensity whilst mitigating sample damage. Higher accumulations (number of spectral averages) resulted in better signal to noise ratios, which was deemed important if spectral changes between similar samples were small. Increasing the number of accumulations was found to be an effective way of improving the spectra without increasing either acquisition time or decreasing the ND filter, both of which frequently resulted in sample decomposition. Therefore, 20 accumulations were collected for every spectrum.

Some samples revealed very fluorescent spectra that would have benefitted from reduced acquisition times, whereas other samples suffered from reduced spectral intensity and would have benefitted from increased acquisition times, but the overarching aim was to maintain the instrumental parameters for the reasons previously explained. Whilst changing acquisition

times across samples would not have affected peak positions or relative peak intensities, it might have decreased the likelihood of discriminating between samples, had their spectral profiles been very similar, and more importantly may have compromised multivariate analyses by introducing or minimising true sample variance.

A suitable spectral range was also decided upon. The starting wavenumber shift had to be low enough such that all peaks could be captured, but not include the stray Rayleigh scattered light at 0 cm^{-1} , as this provided no benefit. Due to the high-quality edge filters supplied with the instrument, peaks could be seen as low as $\sim 75\text{ cm}^{-1}$. No spectral peaks were seen beyond 3100 cm^{-1} across any of the samples, so this was chosen as the wavenumber maximum. Consequently, a spectral range of $30\text{-}3100\text{ cm}^{-1}$ was used in all analyses.

There was no discernible benefit to altering either the slit width or the confocal aperture diameter (hole size). Furthermore, depth profiling was not required as the samples were not layered. Hence, these were used with the standard settings of $100\text{ }\mu\text{m}$ and $800\text{ }\mu\text{m}$ respectively.

The decision was made not to use either of the denoising functions available in the LabSpec 6 software. Again, this decision was taken for two reasons: firstly, any spectral smoothing might inadvertently remove minor peaks that could prove vital for sample discrimination, and secondly, there was no information given on the type and extent of this “*denoising*” process, which meant that ascertaining how the algorithm had treated the data was impossible.

During parameter optimisation there was no indication for SERS enhancement, as all samples gave good to excellent Raman spectra, denoted by intense and well-defined peaks. This was fortunate as it meant that there would be no sample preparation, the integrity of the sample could be preserved, sample contamination could be minimised, and potential sample alteration could be mitigated; all of which are of paramount importance for the forensic analysis of trace evidence.

2.4.2 Sample preparation and measurement

The cosmetic sample selections for each part of the project were chosen for a variety of reasons; these are discussed at the beginning of the relevant Chapters. Raw cosmetic components ($n=65$) were also purchased from various suppliers so that characteristic spectral peaks could be assigned. The list of raw cosmetic components is given in the appendix.

All glass microscope slides were cleaned with isopropyl alcohol (IPA) and lint-free tissue before use. In hindsight, stainless steel slides or aluminium SEM stubs might have provided a better substrate than glass, as both are generally considered Raman inactive, but no obvious interference from the glass was seen in any of the spectra. A small amount of each cosmetic

sample (approximately 1 cm² diameter) was deposited onto these precleaned slides and then stored in the dark, under ambient conditions (air conditioned, temperature-controlled room at 19 °C) for a minimum of 14 days. Powdered samples were gently pressed down onto the slide to a depth of approximately 1mm, to obtain a flat surface for microscope focusing.

The decision to dry the samples prior to analysis, was made for two reasons. Firstly, in the hope that the samples would better represent casework samples, and secondly because it gave the liquid samples time to dry. This meant they did not warm up under the laser light as much and then start moving out of microscopic focus, which is critical for obtaining spectra with good signal to noise ratios. In hindsight, it would have been preferable to acquire spectra from all samples immediately upon deposition, so that baseline spectra could be obtained for reference purposes. This realisation was only made at the end of the project, so unfortunately time did not permit reanalysis of the large dataset.

Colour photomicrograph images were obtained for each dried sample using the video acquisition mode of the Raman microscope. All images were taken using x50 magnification and no subsequent image processing was carried out. This yielded images that represented approximately a 100 µm² area of each sample. Raman spectra were acquired immediately after the photomicrograph images. A new comparison microscope was purchased during the course of this thesis: a Projectina Vision X. This microscope was used exclusively to provide the colour photomicrograph images for Chapter 5. Varying magnification settings were used dependent on the desired outcome; these are detailed further in Chapter 5.

Calibration against a silicon wafer at 520.6 cm⁻¹ was performed at the beginning of every session. Each sample was analysed in five random areas to check for repeatability and sample heterogeneity. Where samples contained obvious flakes of pigment, spectra were taken of both the general matrix and of the flake specifically, to compare and contrast the data. The final acquisition parameters decided upon for all analyses throughout this thesis is given in Table 2.1.

Some of the samples exhibited spectral fluorescence; if the baseline counts were reducing significantly in real time, this indicated a fluorescent response. Where this was discovered, attempts were made at minimal photobleaching, so as not to cause sample photodecomposition (maximum laser power at the sample was measured as 15 mW). The sample was irradiated for no longer than ten minutes and was often only around five minutes duration. The efficacy of the technique was assessed using the “*Real Time Display*” function and was stopped when the baseline intensity remained static. Photobleaching was found to be effective in minimising background slopes and spectral “*stepping*” in all sample types without exception. This is contrary to the findings by Salahioglu¹⁸ who found no improvement

in the Raman spectra of lipstick samples, despite up to three hours of continuous photobleaching. It is important to note that photobleaching of casework samples (dependent on the amount of sample available) should be performed after all other acquisition parameters have been exhausted, in the event of sample damage.

Table 2.1: *Final Raman spectroscopic acquisition parameters used for all analyses throughout this thesis.*

Instrument parameter	Selected setting
Laser	Red 632 nm
Rayleigh filter	Edge
Grating	600 lines/mm
ND filter	10% (blocking 90% of laser light)
Microscope objective	x50 magnification
Slit width	100 μm
Confocal hole diameter	800 μm
Spectral range	30-3100 cm^{-1}
Acquisition time	2 seconds
Accumulations	20
Autofocus/auto exposure	Off
Binning	1
Spike filter	Multiple accumulations
Denoiser	Off
ICS correction	On
Replicates	5

After every spectral acquisition, each sample was microscopically checked for the presence of darkened areas that would indicate laser damage. If sample degradation was identified – this was almost always accompanied by an odd-looking spectrum that indicated a chemical change had occurred – the focus was moved to a fresh area of the sample and the spectrum retaken, this time without any photobleaching. This resulted in some spectra exhibiting “stepping” in the baseline, indicating residual fluorescence within the sample, but was accepted as a compromise to sample damage. It may have been the case that this residual fluorescence could have been mitigated by using one of the other laser wavelengths, however, the overarching aim was to maintain identical acquisition parameters so that sample differences could be more easily determined.

2.4.3 Spectral preprocessing

Horiba's LabSpec 6 software was used for spectral acquisition and comparison, and for automatic cosmic ray removal via the "*multiple spike filter*" algorithm. Spectra were exported in GRAMS file format and imported into Aspen Technology's (previously Camo Analytics) Unscrambler X 11.0 multivariate data analysis software for all spectral preprocessing and chemometric analysis. No spectral libraries were consulted as none were found to be relevant, hence the purchase of raw cosmetic ingredients and their analysis using the same spectral parameters applied to the cosmetic samples.

Initially, all five replicate spectra were averaged using a reduction transformation in the Unscrambler software in order to obtain a single representative spectrum and make visualisation of the data easier. However, it was decided that this would create an artificial spectrum that would not be encountered experimentally. It would also hinder any assessment of sample heterogeneity, which would later prove critical for interpretative frameworks, and prohibit the evaluation of experimental repeatability and reproducibility. This decision was taken despite Salahioglu *et al.* and other researchers using the averaging method^{19–21}. Had the data reduction transformation been adopted, there would have been no distinction between the spectrally homogeneous (Chapter 3) and heterogeneous (Chapter 4) samples. Nonetheless, in a casework scenario it may be preferable to perform spectral averaging due to the simplicity of the results thereafter, but this would need to be assessed according to the circumstances of the trace and the context in which it is found.

The predominant aim when considering preprocessing was to minimise its use, to ensure that the data remained as original as possible, and that modifications were only carried out if there was evidence to suggest it was warranted. It is necessary to eliminate day-to-day instrumental variation via preprocessing techniques, but essential to retain spectral details that relate to genuine chemical information. Indeed, this forms the basis of sample discrimination and subsequent classification.

Descriptive statistics were computed for the initial five samples used in the parameter optimisation experiments, and scatter plots viewed. All samples displayed an additive baseline shift that required baseline subtraction to mitigate the offset. The characteristic "*fanning*" effect was also observed, indicating the presence of multiplicative scatter effects. These tend to be caused by varying particle sizes or differing surface properties and are corrected by a baseline division.

In an attempt to minimise the amount of preprocessing, a spectral transformation capable of addressing both additive and multiplicative scatter effects was chosen. This excluded typical

spectral transformations such as baseline correction, de-trending and normalisation but included multiplicative scatter correction (MSC), extended multiplicative scatter correction (EMSC) and standard normal variate (SNV). SNV was found to be the most effective transformation via inspection of the resultant spectra. SNV essentially combines a baseline correct with a normalisation by subtracting the mean and dividing by the standard deviation for each spectrum. Because each spectrum is treated in exactly the same way, spectral differences between samples can be preserved and there is no degradation in the signal to noise ratio. First and second order derivatives were found to decrease the signal to noise ratio and also made visualisation of the spectra difficult and were therefore not used.

As explained in Section 2.4.1, there was no desire to smooth the data as the signal to noise ratio was already excellent. There was also no need to make the spectra more visually appealing as the ultimate goal across all Chapters in this thesis was for the chemometric differentiation of samples, not the visual discrimination of spectra. In addition to the denoising algorithm in Labspec 6, there were various smoothing transformations available in the Unscrambler software, however, these were not used as they may also remove weak peaks of interest, thereby decreasing discrimination potential.

Initial comparison of sample spectra was carried out visually, to check for focusing accuracy and to provide preliminary analyses. Nonetheless, for a more objective discrimination, and to provide numerical measures of uncertainty, the samples were compared using chemometric techniques as described above.

2.5 References

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Chapter Three: Assessment of the chemical variability within facial cosmetics using Raman microspectroscopy and chemometrics – a forensic “*market survey*”

3.1 Introduction

The initial aim of this Chapter was to assess whether or not Raman spectroscopy was well suited to the analysis of facial cosmetic samples, both in terms of obtaining good quality spectra (well defined peaks with good signal to noise ratio and minimal fluorescence), and also in terms of reproducibility and repeatability. Thereafter, the level of chemical variability among the samples was assessed. Chemometric techniques were employed for the discrimination and classification of the samples; trends and sample groupings were explored using different category variables.

3.2 Experimental

Unfortunately, despite an extensive literature review^{1–16} and several practitioner communications^{17–24}, no specific protocols for the collection of cosmetic samples within a forensic context could be identified, nor were any protocols for the subsequent analytical workflow identified. Therefore, an experimental method was devised that sought to maximise data acquisition and minimise observational and instrumental errors. In hindsight, a Design of Experiments (DOE) method would have been beneficial for parameter optimisation and sampling methods, but this represents a further research opportunity.

3.2.1 Samples

Facial cosmetic samples were purchased from a variety of UK outlets over the course of the project; a full list is given in the appendix (Table A2). The sample population (n) used for this study consisted of 126 new products that comprised 77 foundations, 27 concealers, 16 tinted moisturisers (including BB and CC creams) and six finishing powders.

The decision to include these four product types, rather than foundation alone, was based on two observations. Firstly, that cosmetic wearers tend to layer their products, and hence any traces transferred to a substrate are likely to contain a mixture of those products, and secondly, that consumers may not use a product in the manner that the manufacturer intended. For example, an acne sufferer may choose to substitute foundation for concealer, as these tend to have a higher pigment content (and often increased oil resistance) and therefore offer more coverage, whereas a younger person may choose to only wear a tinted

moisturiser with added SPF instead of a foundation, to achieve a natural finish and to protect their skin from UV radiation.

The sample population was deliberately chosen to represent as wide a range of facial cosmetics as possible given budget constraints and to minimise sampling errors. The sample population spanned 93 different product ranges across 48 different brands, ranging from the budget market (average product cost £1.00), through to the mainstream market (average product cost £12.00) and up to the premium market (average product cost £38.00).

The samples encompassed a range of different formulations: 85 liquids (including gel consistencies), 18 loose powders (predominantly mineral based), five pressed powders (compact style), 15 creams (either stick based or compact style), and three mousses (aerated cream/liquid consistency).

A further 65 raw cosmetic ingredients that are frequently encountered in formulations were also purchased and analysed so that, where appropriate, comparisons could be made between the samples and their constituent parts. The list of raw ingredients is given in the appendix in Table A1.

All samples were prepared, analysed and characterised as described in the experimental section of Chapter 2.

3.3 Results and discussion

3.3.1 Preliminary considerations

From the outset of Raman analysis, it was clear that the mineral particulate sizes and morphologies within some of the samples would give rise to sampling difficulties, as some of the pigment particles were larger than the incident laser spot size (1.4 μm). Therefore, the Raman signal would only be focused upon one particular chemical component and the resultant spectrum not be representative of the sample as a whole. This was confirmed once Raman analysis had been carried out; some of the visually heterogeneous samples caused inconsistencies across their replicate spectra, which varied considerably. Across the entire dataset, some samples were found to be only slightly spectrally heterogeneous, i.e., differing only by the presence or absence of a single peak, or differences in relative intensities of the same peaks; whereas other samples were significantly heterogeneous and could display totally different spectra across their surface, despite only moving the focal point slightly (sometimes in the order of $\sim 50 \mu\text{m}$).

It was initially thought that only the loose and pressed powder samples would display heterogeneous spectra due to particle size, but it was discovered that the liquids too could

suffer from sampling heterogeneity issues. Projecting significantly different replicate spectra onto a PCA would result in disparate scores and difficult interpretation. Hence, all samples were separated based on their apparent spectral heterogeneity (visual heterogeneity was also assessed but not used as the criterion for selection). Interestingly, some samples that appeared visually homogeneous gave inconsistent replicate spectra, and some samples that appeared visually heterogeneous gave spectra with good repeatability. This indicated that the relative particulate sizes were an important contributory factor for good spectral repeatability but not the only factor. Furthermore, it meant that the degree of spectral heterogeneity could not be predicted purely by viewing the sample microscopically and there must be factors to consider other than simply the presence or absence of large pigment particles, as these are not always indicative of which components in the sample will interact with the Raman laser.

This Chapter focuses exclusively on those samples that were assessed as being **spectrally** homogeneous formulations, but not necessarily **visually** homogeneous. Two examples of spectrally homogeneous foundations are given in Figures 3.1 and 3.2 below. The spectrally heterogeneous samples are covered in Chapter 4 and examples of these spectra are given in Figures 3.3 and 3.4. Contrary to prediction, the majority of the spectrally homogeneous samples (91 out of 126) were assessed as having a heterogeneous visual appearance microscopically. Therefore, it can be concluded that visual observations do not necessarily correlate with what is seen spectrally. It should be emphasised that this selection method was carried out in a subjective manner and was only intended to serve as a tool to aid further analysis. It was neither definitive nor exhaustive, but merely an indication of the sample's composition.

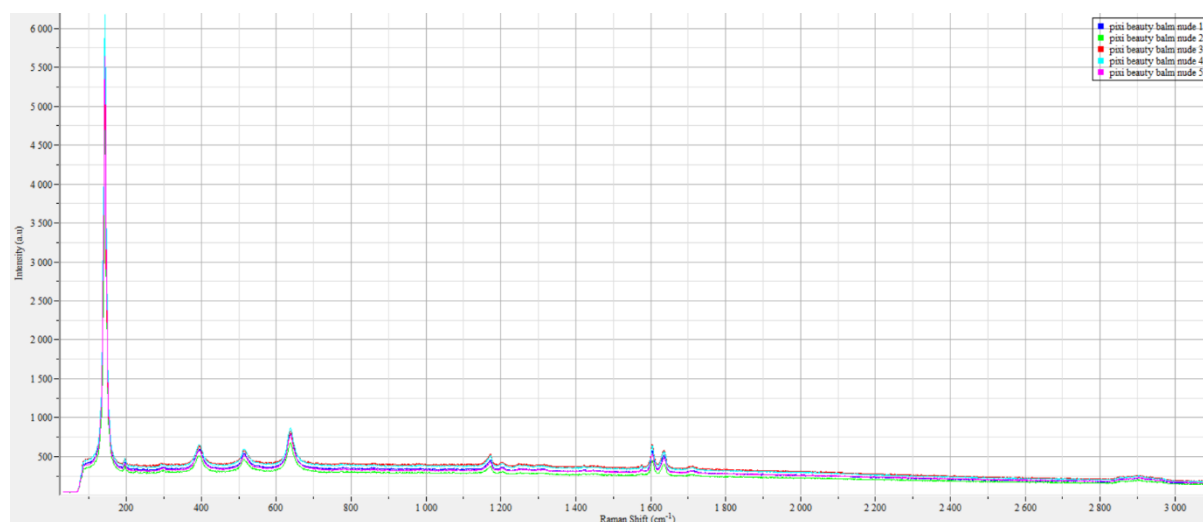


Figure 3.1: Raman spectra (unprocessed) of Pixi Beauty Balm High Coverage Foundation in shade No.2 Nude (liquid formulation), exhibiting spectral homogeneity across the five replicates.

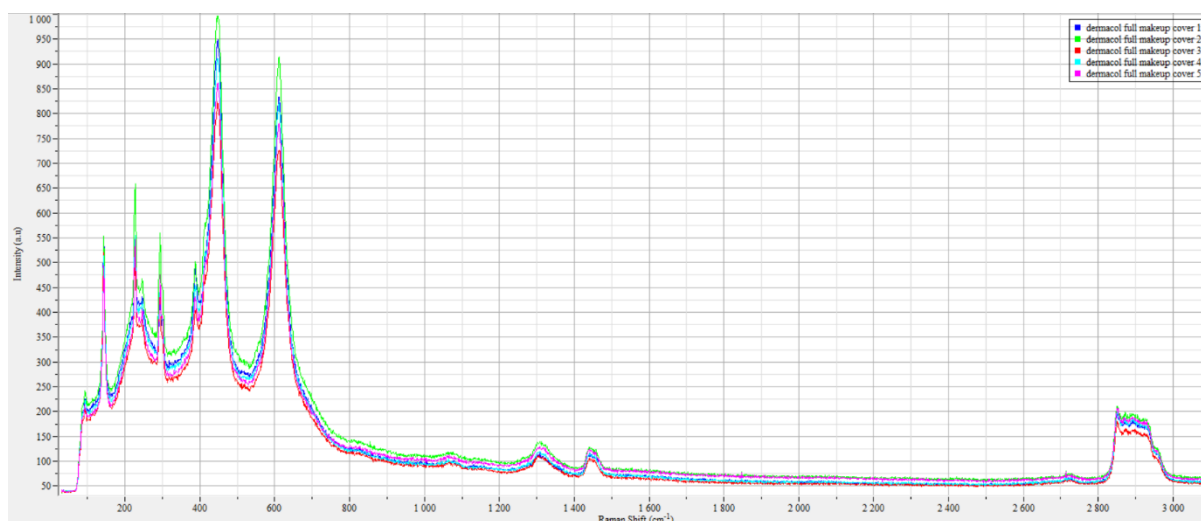


Figure 3.2: Raman spectra (unprocessed) of Dermacol Full Makeup Cover Foundation in shade 211 (thick cream formulation), exhibiting spectral homogeneity across the five replicates.

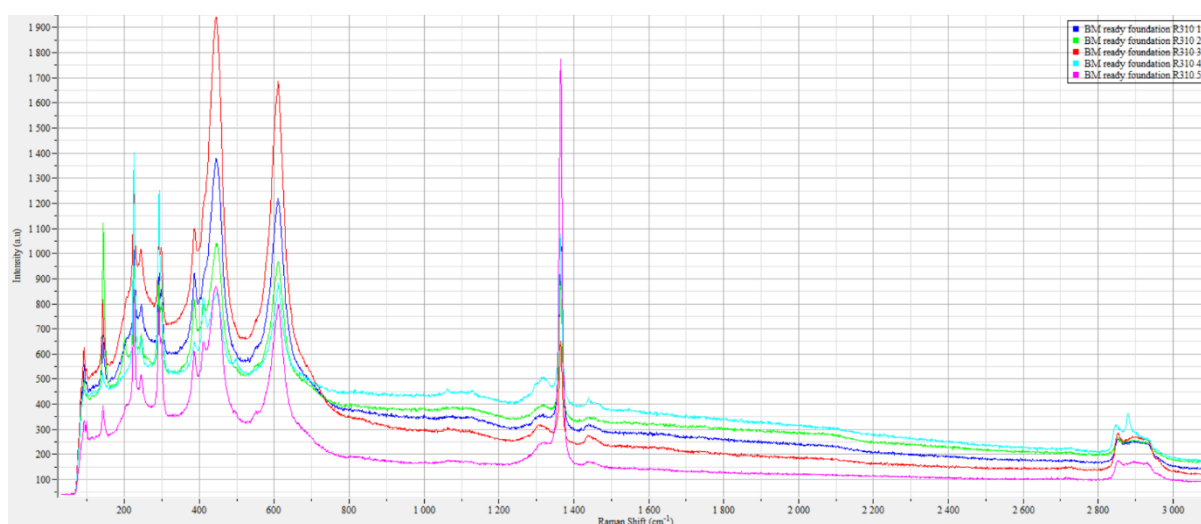


Figure 3.3: Raman spectra (unprocessed) of BareMinerals Ready Foundation in shade R310 (pressed powder formulation), exhibiting spectral heterogeneity across the five replicates.

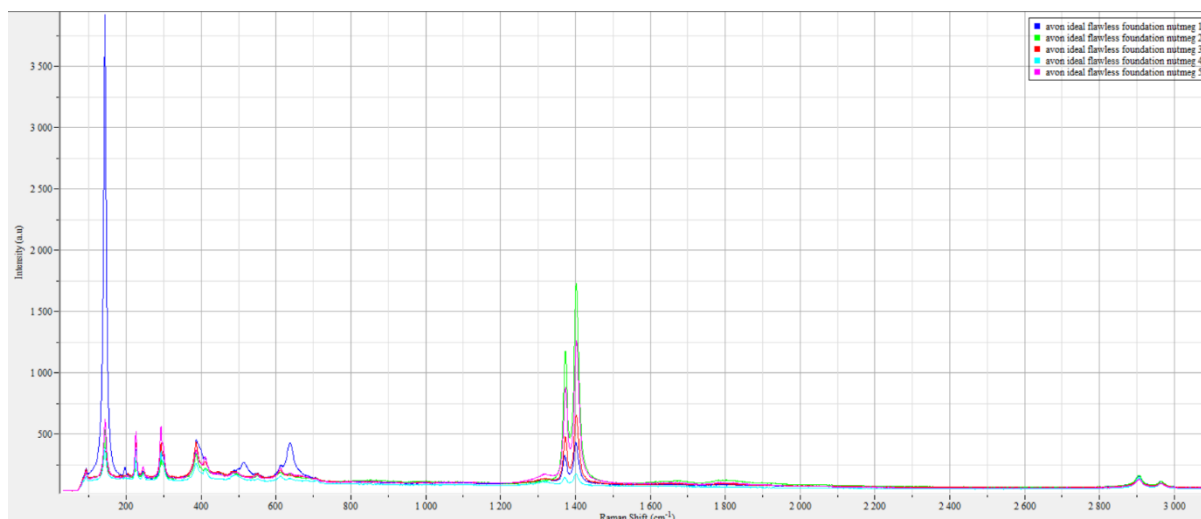


Figure 3.4: Raman spectra (unprocessed) of Avon Ideal Flawless Foundation in shade Nutmeg (liquid formulation), exhibiting spectral heterogeneity across the five replicates.

3.3.2 Characterisation using optical microscopy

3.3.2.1 Foundations

Visual microscopic inspection of the foundation samples gave an initial level of discrimination. The most notable differences were the colours of the sample matrices (i.e. the general liquid medium) and the colour, size, morphology and distribution of pigment particles (i.e. the solids suspended within the liquid medium) as can be seen in Figure 3.5.

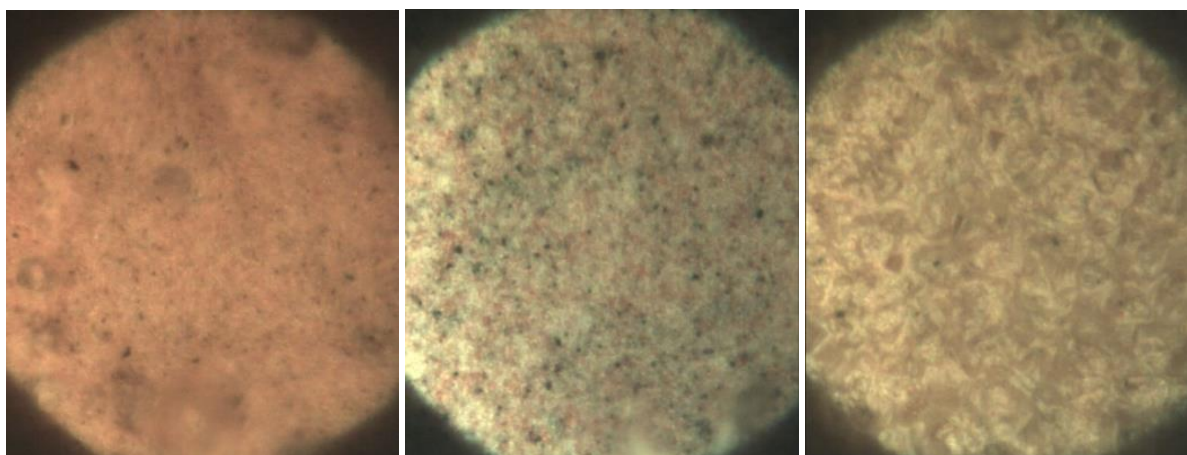


Figure 3.5: Colour photomicrograph images of spectrally homogeneous foundations illustrating differences in matrix colour and pigment particles, (L-R) Giorgio Armani Luminous Silk Foundation in shade 8.25, Laboratoire Actiderm Mineral Makeup in shade Bastille, The Ordinary Serum Foundation in shade 1.2N Light Neutral. Samples deposited onto glass microscope slides.

The foundation sample subset (n=77) comprised all five product formulations: 58 liquid formulations, three pressed powders, 11 loose powders, two cream formulations, and three mousse formulations. The pressed powder, loose powder and mousse formulation samples, exhibited differences in their surface topologies that gave rise to focusing difficulties as the sample surfaces were not completely level, denoted by the visually blurred areas in the photomicrograph images, as is illustrated in Figure 3.6.

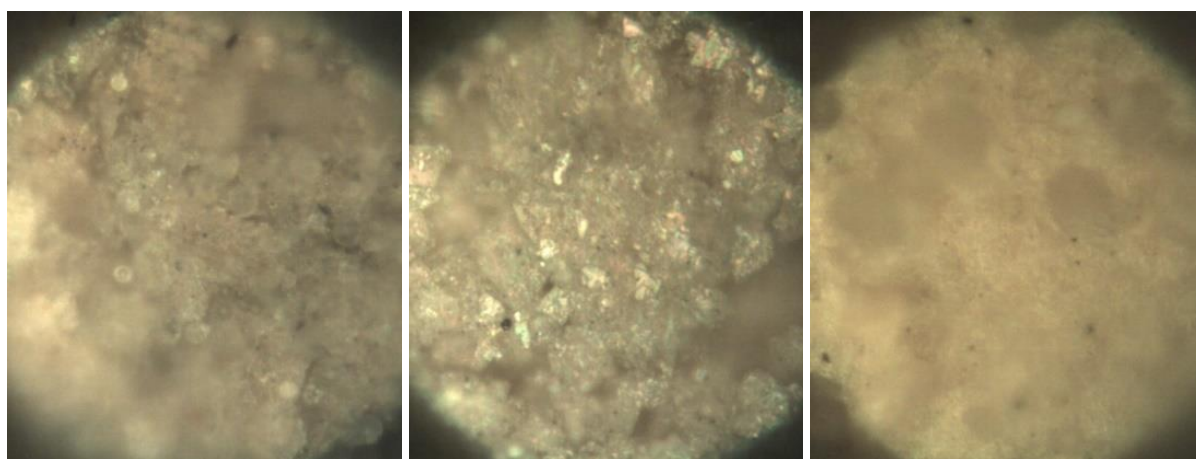


Figure 3.6: Colour photomicrograph images of foundations with varying surface topologies that gave rise to focusing difficulties, (L-R) JML Mineral Magic Compact Foundation (pressed powder formulation), Collection 2000 Perfecting Minerals Foundation in shade 3 Natural Beige (loose powder formulation), Rimmel Lasting Matte Foundation in shade 010 Warm Porcelain (mousse formulation). Samples deposited onto glass microscope slides.

A visual distinction can be made between natural mica flakes and those of borosilicate glass owing to their different morphologies, however, synthetic mica (synthetic fluorophlogopite) is visually consistent with the naturally occurring mica, and can only be differentiated by its fluorine content, via elemental analysis. Mica flakes appear in a variety of shapes and sizes, as illustrated in Figure 3.7, but are comparatively much smaller overall than borosilicate glass flakes. Furthermore, mica flakes tend to display rounded, irregular edges, whereas borosilicate glass flakes have smooth, sharp, well-defined edges.

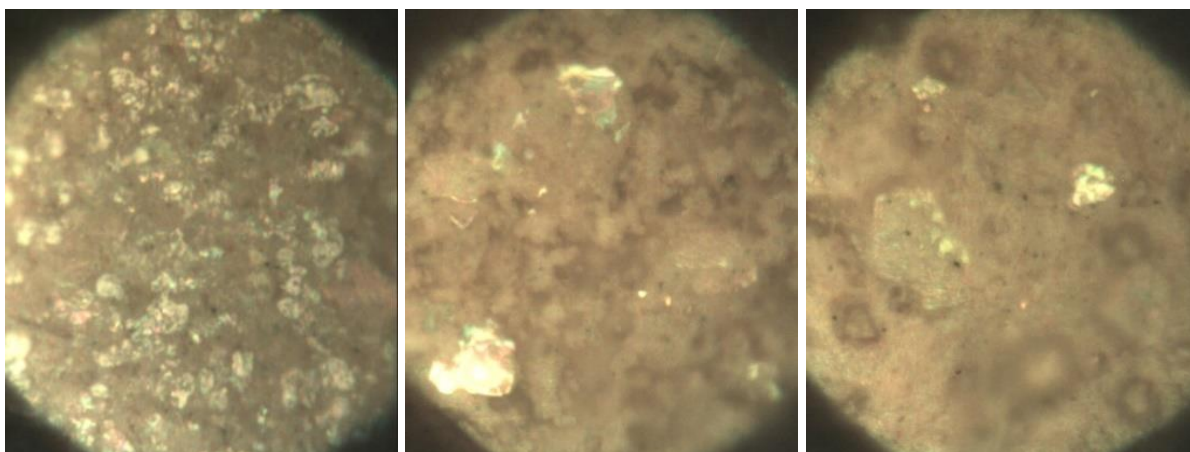


Figure 3.7: Colour photomicrograph images of mica-containing foundations, illustrating differences in flake size and shape, (L-R) BareMinerals Original Foundation in shade Fair Ivory, Astor Lift Me Up Foundation in shade 100, Maxfactor Radiant Lift Foundation in shade 040 Light Ivory. Samples deposited onto glass microscope slides.

Another feature that can be observed, predominantly in foundation samples, is the presence of spherical particles (Figure 3.8). These may be silica, polymethyl methacrylate (PMMA), acrylic, or polyester based. Their purpose is to fill lines and wrinkles on the skin to give a smoother, more even complexion, similar to using Polyfilla to fill in plaster cracks on walls. Microscopically, it is very difficult to discriminate between silica and PMMA based particles, however, they can be readily identified using FTIR spectroscopy. Spherical particles may also be present as a component encapsulation method – similar to biological vesicles – to control the release of active ingredients or provide certain sensory properties. Lecithin can also be used to create liposomes in cosmetics, which function in the same way²⁵.

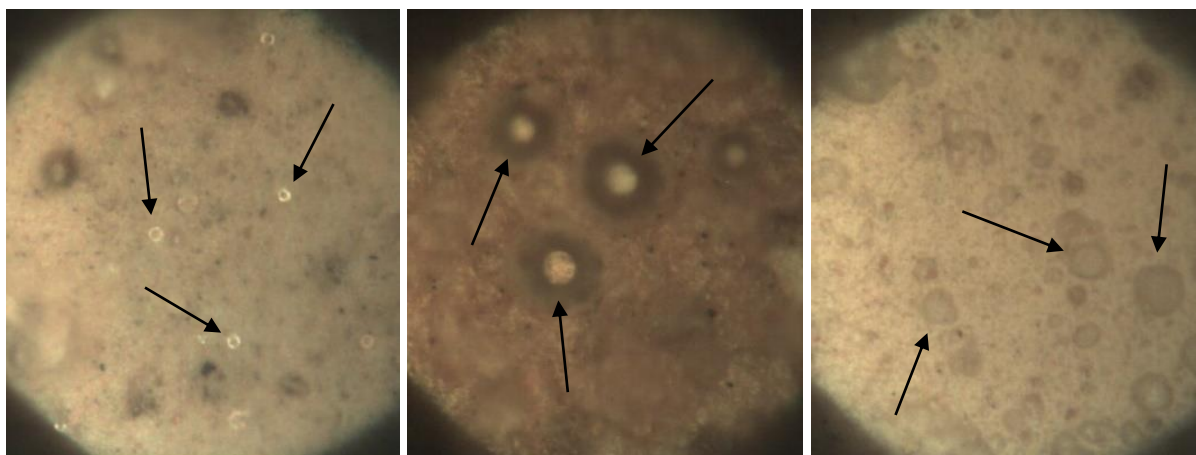


Figure 3.8: Colour photomicrograph images of foundations that contain various different spherical particles (selection marked with black arrows), (L-R) Diorskin Star Studio Makeup in shade 020 Light Beige, Maxfactor Facefinity 3 in 1 Foundation in shade 050 Natural, MAC Studio Sculpt Foundation in shade NC25. Samples deposited onto glass microscope slides.

3.3.2.2 Concealers

The spectrally homogeneous concealer set (n=27) comprised 11 liquid formulations, three loose powders, and 13 cream formulations. None of the concealers included in this part of the study were pressed powder or mousse formulations, and hence, made focusing the laser spot much easier. The majority of the concealers were visually similar and tended to have a more consistent appearance, with less apparent pigment particles than the foundations, as is demonstrated in Figure 3.9.

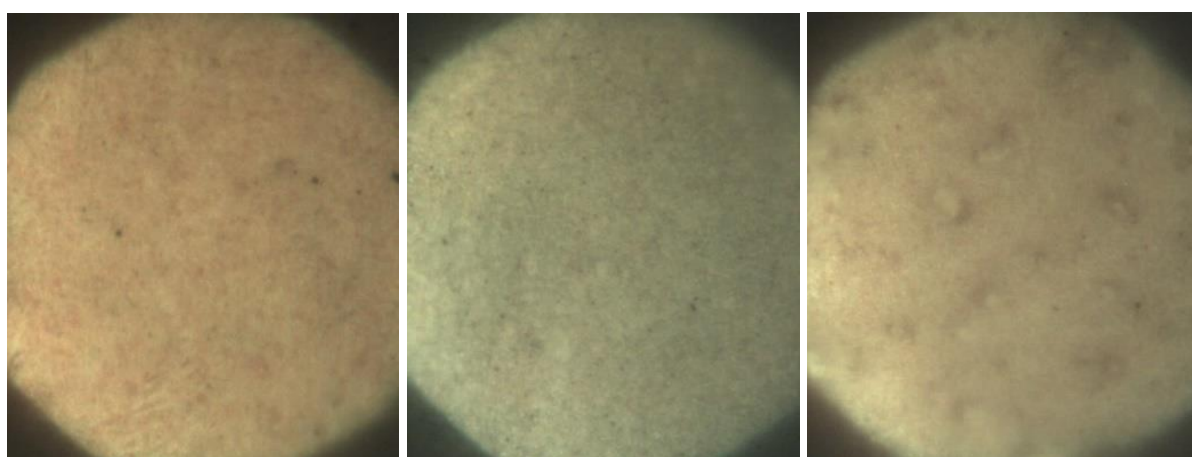


Figure 3.9: Colour photomicrograph images of spectrally homogeneous concealers, demonstrating consistent appearances and lack of obvious pigment particles, (L-R) Body Collection England Concealer in shade Medium, Lacura CC Stick Concealer in shade Peach, Oriflame Love Nature Corrective Stick in shade Beige. Samples deposited onto glass microscope slides.

As was the case with some of the foundation samples, some of the concealers also contained spherical particles as can be seen in Figure 3.10, although less variation in their sizes, colour or appearance was exhibited.

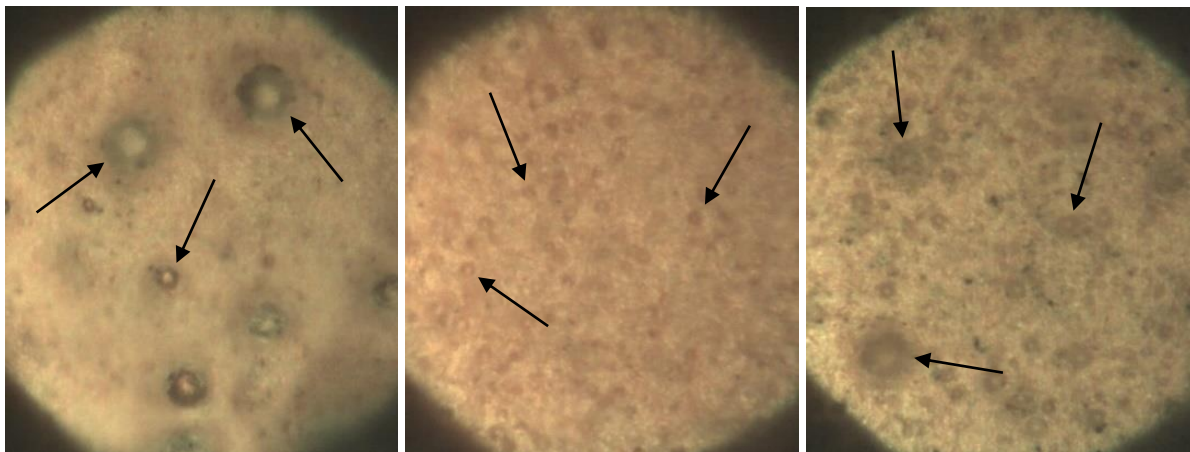


Figure 3.10: Colour photomicrograph images of concealers that contain various spherical particles (selection marked with black arrows), (L-R) Maybelline Fit Me Concealer in shade 15 Fair, Rosie by Autograph Amazing Skin Secret Concealer in shade 1, Pixi Pat Away Concealing Base in shade 3 Warm. Samples deposited onto glass microscope slides.

A minority of the concealers contained mica flakes, as is shown in Figure 3.11, but these were all identified as being specifically under-eye concealers as opposed to blemish concealers. An under-eye concealer is a targeted cosmetic, formulated to brighten the skin and counteract the appearance of dark circles, whereas a traditional concealer is designed to mask imperfections and is essentially a higher coverage, more highly pigmented version of a foundation. Concealers tend to contain fewer reflective pigments than do foundations, as these particles highlight and draw attention to unwanted areas of the skin. Therefore, concealer formulations tend to give a solid block colour and matte finish.

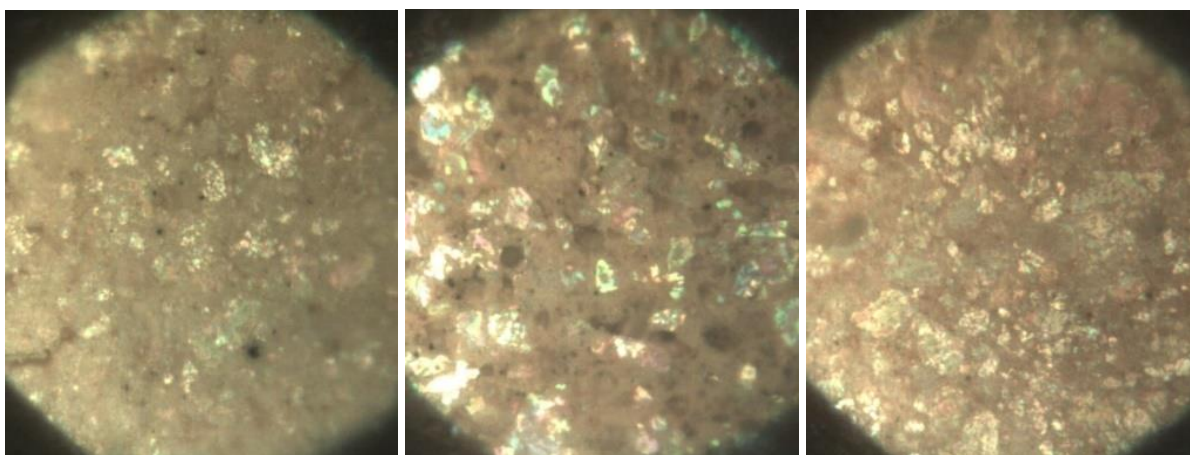


Figure 3.11: Colour photomicrograph images of mica-containing concealers, (L-R) BareMinerals Well Rested Eye Brightening Concealer (no shade specified), Barry M Flawless Light Reflecting Concealer in shade 2 Medium, BareMinerals Multitasking Concealer in shade Bisque. Samples deposited onto glass microscope slides.

3.3.2.3 Tinted moisturisers

The spectrally homogeneous tinted moisturisers (n=16) consisted exclusively of liquid formulations. As was the case with the concealers, the majority of these samples were visually consistent and unremarkable as can be seen in Figure 3.12.

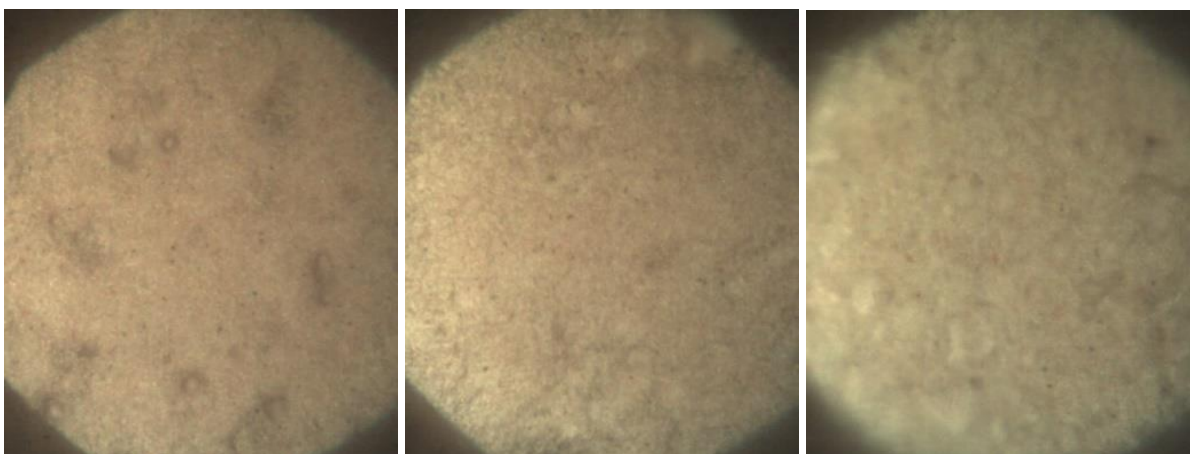


Figure 3.12: Colour photomicrograph images of spectrally homogeneous tinted moisturisers that also exhibit good visual homogeneity, (L-R) Olay Total Effects Touch of Foundation in shade Medium, No.7 Triple Protection Tinted Moisturiser in shade Fair, Osiris Avise BB Cream in shade Light. Samples deposited onto glass microscope slides.

Four of the samples were found to contain mica flakes, three of which are shown in Figure 3.13. The mica flakes in these samples were numerous and widespread, giving a heterogeneous appearance. However, the other mica-containing tinted moisturiser – Avon

Ideal Flawless CC Cream – did not exhibit the same number or distribution of flakes. As is demonstrated in Figure 3.14, this sample was visually homogenous on first glance but a thorough microscopic search revealed randomly sized, shaped, and coloured mica flakes. It is important to note here that the Raman spectroscopic analysis of visually heterogeneous samples such as this Avon product, was deliberately carried out on both the particles specifically and the matrix more generally, to check for spectral differences.

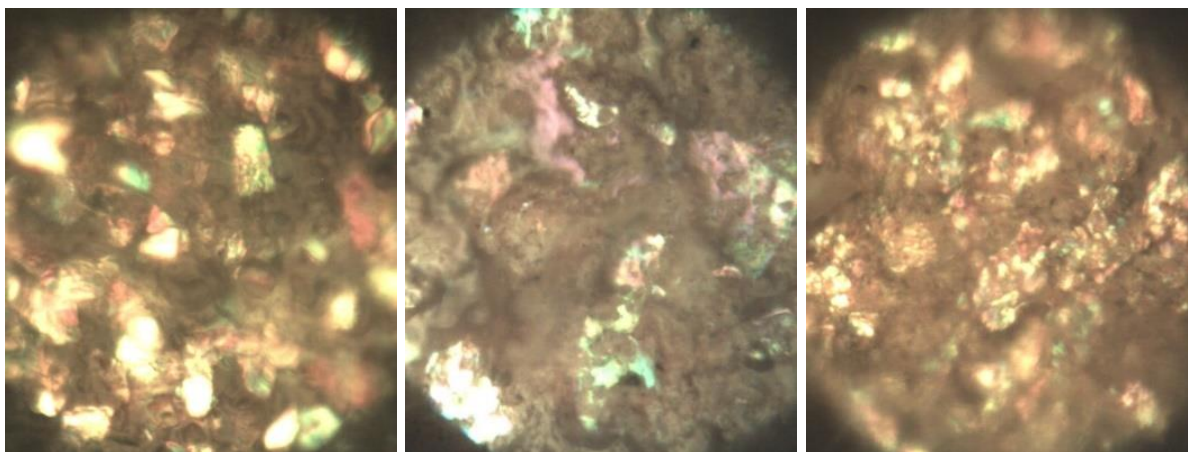


Figure 3.13: Colour photomicrograph images of mica-containing tinted moisturisers, (L-R) Elemis Glow Priming Moisturiser (no shade specified), M&S Luminescent Tinted Moisturiser in shade Medium, Vita Liberata Instant HD Skin Finish in shade Medium. Samples deposited onto glass microscope slides.

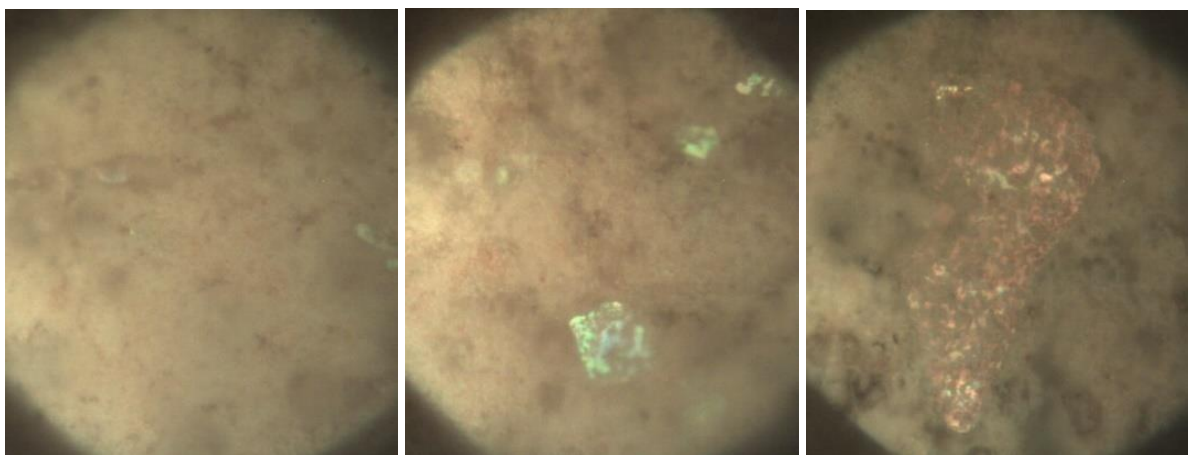


Figure 3.14: Colour photomicrograph images of tinted moisturiser sample Avon Ideal Flawless CC Cream in shade Ivory, showing (L-R) the general sample matrix, some green coloured small-medium sized reflective particles, and a large red coloured reflective particle. Sample deposited onto glass microscope slide.

3.3.2.4 Finishing powders

Only six finishing powders from the initial sample set were found to be spectrally homogeneous, the other 13 were categorised as spectrally heterogeneous and are discussed in Chapter 4. Of these six finishing powders, four were loose powders and two were pressed powders. As was the case with the other product types, the solid formulations were much more difficult to focus than the liquid and cream formulations owing to variations in their surface topologies. Figure 3.15 illustrates the varying levels of focus achieved with pressed powders, compared with loose powder formulations.

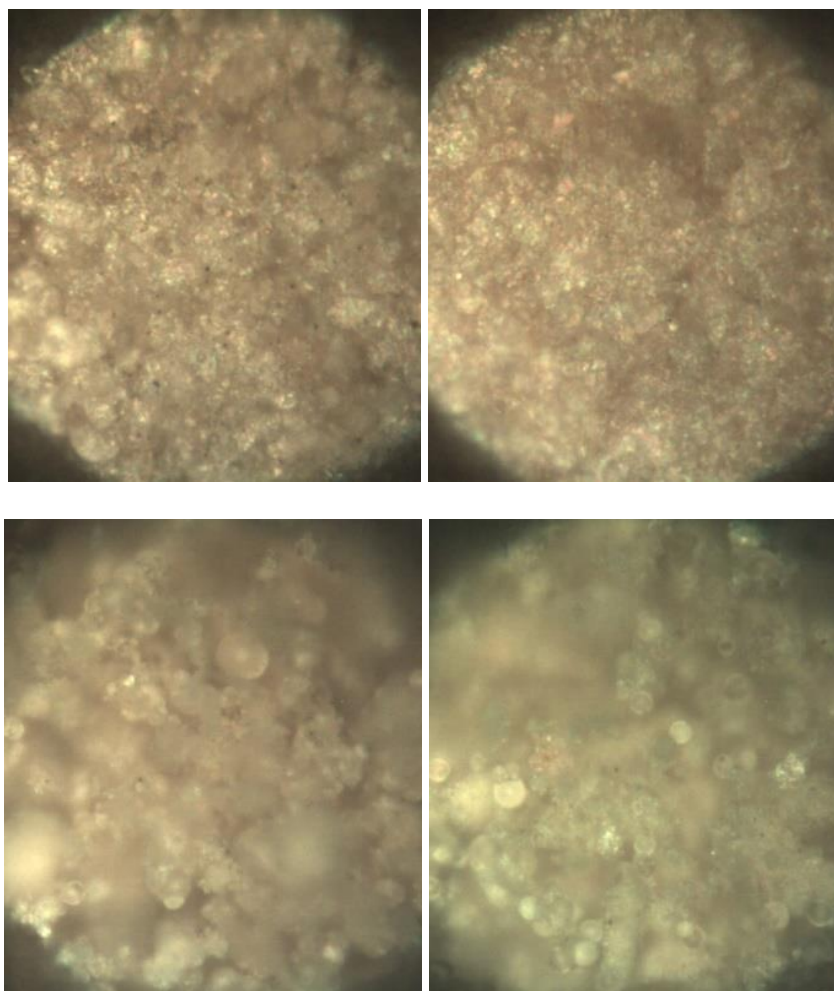


Figure 3.15: Colour photomicrograph images of spectrally homogeneous finishing powders, illustrating focusing differences between pressed and loose powder formulations, (top row L-R) Avon Pressed Powder in shade Beige (pressed powder), Ocuz Compact Powder in shade P03 (pressed powder), (bottom row L-R) BareMinerals Under Over Priming-Finishing Powder (no shade specified) (loose powder), BareMinerals Colour Correcting Mineral Veil (no shade specified) (loose powder). Samples deposited onto glass microscope slides.

As is evident from the images, all six finishing powders displayed visual heterogeneity despite being spectrally homogeneous. One can assume that the particulate sizes were sufficiently small such that the laser spot size (1.4 μm) did not focus solely upon one component.

The two loose finishing powders labelled as translucent shades had quite different appearances, as is shown in Figure 3.16. Upon reviewing the ingredients lists for both products it transpired that the predominant ingredient in the Pixi Quick Fix Powder (as indicated by its position in the list) was mica, followed by modified corn starch, with a minimal quantity of titanium dioxide. However, the BareMinerals sample was mainly corn starch (a mixture of standard corn starch and modified corn starch) with a minimal quantity of the three iron oxide pigments (red CI77491, yellow CI77492, and black CI77499) but neither mica nor titanium dioxide.

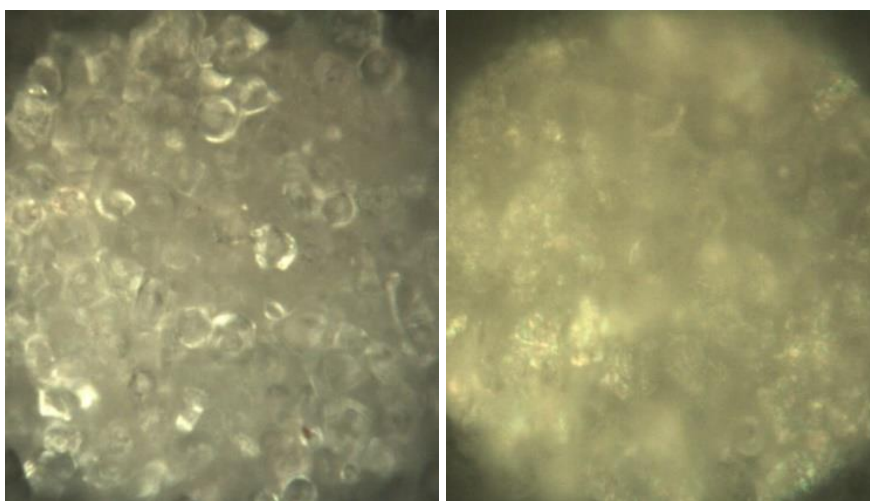


Figure 3.16: Colour photomicrograph images of spectrally homogeneous translucent finishing powders, (L-R) BareMinerals Original Mineral Veil in shade Translucent (corn starch based), Pixi Quick Fix Powder in shade Translucid (mica based). Samples deposited onto glass microscope slides.

3.3.3 Characterisation using Raman spectroscopy

Raman spectroscopic analysis of all samples demonstrated that the most discriminating features were the titanium dioxide polymorph, red iron oxide (Fe_2O_3), yellow iron oxide ($\text{FeO}(\text{OH})$) or black iron oxide (Fe_3O_4) content, and the organic or silicone profile. Titanium dioxide has three main polymorphs: anatase, rutile, and brookite. Rutile is the most abundant and most stable polymorph; anatase and brookite are both metastable forms that convert irreversibly to rutile under certain temperature and pressure conditions. All the samples analysed in this Chapter contained either the anatase or rutile polymorphs (or a combination of the two), but no samples were found to contain the brookite polymorph. Foundation samples

were found to be predominantly anatase based, whereas the concealer sample population demonstrated a heavier reliance on the rutile polymorph. It is assumed that this may be due to rutile's slightly increased hardness as per the Mohs scale, increased density, and increased adhesion²⁶. This would be beneficial within a concealer formulation as it may give the product increased persistence on the skin. Rutile also has a higher opacity and a slightly more yellow tone than anatase²⁶, both of which would be desirable characteristics for a concealer. It may also simply be to save costs as rutile is cheaper to purchase than anatase.

Unsurprisingly, tinted moisturiser spectra tended to display a higher organic/silicone content, consistent with the purpose of the product. Finishing powders did not feature titanium dioxide as strongly, with half of these products containing neither polymorph and instead relying upon corn starch or talc. This is also consistent with the product's intended purpose of absorbing oil and smoothing the skin's appearance, as no pigment would be required to fulfil these properties.

Where samples displayed **visual** heterogeneity via randomly distributed mica flakes or other particles, such as those seen in Figure 3.14, spectra were deliberately taken of both the matrix and of the specific particles to assess for **spectral** heterogeneity. In some cases, the particle and the general matrix revealed similar spectra, in others, the spectra were noticeably different. For the purposes of displaying representative spectra and for creating the chemometric models in Section 3.3.5, only the general matrix spectra were included. Where spectra taken of particles was seen to be different to the general matrix, these always showed a strong anatase response, suggestive of a titanium dioxide-coated interference pigment particle.

It is important to note that cosmetic ingredients are listed in decreasing order of concentration^{27–29} or weight but no specific amounts are given, unless an ingredient is listed as “*active*”, such as is the case when titanium dioxide is used as a physical sunscreen rather than a white pigment. Thus, products with identical ingredient lists may have very different compositions, even if those ingredients appear in the same order. Furthermore, due to the stringent legislation governing cosmetic ingredients, commonly encountered substances such as talc or rice starch may have been chemically treated so they are suitable for application to the skin. This means that an ingredient in a formulation may be a different version to what is expected and therefore behave unexpectedly when analysed.

In addition to the product's purpose, its desired properties and/or the effect on the skin need to be considered in the context of raw components. These will dictate the ingredients used in the formulation and the relative proportions of each. Moreover, the product's mode of

application will also influence its formulation, i.e. a pressed powder applied with a brush compared to a liquid applied with a sponge.

Ingredient lists for cosmetic products are often extensive and complex. Some of these ingredients will show in a Raman spectrum, others will not, dependent upon the properties of the chemical and its relative abundance (which we do not know). This complicates the interpretation of resultant spectra.

3.3.3.1 Foundations

The foundation samples ($n=77$) encompassed 55 different product lines from 35 different brands, spanning a range of shades from “ivory” to “dark”. As mentioned in the previous Section, all five different formulations were represented in this sample subset.

The majority of the foundation samples contained the anatase polymorph of titanium dioxide ($n=56$) and examples of these Raman spectra are given in Figure 3.17. Examples of rutile dominant Raman spectra are shown in Figure 3.18, while those containing a mixture of the two polymorphs are shown in Figure 3.19. A single foundation sample contained no discernible titanium dioxide and this is shown in Figure 3.20.

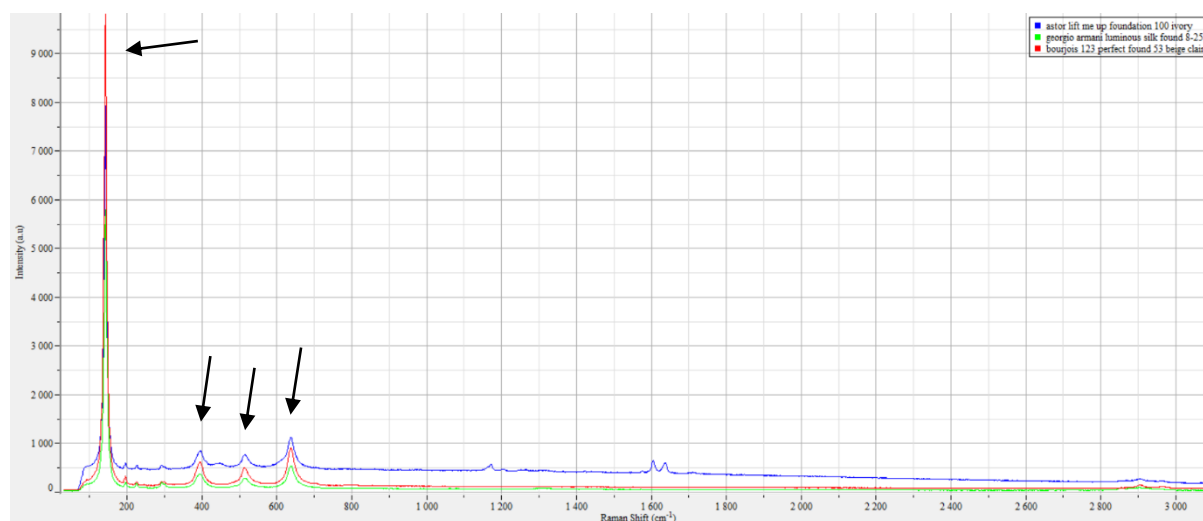


Figure 3.17: Raman spectra (unprocessed) of Astor Lift Me Up Foundation in shade 100, Giorgio Armani Luminous Silk Foundation in shade 8.25, and Bourjois 123 Perfect Foundation in shade 53 Light Beige, examples of anatase-containing foundations. Black arrows mark the intense “lattice” peak at 143 cm^{-1} and the characteristic triplet of peaks at 395 cm^{-1} , 515 cm^{-1} , and 637 cm^{-1} .

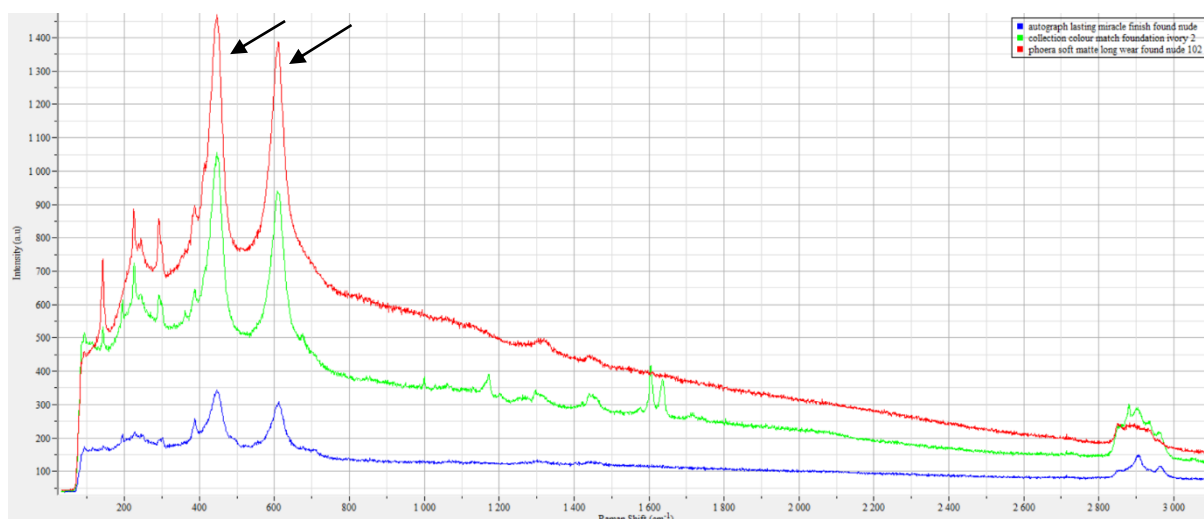


Figure 3.18: Raman spectra (unprocessed) of Autograph Lasting Miracle Foundation in shade Nude, Collection Colour Match Foundation in shade Ivory 2, and Phoera Soft Matte Long Wear Foundation in shade Nude 102, examples of rutile-containing foundations. Black arrows mark the characteristic doublet of peaks at 447 cm^{-1} , and 610 cm^{-1} .

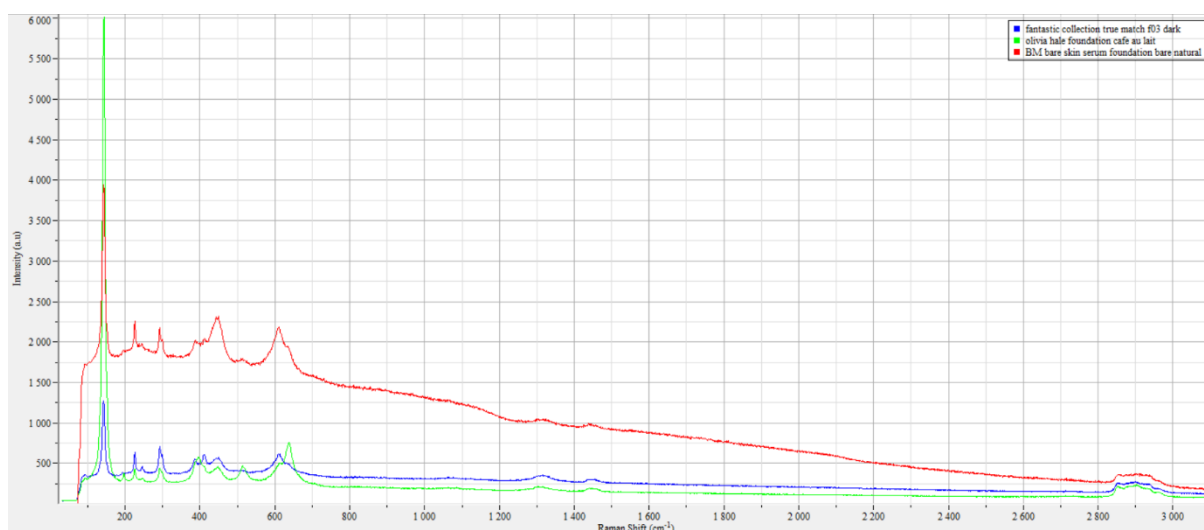


Figure 3.19: Raman spectra (unprocessed) of Fantastic Collection True Match Foundation in shade F03 Dark, Olivia Hale Foundation in shade Café au Lait, and BareMinerals BareSkin Serum Foundation in shade Bare Natural, examples of anatase/rutile-containing foundations.

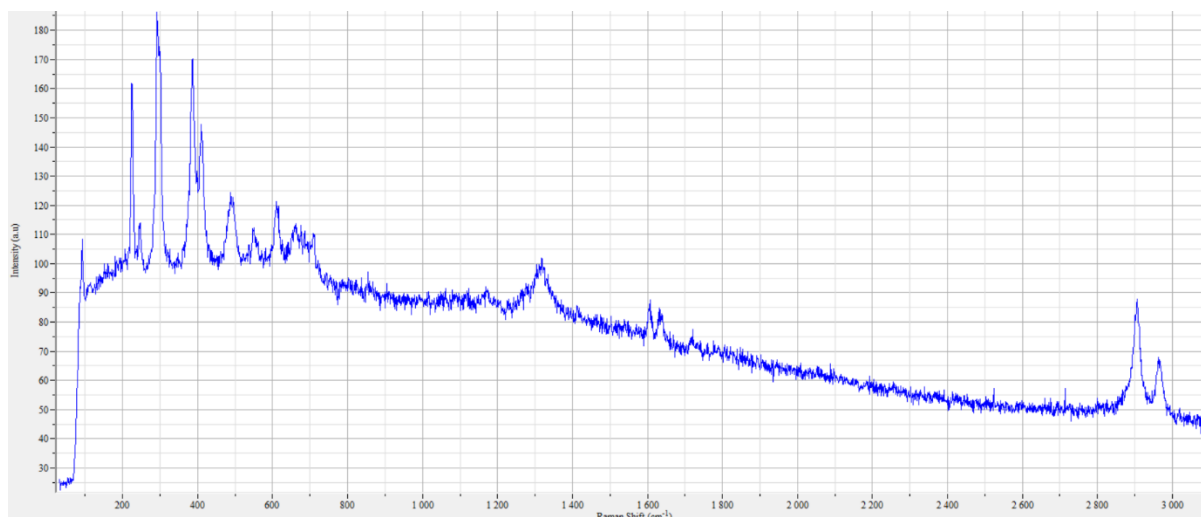


Figure 3.20: Raman spectrum (unprocessed) of Primark My Perfect Colour Matte Foundation in shade Amber Honey, an example of a foundation that contains neither titanium dioxide polymorph.

As can be seen in Figure 3.17, anatase-containing foundations present an extremely intense peak at 143 cm^{-1} , often referred to as the “*lattice*” peak, and these dominate the spectra³⁰. The characteristic triplet of peaks seen thereafter occur at 395 cm^{-1} , 515 cm^{-1} , and 637 cm^{-1} respectively. The response from the “*lattice*” peak is approximately one order of magnitude larger than the next most intense spectral peak (the 637 cm^{-1} peak) and this overwhelms the spectrum.

The result is that many of the anatase containing foundations appear at first glance to be very similar, however, upon zooming into the baseline many different spectral features become apparent, and samples can be visually differentiated from one another. Figure 3.21 demonstrates that multiple spectral peaks were not visible initially but became clear once zoomed into the baseline. Consequently, it was possible to see that the Clinique foundation has a small peak at 3052 cm^{-1} , which is indicative of an aromatic compound, a sharp peak at 1000 cm^{-1} indicative of a silicate and a broader peak centred at 1300 cm^{-1} indicative of red iron oxide (Fe_2O_3). Conversely, the Bourjois foundation has a peak at $\sim 1400\text{ cm}^{-1}$ that is absent in all the Clinique replicate spectra.

This approach was used during spectral acquisition to both assess the spectral peak positions/intensities that would otherwise not have been readily visible, and also to check for residual fluorescence in the baseline.

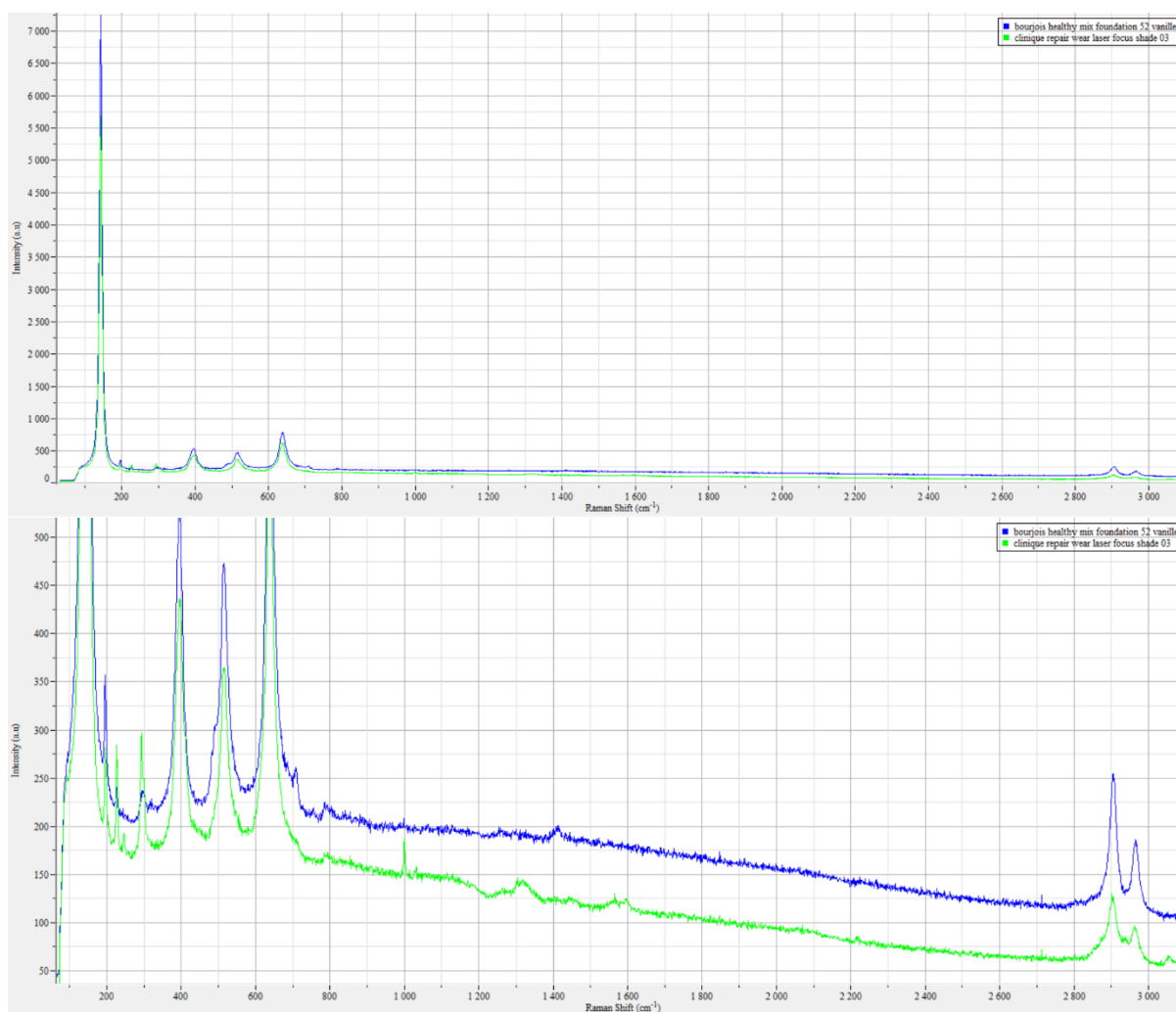


Figure 3.21: Raman spectra (unprocessed) of Clinique Repair Wear Laser Focus Foundation in shade 03 and Bourjois Healthy Mix Foundation in shade 52 Vanille (top), and magnified to show visible peak differences in the baseline, between the two samples (bottom).

This problem was not encountered among the rutile containing foundations, as there is no intense “lattice” peak associated with the rutile spectrum³⁰. Instead, the characteristic Raman spectral profile for rutile is a double peak, occurring at 447 cm⁻¹ and 610 cm⁻¹. The overall spectral peak intensities for rutile containing foundations are markedly lower, as is evident from the intensity axes in Figure 3.18, meaning weaker peaks elsewhere in the spectra are more readily discernible.

A small minority of foundation samples contained a mixture of anatase and rutile as shown in Figure 3.19. Where this mixture was detected, the samples all derived from a budget range, leading one to assume that the manufacturers of cheaper products use a non-specific titanium dioxide blend in order to keep costs to a minimum.

A caveat to this is the BareMinerals Bareskin Foundation in shade Bare Natural, which retails in the premium market. What is particularly interesting though, is that the three other BareMinerals Bareskin Foundations (in shades Bare Linen, Bare Satin and Bare Ivory) analysed within this dataset, do not exhibit this titanium dioxide polymorph mixture as can be seen in Figure 3.22. Upon reviewing the sample packaging, it was discovered that the Bare Linen, Bare Satin and Bare Ivory foundations were original bottled samples but the Bare Natural foundation was obtained as a promotional tester packet.

The reason for this chemical composition difference is unclear. It may be due to them being manufactured at different times (formulation changes), or in different factories, or perhaps even in different countries. It may be that tester samples are not made to the same standard as the full-sized commercial product, or it may simply be that the darker the shade becomes, the more rutile is added to the composition. Given that the Bare Natural sample is the darkest shade within the series that is represented in this dataset, further research would be needed to ascertain whether or not there is any basis to this assertion. Furthermore, the conversion of anatase to rutile is reported to take place under relatively low temperature and therefore it may be the case that the integrity of this sample's packaging was compromised, allowing the polymorph conversion to take place. As there was neither an expiry date nor a manufactured date on the tester packet, its age cannot be verified.

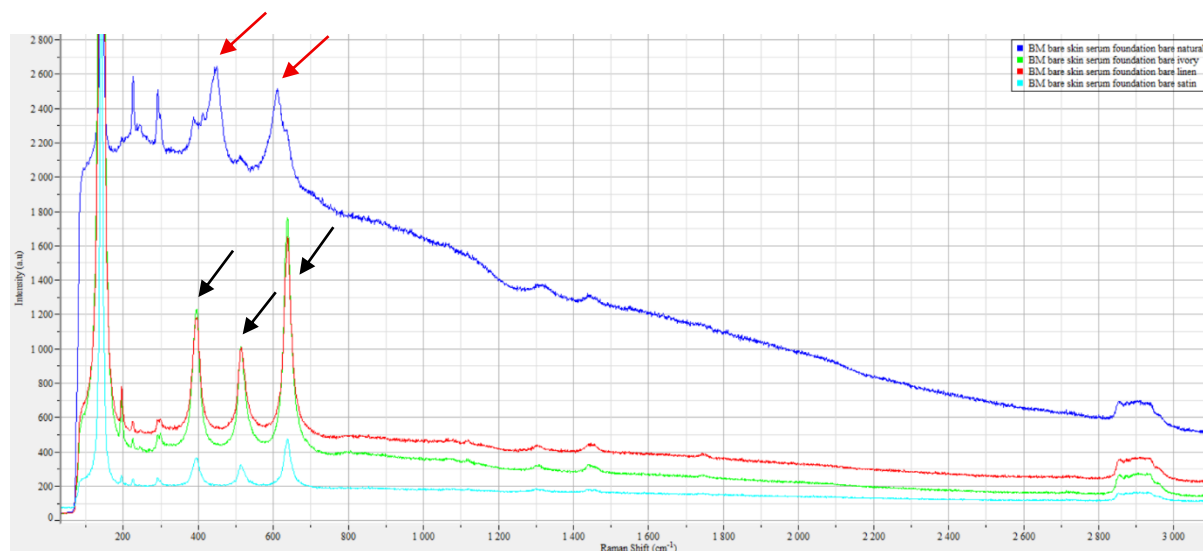


Figure 3.22: Raman spectra (unprocessed) of BareMinerals Bareskin Foundations in shades Bare Ivory, Bare Linen, Bare Natural, and Bare Satin, showing a clear difference in the titanium dioxide polymorph used in these formulations. Black arrows mark the anatase triplet of peaks at 395 cm^{-1} , 515 cm^{-1} , and 637 cm^{-1} ; the red arrows mark the rutile doublet of peaks at 447 cm^{-1} , and 610 cm^{-1} .

Only one foundation sample was discovered to contain no titanium dioxide – Primark My Perfect Colour Matte Foundation (Figure 3.20) – yet the ingredients list states “*may contain (+/-) CI 77891 (Titanium Dioxide)*”. Nevertheless, this foundation is a relatively dark shade, therefore it is reasonable to assume that the amount of titanium dioxide (as a white pigment) used in its formulation is very small, and therefore those peaks may have been obscured by other, more abundant components. The predominant mineral ingredient for this sample is listed as talc, but the reference spectrum taken of talc does not seem to support this assertion either. This may be due to the fact that the talc used as a reference sample was not of cosmetic grade, which is the highest purity of all talc grades and is mined specifically from asbestos-free sites. However, this would only be relevant if cosmetic talc had a substantially different Raman spectrum to that of standard lab-grade talc, which is unlikely. Moreover, none of the four main characteristic group frequencies of talc as given by Socrates ($445\text{--}455\text{ cm}^{-1}$, $530\text{--}540\text{ cm}^{-1}$, $665\text{--}675\text{ cm}^{-1}$, and $1005\text{--}1030\text{ cm}^{-1}$) match with the peaks in this sample’s spectrum³⁰. Nonetheless, what must also be borne in mind is that the mixture itself confers a specific pattern that might not be apparent when viewed as component molecules.

The reference spectrum that matches most closely with this product’s spectrum is that of the bronze mica reference sample as can be seen in Figure 3.23. This sample was purchased from Making Cosmetics and is listed as natural muscovite (CI 77019) coated with red iron oxide (CI 77491). As is shown in Figure 3.23, the spectra for this Primark foundation and the mica bronze reference share common peaks at 225 cm^{-1} , 246 cm^{-1} , 292 cm^{-1} , 298 cm^{-1} , 410 cm^{-1} , 490 cm^{-1} , 612 cm^{-1} , and 1318 cm^{-1} .

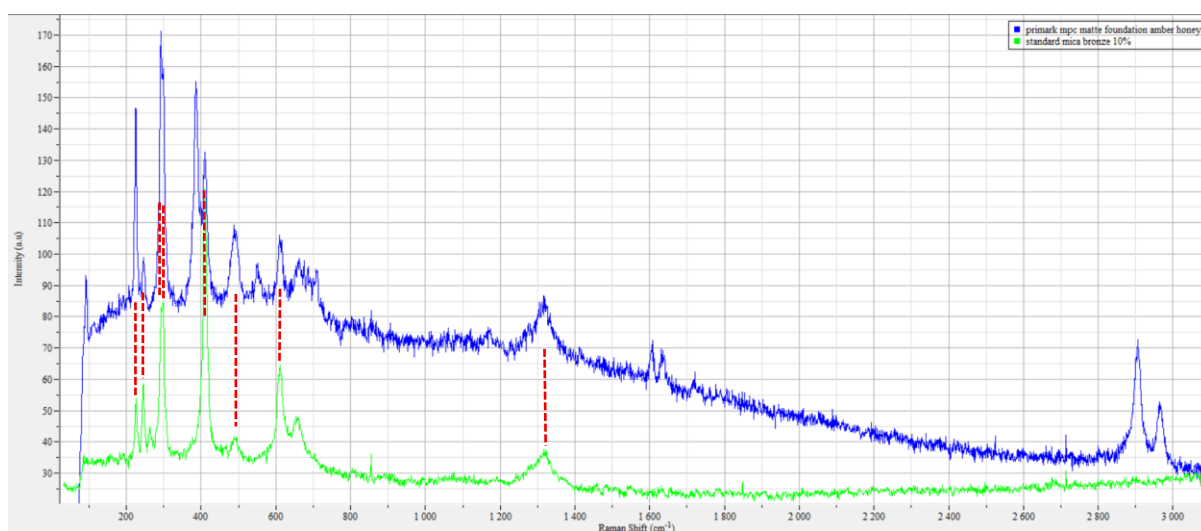


Figure 3.23: Raman spectra (unprocessed) of Primark My Perfect Colour Matte Foundation in shade Amber Honey and the bronze mica reference standard, demonstrating common peaks at 225 cm^{-1} , 246 cm^{-1} , 292 cm^{-1} , 298 cm^{-1} , 410 cm^{-1} , 490 cm^{-1} , 612 cm^{-1} and 1318 cm^{-1} .

3.3.3.2 Concealers

The concealer samples (n=27) represented 15 different brands, spanning 21 product ranges and covering shades from “ivory” to “dark”. As mentioned in Section 3.3.2.2, the concealers were represented by only three formulations: liquids, loose powders, and creams, however, the proportion of cream formulations within this sample set (n=13) is noteworthy. This is presumably due to the increased coverage that is preferable for this type of product. None of the concealers included in this part of the study were pressed powder or mousse formulations.

As mentioned at the beginning of Section 3.3.3, the concealers relied more heavily upon the rutile polymorph, with 11 of the samples showing rutile content, 15 showing anatase content, and a single sample showing a mixture of the two polymorphs (Figure 3.24). Discussions with several cosmetic companies have indeed confirmed the assumption that the choice of polymorph is deliberate and relates to both the product’s function and its desired effect. Nevertheless, communication with Glorious Brands – the parent company that manufactures the Pur Disappearing Ink Concealer included in this sample set (Figure 3.25) – revealed some surprising results from their in-house study³¹.

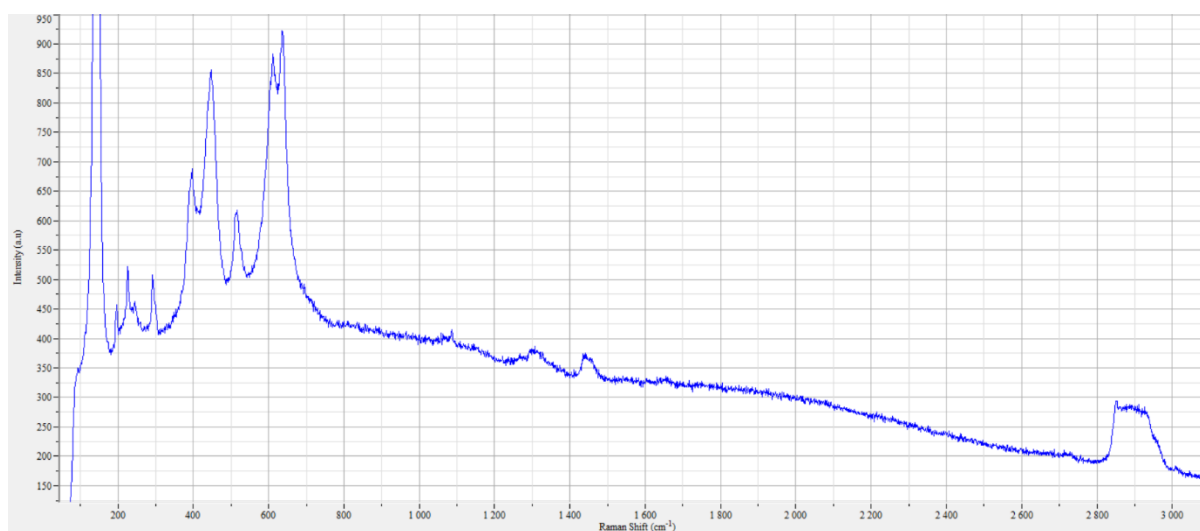


Figure 3.24: Raman spectra (unprocessed) of Body Collection England Concealer in shade Medium; the only anatase/rutile mix containing concealer in the spectrally homogeneous concealer sample set.

A representative of the company stated that during their tests, the rutile polymorph was found to wash off skin more easily than the anatase version. This contradicts the assumption that rutile may demonstrate higher persistence due to its slightly higher Mohs scale hardness value. Worryingly, they also report that once applied to the skin and irradiated with UV-light, the anatase polymorph had “*damaged the outermost layer of skin with low ultraviolet light*”, while rutile had “*little effect*” on the skin. They further commented that “*rutile titanium dioxide*

has high UV-light absorption and is non-toxic”, yet the safety data sheets for both polymorphs suggest that neither are toxic^{32,33}.

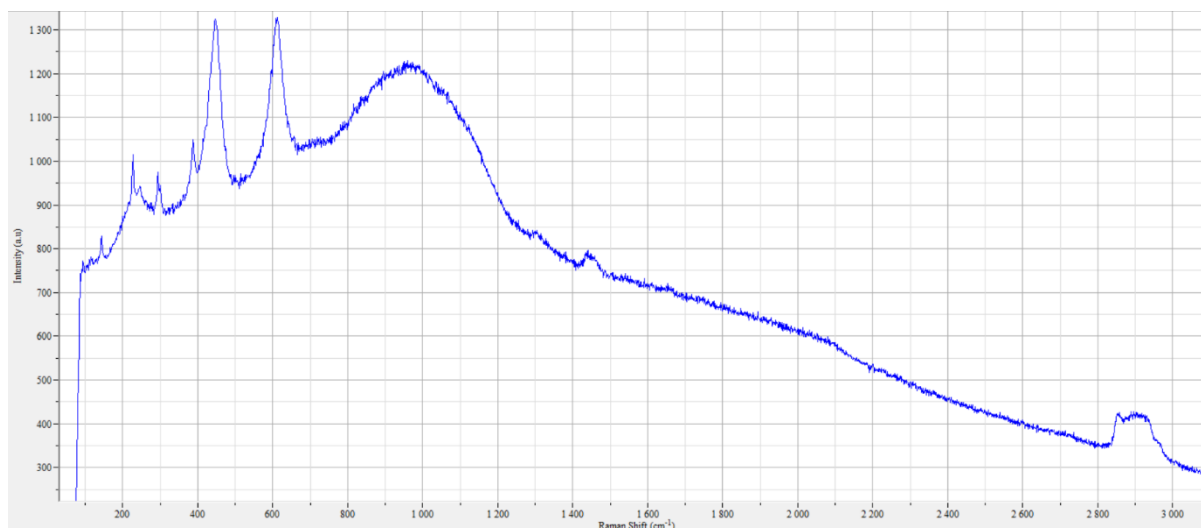


Figure 3.25: Raman spectra (unprocessed) of Pur Disappearing Ink Concealer in shade Light; an example of a rutile-containing concealer.

3.3.3.3 Tinted moisturisers

The tinted moisturisers (n=16) represented 11 product lines from nine different brands, but only represented shades from the light and medium ranges. In addition, the samples were exclusively liquid formulations. With respect to the titanium dioxide polymorph usage, here seven samples contained anatase, eight contained rutile, and one sample contained neither polymorph (Figure 3.26). Unlike the foundation and concealer samples, none of the tinted moisturisers appeared to contain a mixture of the two polymorphs.

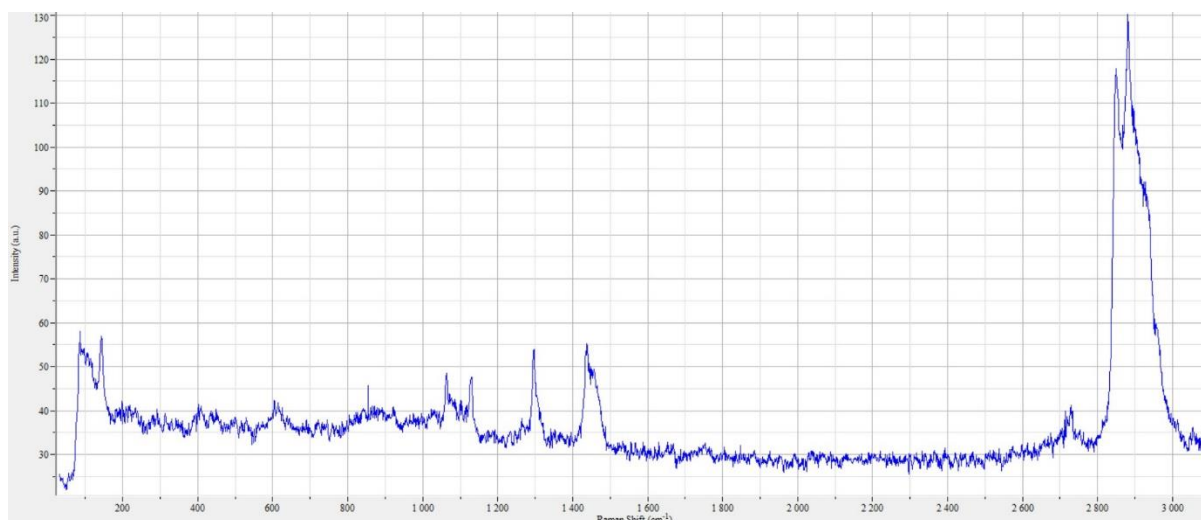


Figure 3.26: Raman spectrum (unprocessed) of Elemis Glow Priming Moisturiser; an example of a tinted moisturiser that contains neither titanium dioxide polymorph.

Fortunately, six of the samples all belonged to the same brand (BareMinerals) and same product line (Complexion Rescue) and differed only by shade. This permitted observations of the influence of pigment concentration to the Raman spectra, across the series of shades. As can be seen in Figure 3.27, all six samples displayed a very intense and broad peak centred at 990 cm^{-1} , consistent with the lecithin reference spectrum. Indeed, when checking the ingredients list for these products, lecithin appeared in position five, although its specific origin was not stated.

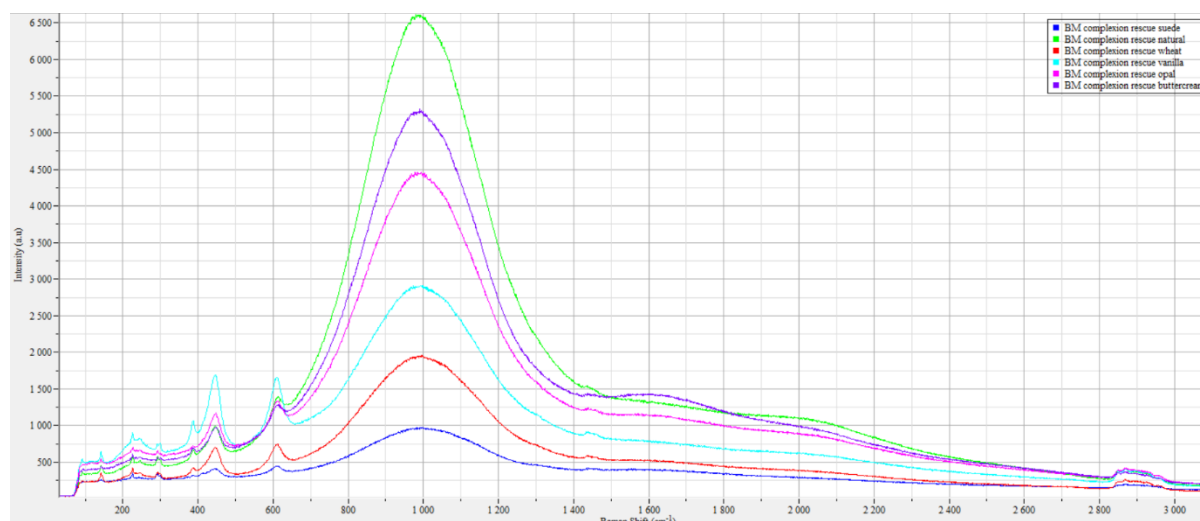


Figure 3.27: Raman spectra (unprocessed) of BareMinerals Complexion Rescue tinted moisturisers in shades *Suede*, *Natural*, *Wheat*, *Vanilla*, *Opal*, and *Buttercream*, depicting differences in lecithin and rutile content.

Lecithin is a mixture of diglycerides of stearic, palmitic, and oleic acids, and is added to cosmetic preparations as an emollient, an emulsifier, a skin penetration enhancer, and an antioxidant³⁴. It can be derived from many different sources including soybean, egg, sunflower seed, maize, rapeseed, and cottonseed³⁵. Unfortunately, the origin of the lecithin used in these tinted moisturisers is not given in the ingredients list so it may be the case that various sources are used and this results in the relative peak intensity differences displayed.

No clear trends could be observed when comparing all 30 spectra except for the relative decrease in the lecithin peak for the shades “*Suede*” and “*Wheat*”, and the additional decrease in the two main rutile peaks for the shade “*Suede*”. These spectral differences cannot be readily explained in terms of pigment concentration as the darkest shade in the series is “*Natural*” and therefore this shade should logically contain the least amount of titanium dioxide.

The tinted moisturiser samples exhibited the most spectral variety of all the product types in this Chapter. Figure 3.28 shows the complex spectrum obtained for the M&S Luminescent Tinted Moisturiser, which shows a highly featured mid-spectral region. Given that tinted

moisturisers are more likely to contain sun protection factor (SPF) than other types of cosmetic, and that physical sunscreens (titanium dioxide and zinc oxide) would display peaks in the lower end of the spectrum, it was thought that these peaks might be related to a chemical sunscreen. Therefore, the reference spectrum for octylmethoxycinnamate (OMC), a UVA/UVB ray sunscreen added to many cosmetics, was obtained and overlaid on the M&S tinted moisturiser spectrum. As can be seen in Figure 3.28, all of the peaks between 1000 cm^{-1} and 1800 cm^{-1} correlate between the spectra. Furthermore, the relative intensities of those peaks are similar, as is the characteristic weak broad peak that immediately follows the peak at 1423 cm^{-1} , and the profile of the response in the CH stretching region (2800-3000 cm^{-1}).

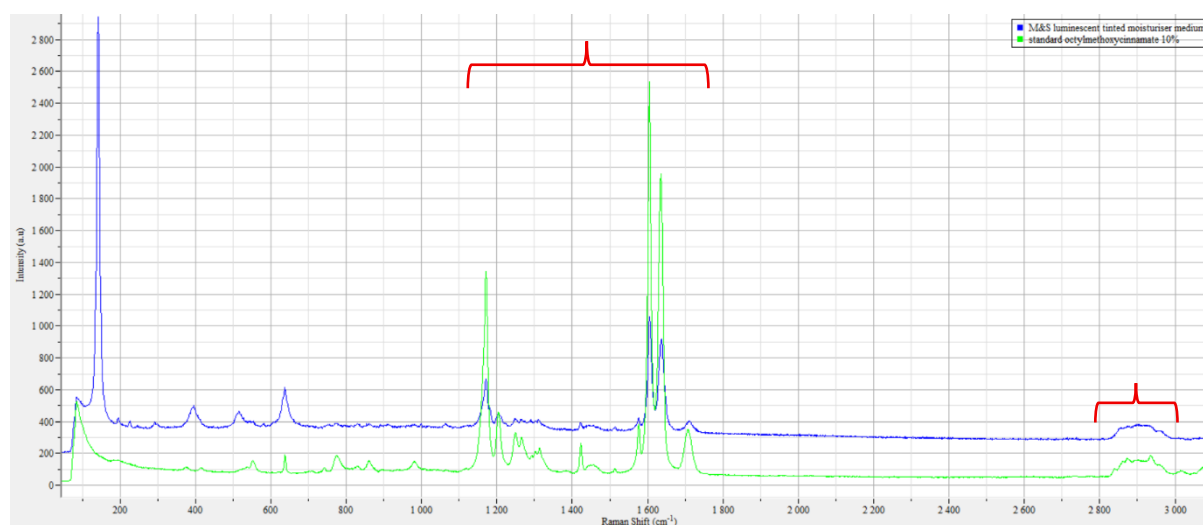


Figure 3.28: Raman spectrum (unprocessed) of M&S Luminescent Tinted Moisturiser in shade Medium; an example of a very unusual and distinctive (anatase-containing) spectrum, overlaid with the Raman spectrum (unprocessed) of octylmethoxycinnamate (OMC) reference standard. Areas with congruent peaks highlighted with red braces.

3.3.3.4 Finishing powders

Only six of the finishing powders analysed in this study were found to be spectrally homogeneous; 13 were found to be spectrally heterogeneous and are dealt with in Chapter 4. The product formulation is the principal reason for this split. As the name suggests, all these products were powders, either loose or pressed. The size and distribution of the powdered particles determined how the laser spot size would interact with the sample, and whether that would result in a largely homogeneous or heterogeneous Raman spectrum. It should be noted that this spectral homogeneity is distinct from visual homogeneity as has been previously discussed.

This sample set comprised six different product lines across four brands. Two of the samples were translucent and four were tinted/coloured. Both of the translucent samples (BareMinerals

Original Mineral Veil and Pixi Quick Fix Powder in Translucid) were found to contain no discernible titanium dioxide at all. Two of the tinted/coloured samples contained anatase, one of them contained rutile, and the remaining tinted sample did not contain any titanium dioxide.

The predominant ingredient for the BareMinerals Original Mineral Veil is listed as corn starch. Unfortunately corn starch was unavailable to purchase from the supplier, so rice starch was selected as the next best option. Plotting this reference spectrum of rice starch against the BareMinerals Original Mineral Veil reveals a striking similarity with correlation between the majority of the peaks, as is demonstrated in Figure 3.29. None of the Raman peaks associated with the five other ingredients that comprise this Mineral Veil product (zinc stearate, magnesium stearate, and the three iron oxides) appear in its spectrum, suggesting that the proportion of these ingredients within the mixture is minimal.

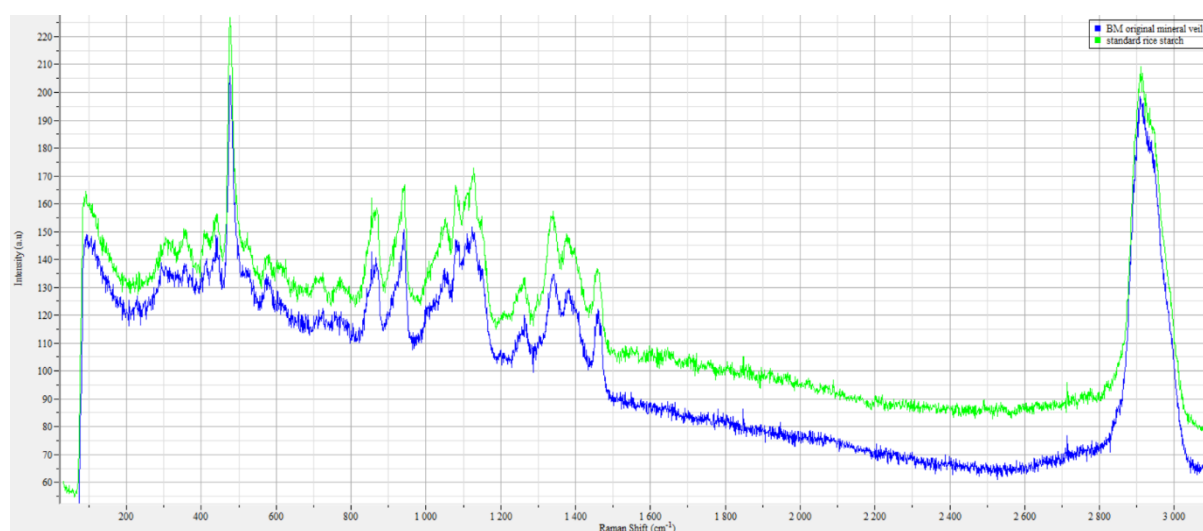


Figure 3.29: Raman spectra (unprocessed) of BareMinerals Original Mineral Veil finishing powder and rice starch reference standard, depicting an almost identical profile.

3.3.4 Exploratory data analysis

The five replicate Raman spectra for each sample were imported into the Unscrambler software as described in Chapter 2.

Firstly, the explained variance plot (or scree plot) was used to assess the optimum number of PCs (Figure 3.30). Typically, between 3-5 PCs is reported in the literature as suitable for ensuring the data structure is modelled sufficiently, without introducing error due to noise³⁶. Nevertheless, for highly variable samples or a large number of samples, it may be suitable to include more PCs than this³⁷. Looking at the explained variance plot it initially seemed that three PCs would be sufficient to model the structure, as this accounted for 89% of the total variance. However, upon inspection of the loadings plots, it became clear that the first seven

PCs (98% of the total variance) all carried useful chemical meaning and were therefore valuable to the model. Given the number of samples in the dataset, the different types of cosmetic products, the differing formulations, and the amount of chemical variability, this is not surprising. Furthermore, the range of PC score values across the samples indicated that there was a lot of variability throughout the data³⁸. Nonetheless, the validation variances track the calibration variances closely, as can be seen in Figure 3.30, indicating the creation of a robust model.

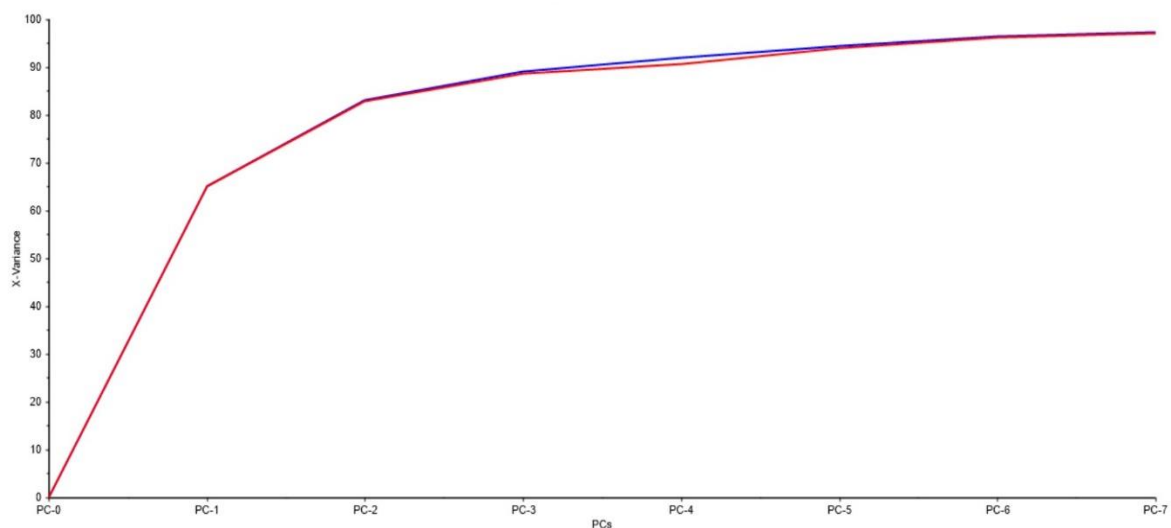


Figure 3.30: Explained variance plot for the spectrally homogeneous sample set ($n=126$), showing 98% of the model variance accounted for in the first seven PCs, the blue trace denotes the calibration variance, the red trace denotes the validation variance.

Due to the large number of samples in the spectrally homogeneous dataset ($n=126$) and the high amount of variability among them, the 3-dimensional PCA scores plots were very difficult to interpret. Subsequently, it was decided that the 3-dimensional plots would be used in the software to help identify trends across three PCs (as these plots could be rotated), but that the simpler 2-dimensional plots would be exported for results purposes.

Sample grouping was performed for each category variable in turn, then various different combinations of PCs were assessed in the scores plots. Figure 3.31 shows the first analysis carried out: a visualisation of the total spread of all the spectrally homogeneous samples, grouped by brand. Some sample clustering is observed with spectral replicates showing good repeatability, but in general the plot is messy and difficult to interpret. This is partly expected given that this model comprises 630 spectra across the 126 samples.

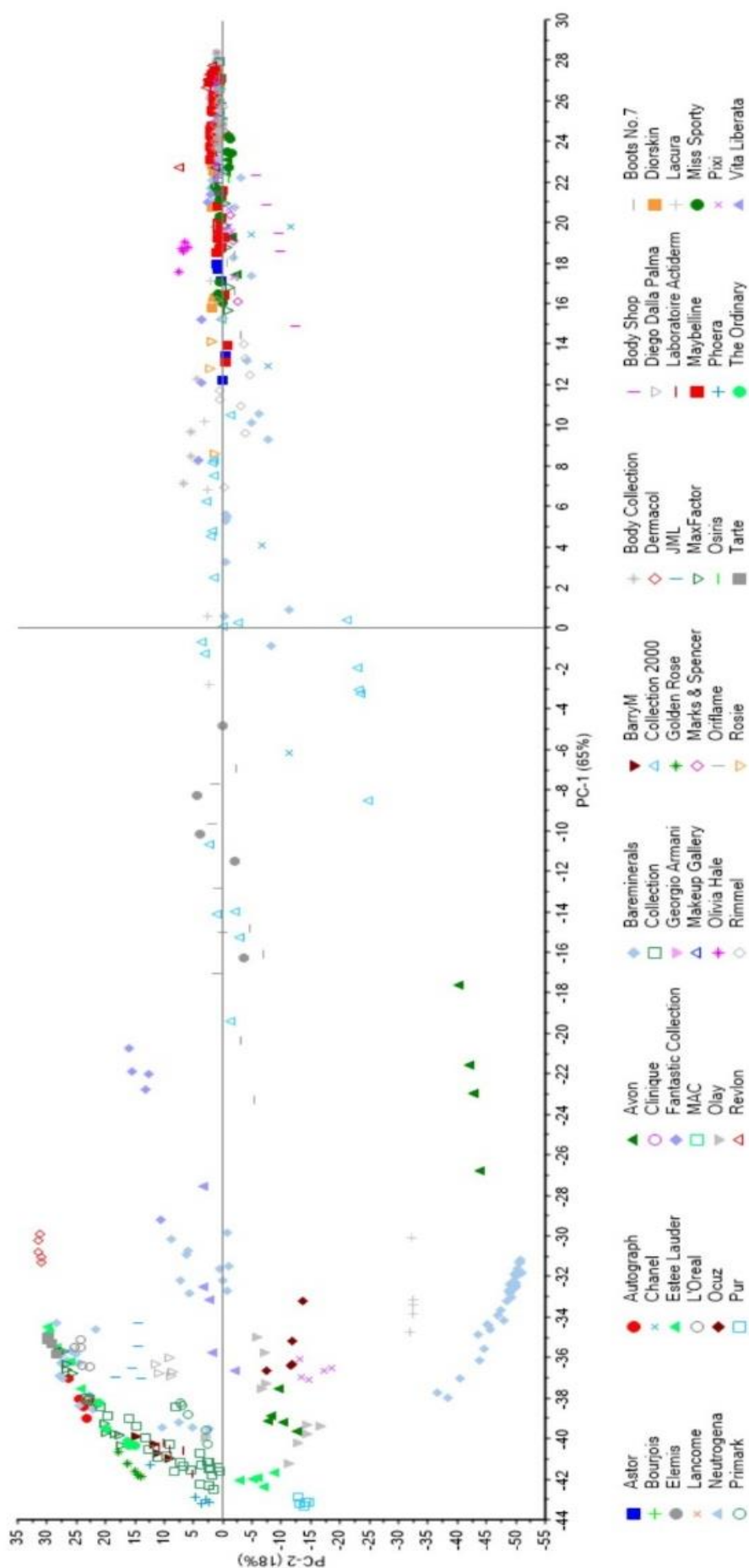


Figure 3.31: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous samples, grouped by brand. The separation across PC1 is due to the predominant titanium dioxide polymorph, with rutile-containing products appearing on the left hand side of the plot and anatase-containing products appearing on the right side.

The overwhelming conclusion that can be reached from these scores, when viewed together with the first loadings plot (Figure 3.32) is that the separation of all samples along PC1 is due to the titanium dioxide polymorph present. The large initial peak in the loadings plot indicates a high weighting assigned to the “*lattice*” peak that we see in anatase containing samples. It can therefore be inferred that samples scoring highly along PC1 are positively, and strongly correlated with anatase content. The opposite is also true; any sample spectrum that does not display a strong “*lattice*” peak will be negatively correlated along PC1 and is likely to contain the rutile polymorph. It is reassuring to note that this is exactly what was seen during visual examination of the Raman spectra earlier.

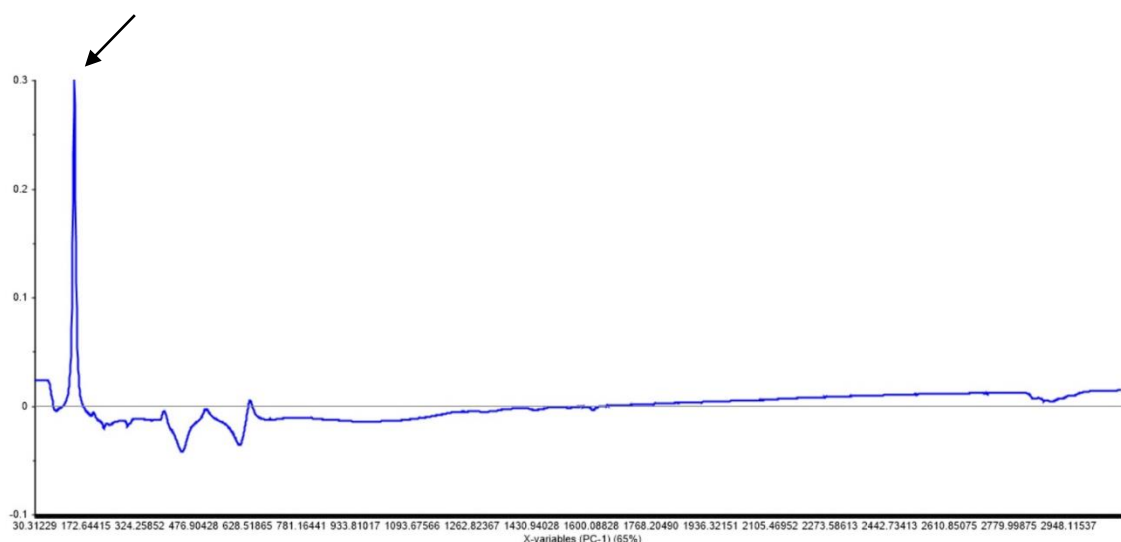


Figure 3.32: PCA loadings plot for PC1 of all the spectrally homogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading. Black arrow denotes the “*lattice*” peak.

As can be seen in Figure 3.33, the separation along PC2 is based not only on rutile content but also on organic content, specifically in the $\sim 1000 \text{ cm}^{-1}$ region. This negative correlation at $\sim 1000 \text{ cm}^{-1}$ is indicative of lecithin content as was seen amongst the BareMinerals Complexion Rescue tinted moisturisers in Section 3.3.3.3 and suggests that lecithin-rich samples tend to contain the rutile polymorph. It is interesting to note that the spread of samples along PC2 in the scores plot is significantly reduced for the anatase containing samples, suggesting that these samples do not contain high levels of these kinds of organic compound.

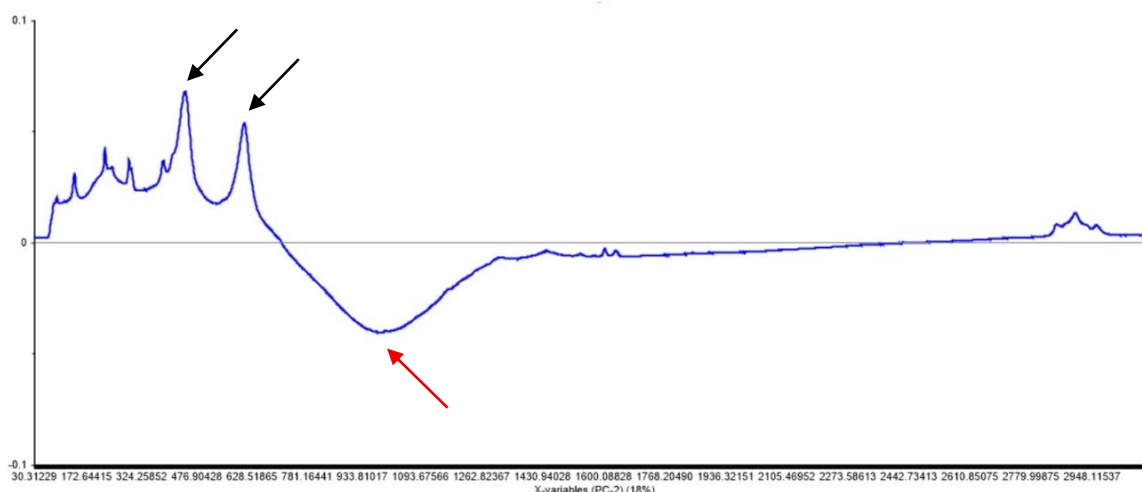


Figure 3.33: PCA loadings plot for PC2 of all the spectrally homogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading. Black arrows denote the rutile peaks; red arrow denotes lecithin content.

Plotting PC1 against PC3 in the same PCA model (Figure 3.34) reveals a different overall trend and one particularly distinct sample, the Elemis Glow Priming Moisturiser. Inspection of the loadings plot for PC3 (Figure 3.35) shows that samples with high positive scores along PC3 are correlated with peaks in the CH stretching spectral region. This is also to be expected as tinted moisturisers tend to be higher in organic content. As can be seen in Figure 3.26, the spectrum for this Elemis sample does indeed have peaks in the CH region, however, the relative intensity of these peaks compared with all the other peaks in the spectrum is what makes it distinctively different from other organic containing samples.

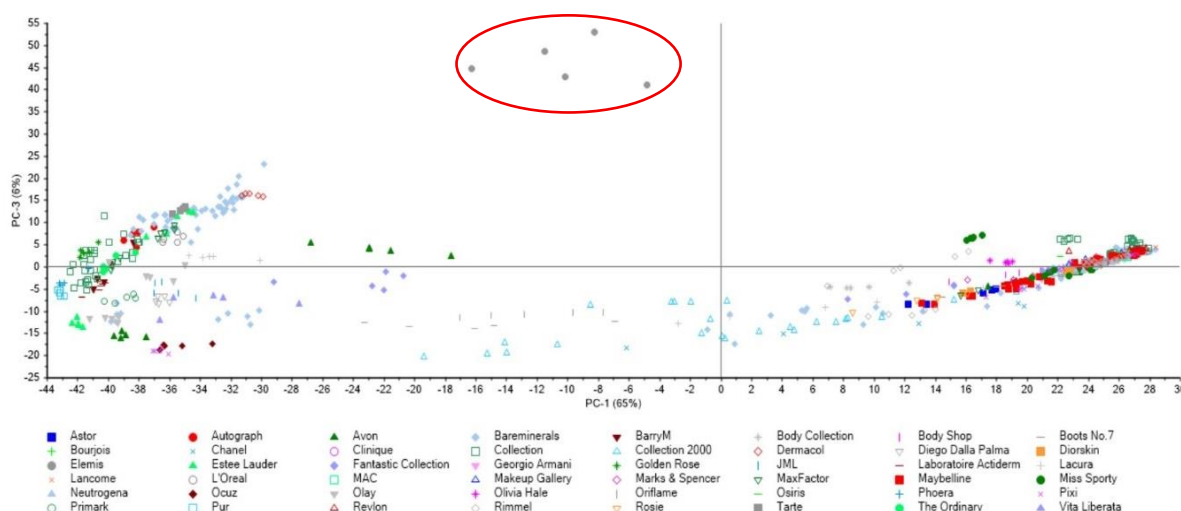


Figure 3.34: PCA scores plot (PC1 vs. PC3) of all the spectrally homogeneous samples, grouped by brand. Replicate spectra for Elemis product highlighted with the red ellipse.

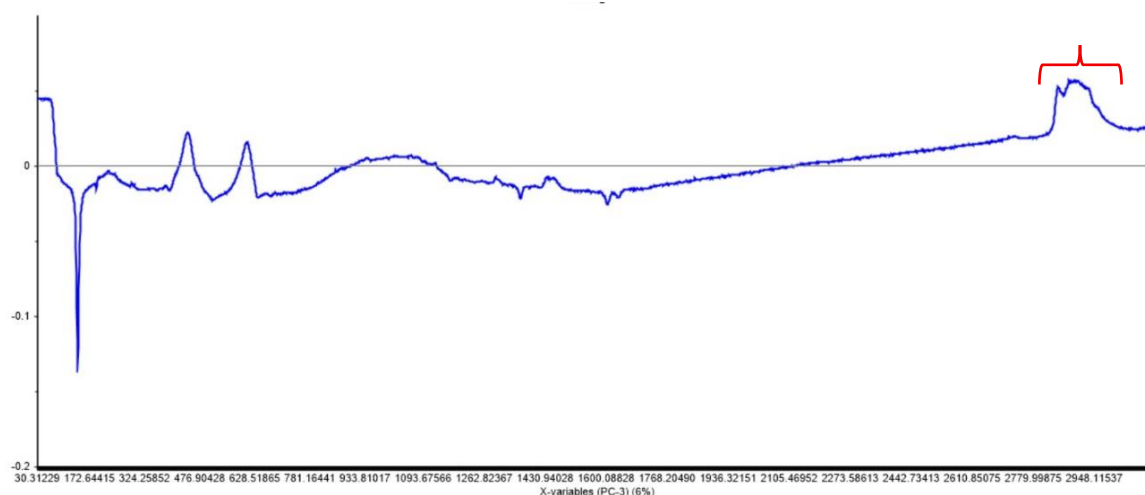


Figure 3.35: PCA loadings plot for PC3 of all the spectrally homogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading. Red brace denotes CH stretching spectral region.

Assessment of PC1 against PC4 (Figure 3.36) seems to invert the pattern seen in the PC1 vs. PC2 plot (Figure 3.31). Examination of the loadings for PC4 (Figure 3.37) shows that the CH region responsible for separation along PC3 now has a negative loading. Unsurprisingly, this forces the Elemis Glow Priming Moisturiser to the opposite side of the scores plot (negative along PC4) and helps to reinforce the link between what we see in the scores plots and the actual chemistry of the samples.

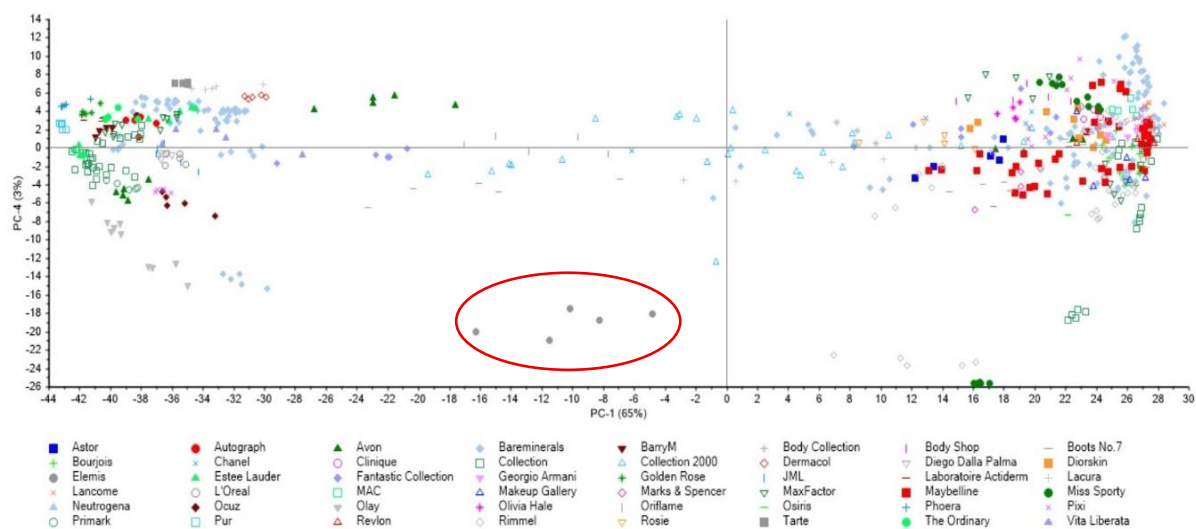


Figure 3.36: PCA scores plot (PC1 vs. PC4) of all the spectrally homogeneous samples, grouped by brand. Replicate spectra for Elemis product highlighted with the red ellipse.

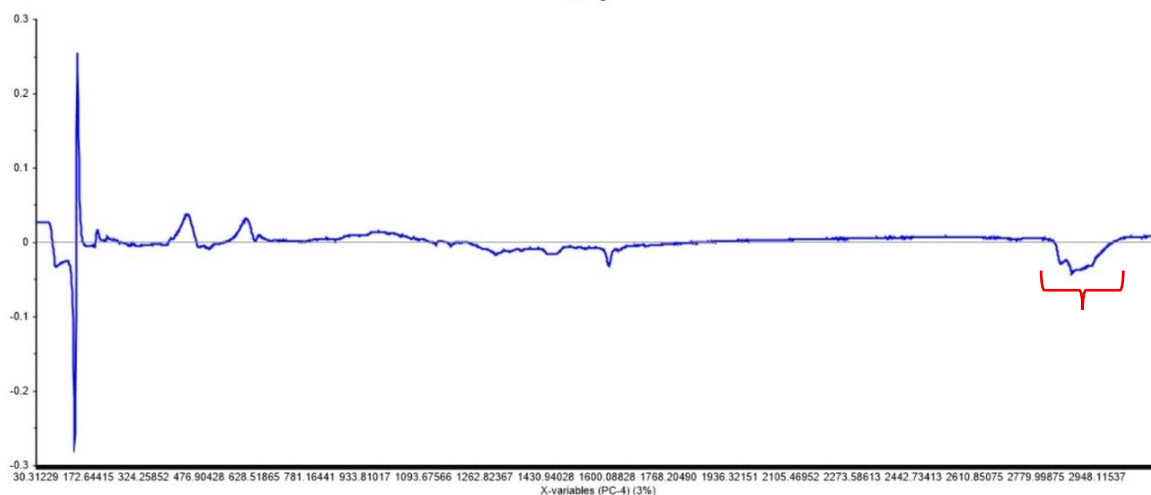


Figure 3.37: PCA loadings plot for PC4 of all the spectrally homogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading. Red brace denotes CH stretching spectral region.

The PC1 vs. PC5 scores plot did not differ substantially to the PC1 vs. PC4 plot, and the corresponding loadings plot for PC5 confirmed that the variables and respective weightings were similar to those seen in PC4. Hence, it was assumed that the loadings would carry little relevant structural information after PC5, but inspection of the scores and loadings for PC6 revealed otherwise. As can be seen in Figure 3.38, further samples have been separated from the main sample clusters and inspection of the PC6 loadings (Figure 3.39) shows that importance is now placed upon the variables in the mid-spectral region, i.e. the C=C double bonds and the C=O carbonyl stretches.

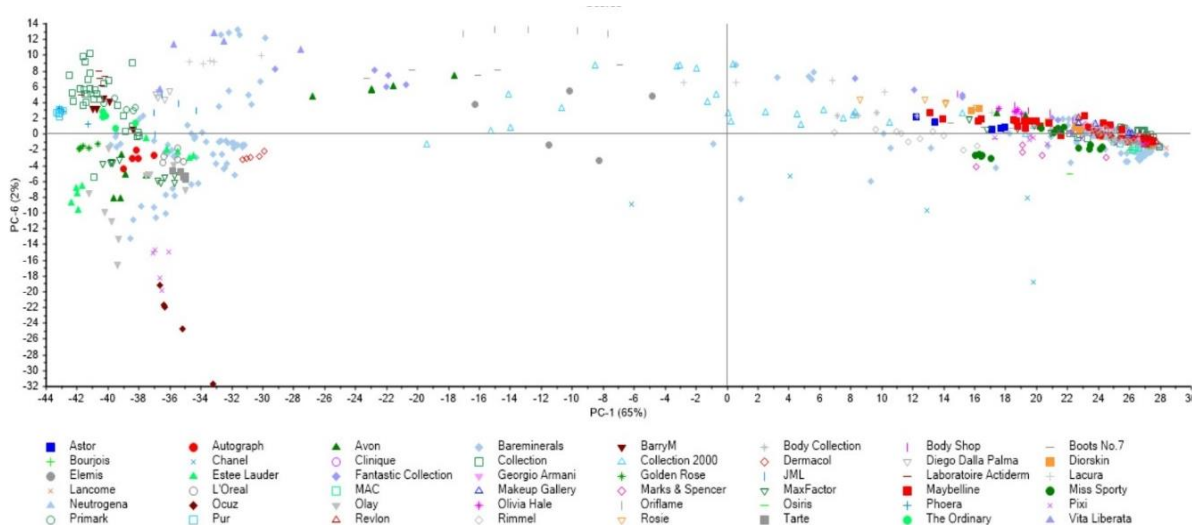


Figure 3.38: PCA scores plot (PC1 vs. PC6) of all the spectrally homogeneous samples, grouped by brand.

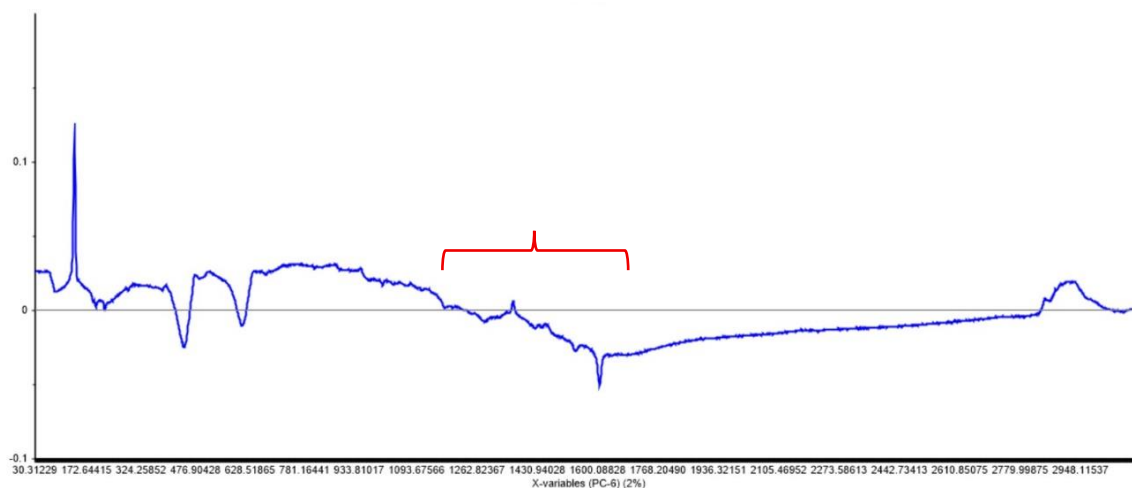


Figure 3.39: PCA loadings plot for PC6 of all the spectrally homogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading. Red brace denotes C=C double bond and C=O carbonyl stretching spectral region.

Despite PC7 only accounting for 1% of the total variance within the dataset, examination of the loadings (Figure 3.41) reveals that structural information is still being retained and that different mid-spectral range peaks are being isolated³⁹. Indeed, the PC1 vs. PC7 scores plot (Figure 3.40) shows that samples are being further discriminated and therefore the addition of PC7 to the model is valuable.

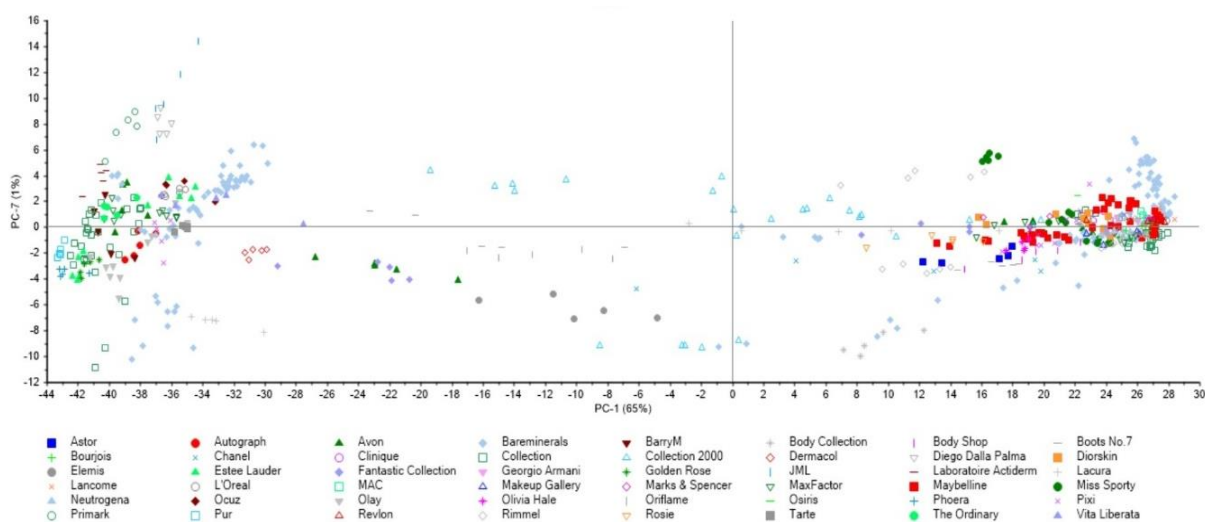


Figure 3.40: PCA scores plot (PC1 vs. PC7) of all the spectrally homogeneous samples, grouped by brand.

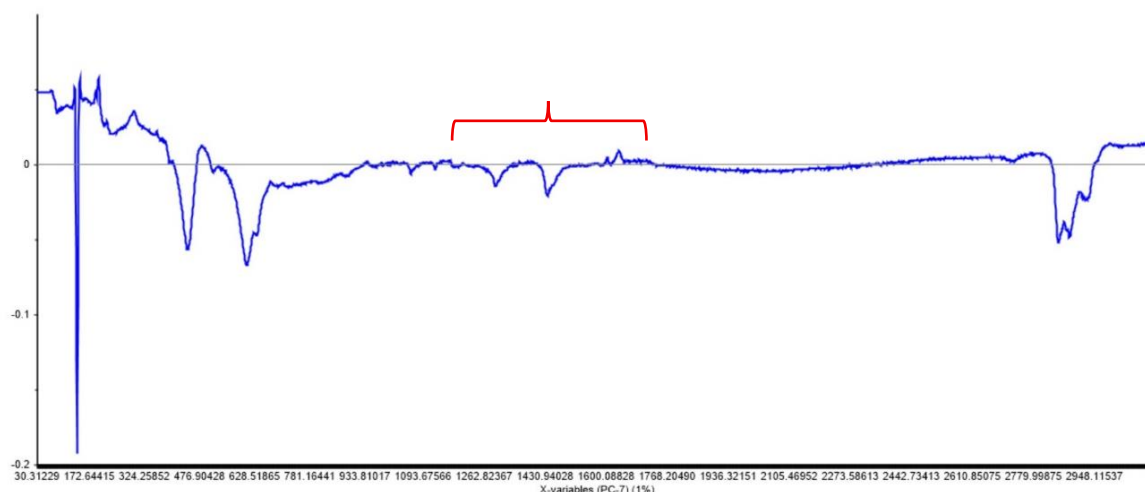


Figure 3.41: PCA loadings plot for PC7 of all the spectrally homogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading. Red brace denotes C=C double bond and C=O carbonyl stretching spectral region.

All the scores plots thus far have included combinations of PC1 against other PCs, yet when PC1 is omitted from the scores plots, totally different trends are visualised. As Figure 3.42 shows, the PC2 vs. PC3 scores plot displays a very different pattern, with the majority of the samples grouped at the origin, rather than at opposite ends of the plot. Nonetheless, the samples are generally symmetrically distributed as is expected⁴⁰. This change in pattern suggested that the discrimination potential of the model might be improved by either truncating out, or down-weighting the variables corresponding to the “lattice” peak.

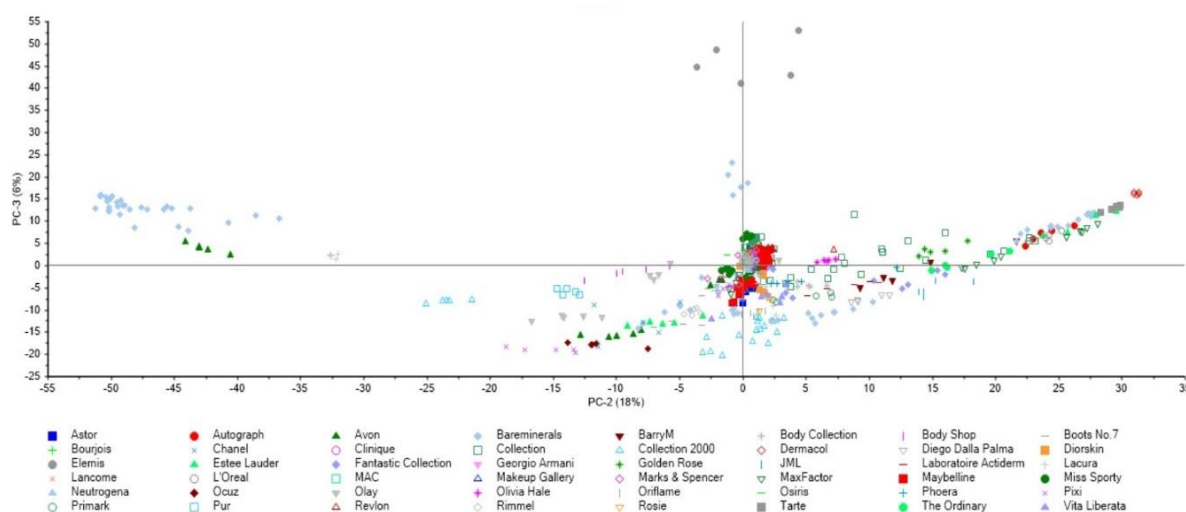


Figure 3.42: PCA scores plot (PC2 vs. PC3) of all the spectrally homogeneous samples, grouped by brand.

Consequently, the variables attributed to the “*lattice*” peak were identified and selected, and the PCA model was recalculated with these selected variables down-weighted⁴¹. The resultant scores plot was surprisingly very similar, which indicated that the “*lattice*” peak was not as important as predicted. Further research confirmed that other cosmetic components also display a “*lattice*” peak in this region, such as bismuth oxychloride. This further highlights the chemical variability and complexity among these types of cosmetics and supports their validity as forensic trace evidence.

Additional sample discrimination is observed when plotting PCs 2 and 4 against one another (Figure 3.43) and some sample replicate clustering is evident. Sample clustering is enhanced when plotting PC2 against PC6, further reinforcing the value of retaining these higher PCs within the model, as is demonstrated in Figure 3.44.

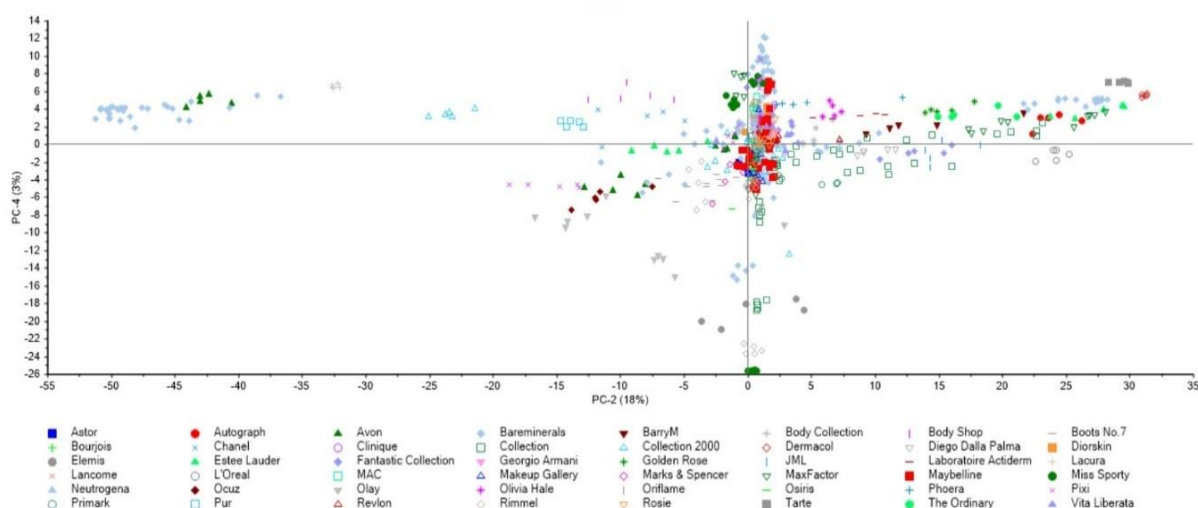


Figure 3.43: PCA scores plot (PC2 vs. PC4) of all the spectrally homogeneous samples, grouped by brand.

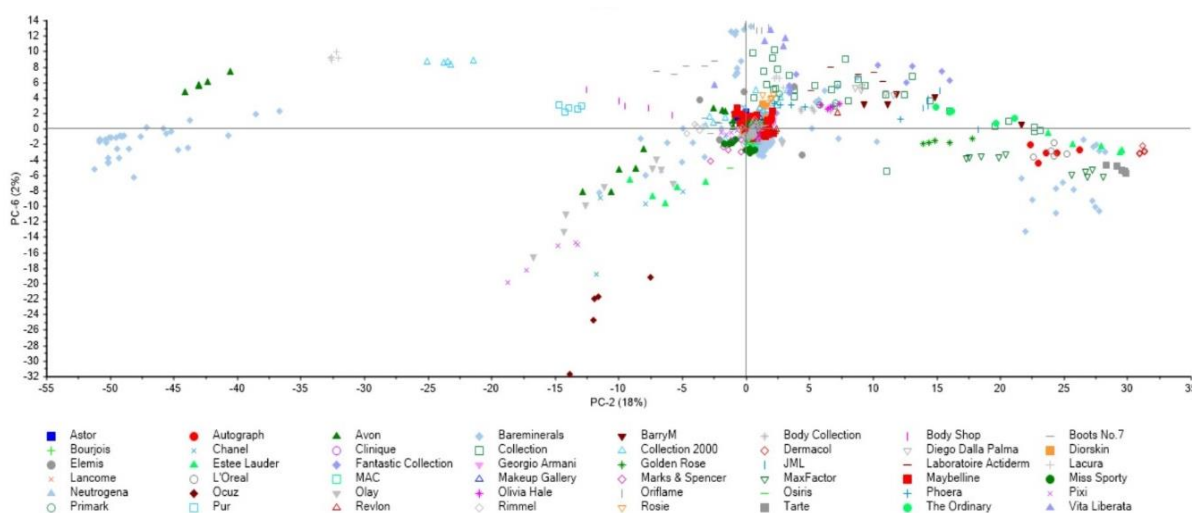


Figure 3.44: PCA scores plot (PC2 vs. PC6) of all the spectrally homogeneous samples, grouped by brand.

Figure 3.45 demonstrates the benefit of including PC7 to the model. The Autograph sample replicates, denoted by the red dots, overlay other samples in the PC2 vs PC3, PC2 vs PC4, and PC2 vs PC6 scores plots, yet the five replicates of this sample are clearly distinct once PC2 is plotted against PC7. The same is true for the Diego Dalla Palma sample, denoted by the grey outlined downward-pointing triangles, in the top right of the plot (Figure 3.45).

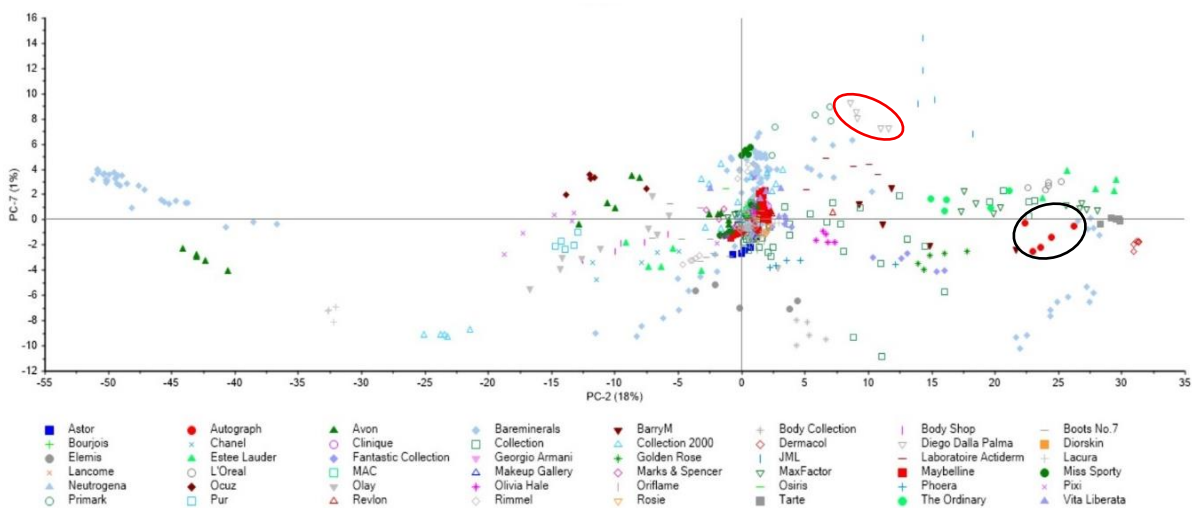


Figure 3.45: PCA scores plot (PC2 vs. PC7) of all the spectrally homogeneous samples, grouped by brand. Replicate spectra for the Diego Dalla Palma product highlighted with the red ellipse; replicate spectra for the Autograph product highlighted with the black ellipse.

The benefit of plotting PC3 against PC4 can be seen in Figure 3.46, the tightly packed sample cluster at the centre of previous scores plots has now been separated out more evenly, allowing further trends and sample clusters to be identified. Plotting PC3 against PC6 permits

the additional discrimination of the Vita Liberata and Oriflame products from the main sample cluster (Figure 3.47), which was not readily identifiable from the PC3 vs PC4 plot (Figure 3.46), demonstrating the utility of retaining structure-containing PCs and inspecting the relevant plots.

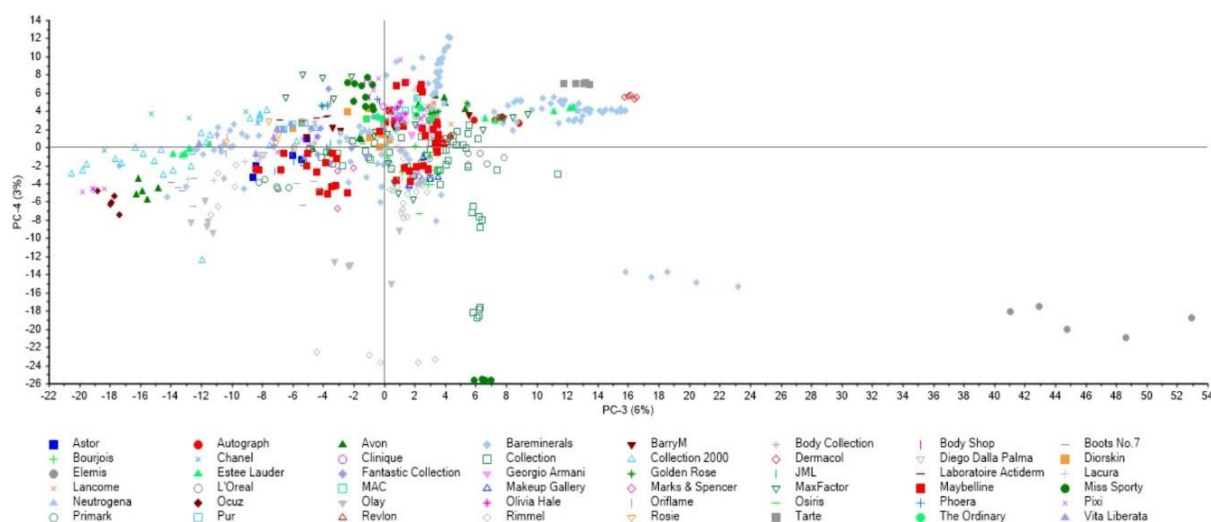


Figure 3.46: PCA scores plot (PC3 vs. PC4) of all the spectrally homogeneous samples, grouped by brand.

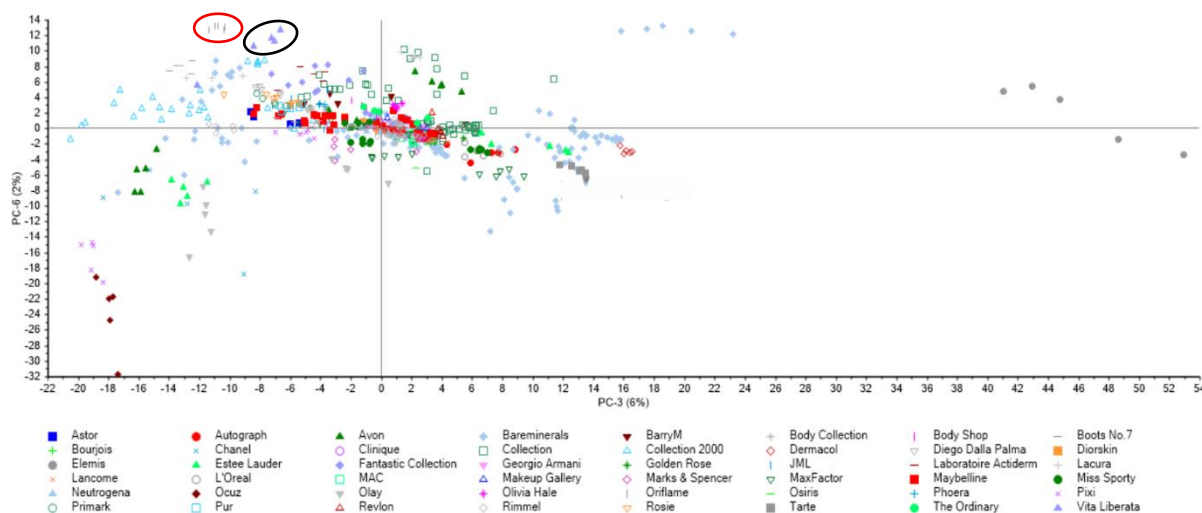


Figure 3.47: PCA scores plot (PC3 vs. PC6) of all the spectrally homogeneous samples, grouped by brand. Replicate spectra for the Oriflame product highlighted with the red ellipse; replicate spectra for the Vita Liberata product highlighted with the black ellipse.

Conversely, plotting PC4 against PC5 displays an X-shaped pattern, indicative of a collinear response, confirming that these PCs are essentially measuring the same variables (Figure 3.48). This was identified earlier when comparing the loadings for PC4 and PC5, and noting

that they showed very similar variables and respective weightings. A collinear response such as this, is unhelpful and should not be used to model the data⁴².

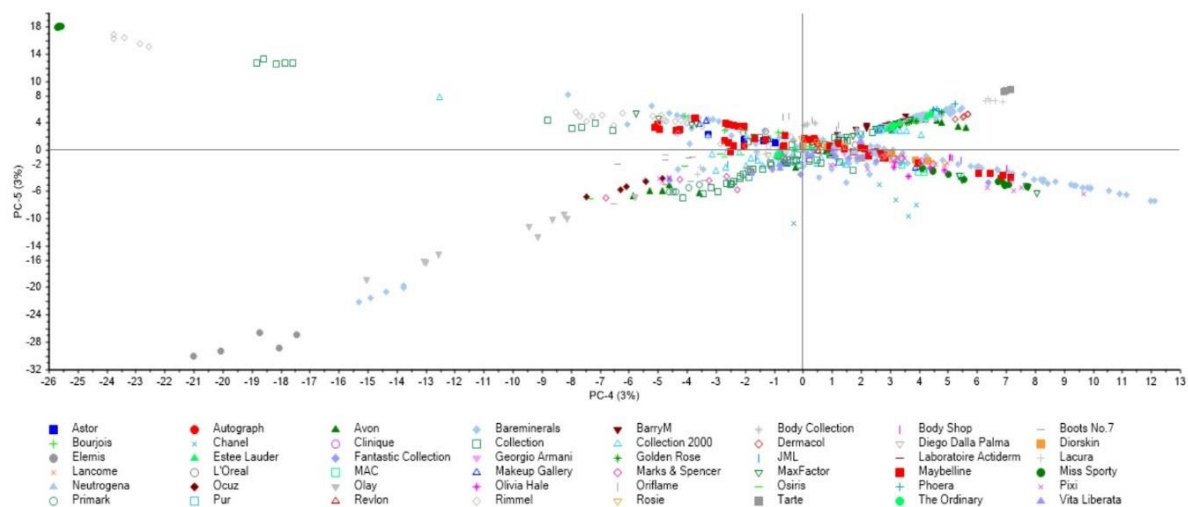


Figure 3.48: PCA scores plot (PC4 vs. PC5) of all the spectrally homogeneous samples, grouped by brand.

In addition to inspection of the scores plots, the influence plots highlight which samples have high leverage, or are highly influential to the model. Inclusion of these “*extreme*” samples helps build a general model, whereas inclusion of only “*typical*” samples would build a robust model³⁹. However, it is important to review these plots, along with the raw spectra to ascertain whether the sample is “*extreme*” or is a true outlier. The position of the sample within the influence plot can guide the decision-making process; samples appearing in the bottom left box are well described by the model, samples appearing in the bottom right box are highly influential to the model and skew the results (this may mean they enhance or perturb the model), samples appearing in the top left box are poorly described by the model (so they are not representative of the general sample population, and samples in the top right box are generally outliers and warrant further checking⁴¹.

As can be seen in Figure 3.49, samples appear in all four quadrants of the influence plot, but upon reviewing their respective spectra, it was decided that none of the samples would be classified as outliers and removed from the model. The reason why some of the samples appeared in the top right box was due to their atypical chemical nature, and not due to an error in the acquisition of the spectra. The removal of some high leverage samples was trialled to see what effect it had on the PCA model. Remarkably, there was little difference between the models; this indicated a high amount of variation within dataset, such that the removal of one sample did not perturb the model.

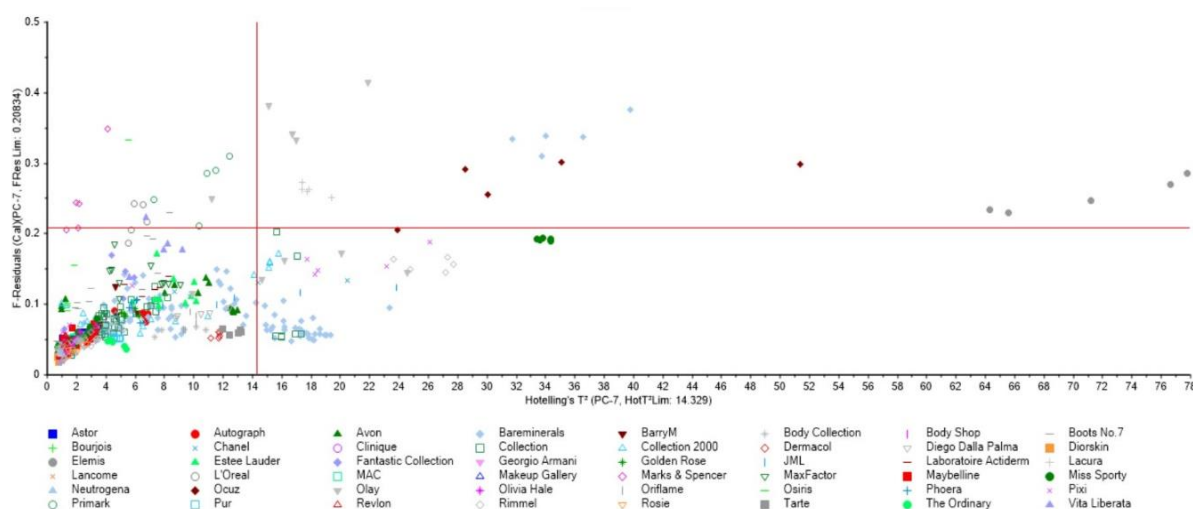


Figure 3.49: Influence plot (PC7) of all the spectrally homogeneous samples, grouped by brand.

After the analyses by brand had been completed, the scores plots were sample grouped by formulation. Figure 3.50 revealed that there were also no trends within this category according to the PC1 vs PC2 scores plot. Samples from all five of the formulation categories were found to have either positive or negative scores along PC1, i.e. none of the formulations used one titanium dioxide polymorph exclusively.

Inspection of the influence plot (Figure 3.51) revealed that all formulations bar the mousses could be regarded as “*extreme*” samples, but again, none were removed from the model as it was decided that they represented accurate data.

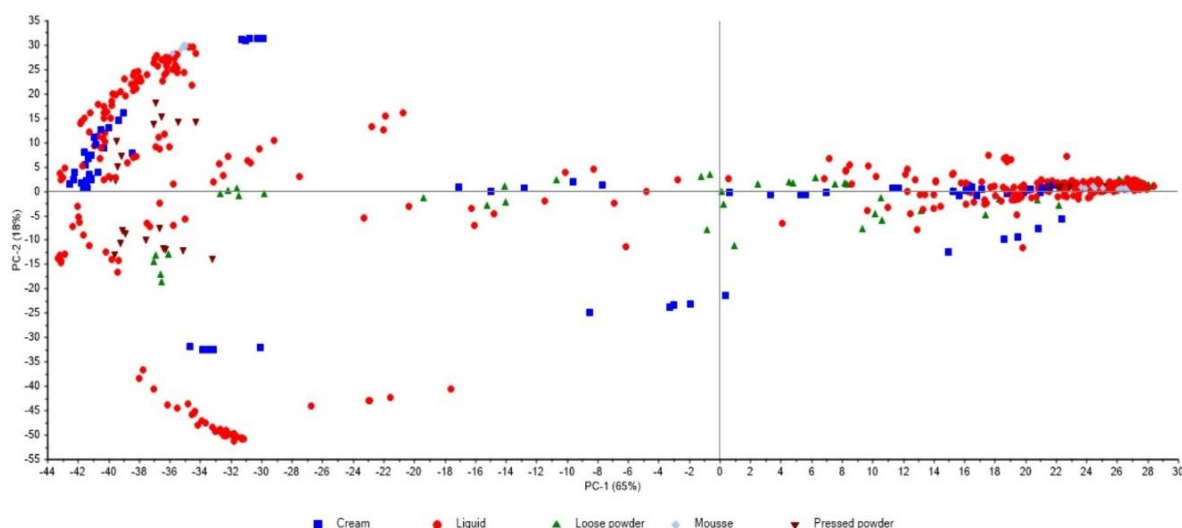


Figure 3.50: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous samples, grouped by formulation.

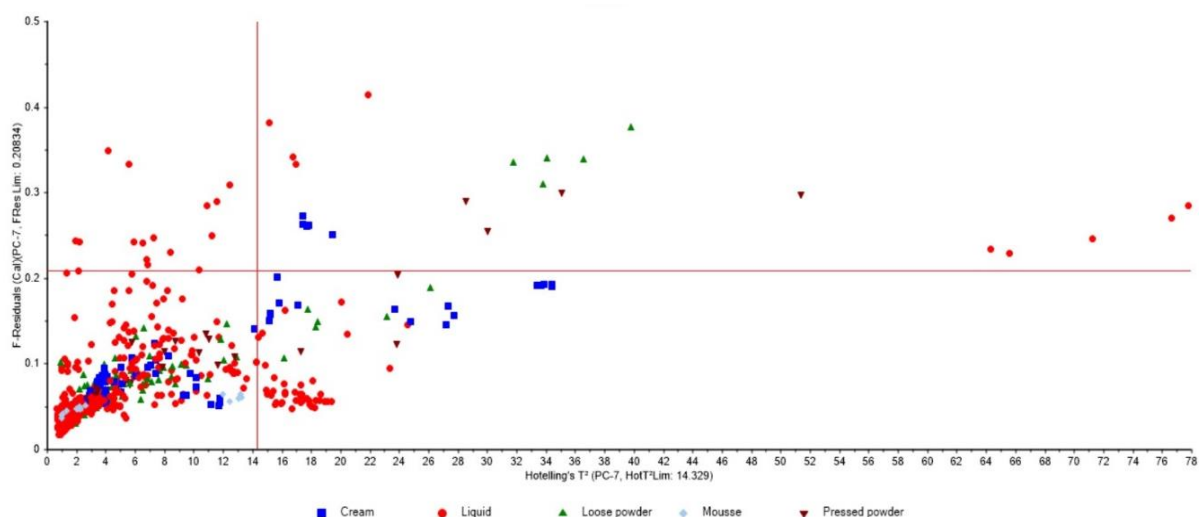


Figure 3.51: Influence plot (PC7) of all the spectrally homogeneous samples, grouped by formulation.

Sample grouping by shade (Figure 3.52) indicated there may be a slight trend with the translucent shaded products. These samples – denoted with the pale blue diamonds – tend to be focused towards the middle of the scores plot, and none of them cross the origin into the positive PC1 area of the scores. No other trends concerning product shade could be ascertained, again reinforcing the complex chemical variability within this dataset.

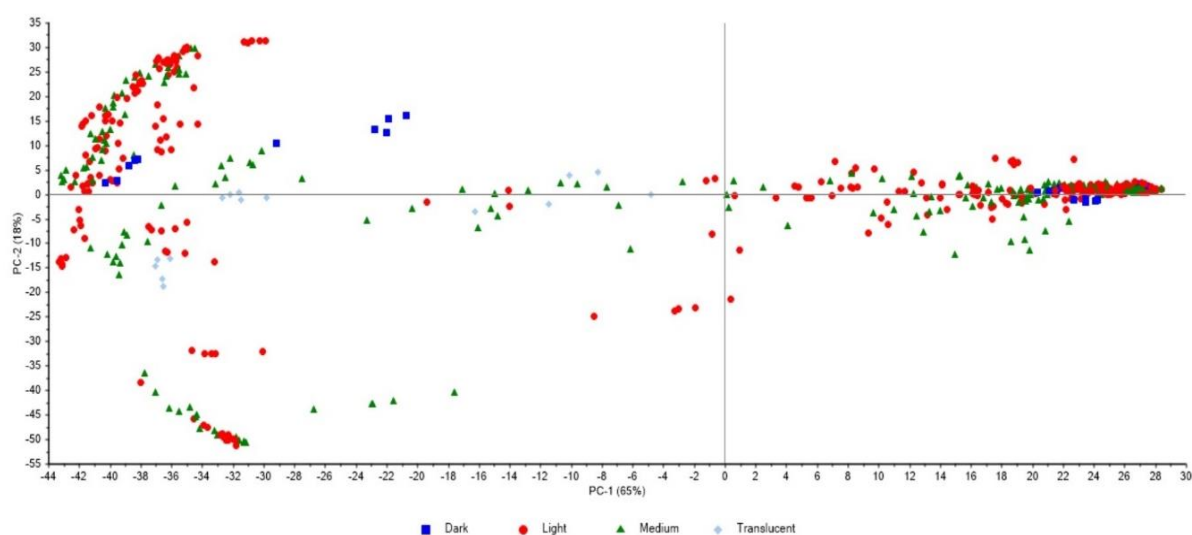


Figure 3.52: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous samples, grouped by shade.

The influence plot relating to shade (Figure 3.53) showed that only a few of the light-coloured samples could be regarded as outliers but that a large proportion of the translucent-shaded products were designated as outliers. Some of the medium-shaded samples were highly

influential to the model, while some of the dark-shaded products were poorly described by the model. This indicates that pigment plays a key role in model construction and the differentiation of samples. The lighter-shaded products were typical of the dataset and were generally well described by the model, whereas the translucent products struggled to be modelled. The medium-shaded products skewed the data and their removal would have perturbed the model, while the dark-shaded products did not follow the general trend of all samples in the model.

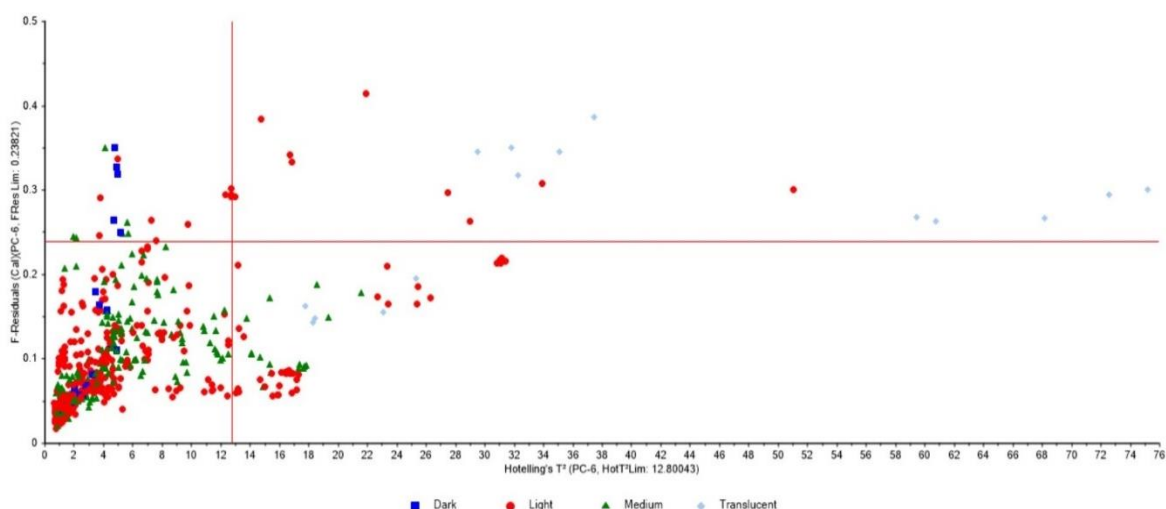


Figure 3.53: Influence plot (PC6) of all the spectrally homogeneous samples, grouped by shade.

Plotting PC2 against PC3 and sample grouping by shade revealed discrimination of two of the translucent products from the main cluster of products (high positive scores along PC3) and a tighter clustering of the dark-shaded samples towards the origin of the plot, but did not permit identification of any further trends (Figure 3.54). Scores plots with all possible PC combinations were viewed by shade and no remarkable trends were identified.

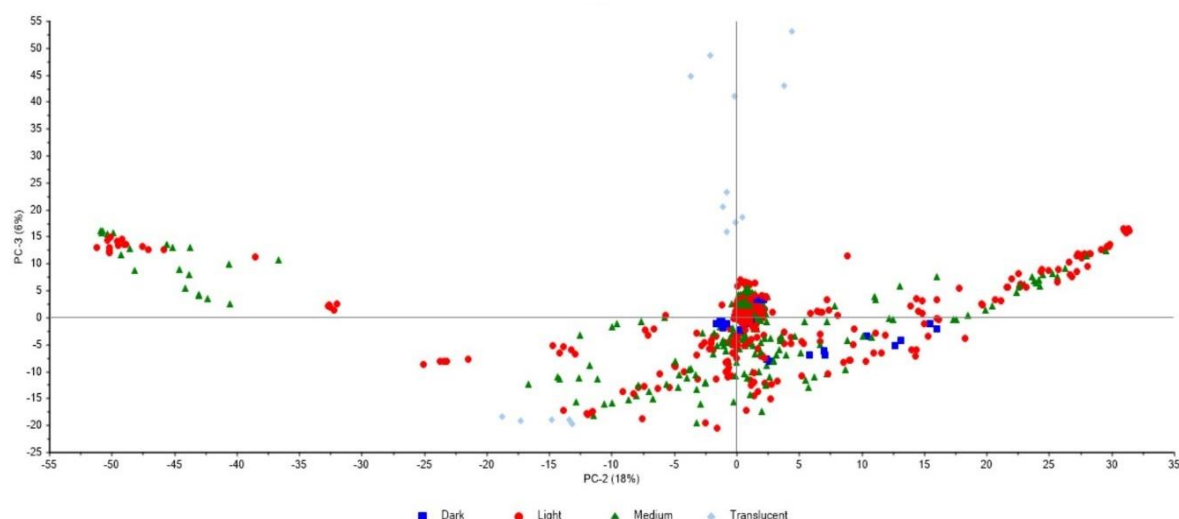


Figure 3.54: PCA scores plot (PC2 vs. PC3) of all the spectrally homogeneous samples, grouped by shade.

The final sample grouping to apply was product type, i.e. foundation, concealer, tinted moisturiser or finishing powder. As can be seen in Figure 3.55, no discernible trends could be detected here either. All four product types featured in the positive and negative areas of both PC1 and PC2, underlining the complex chemistry within the whole dataset.

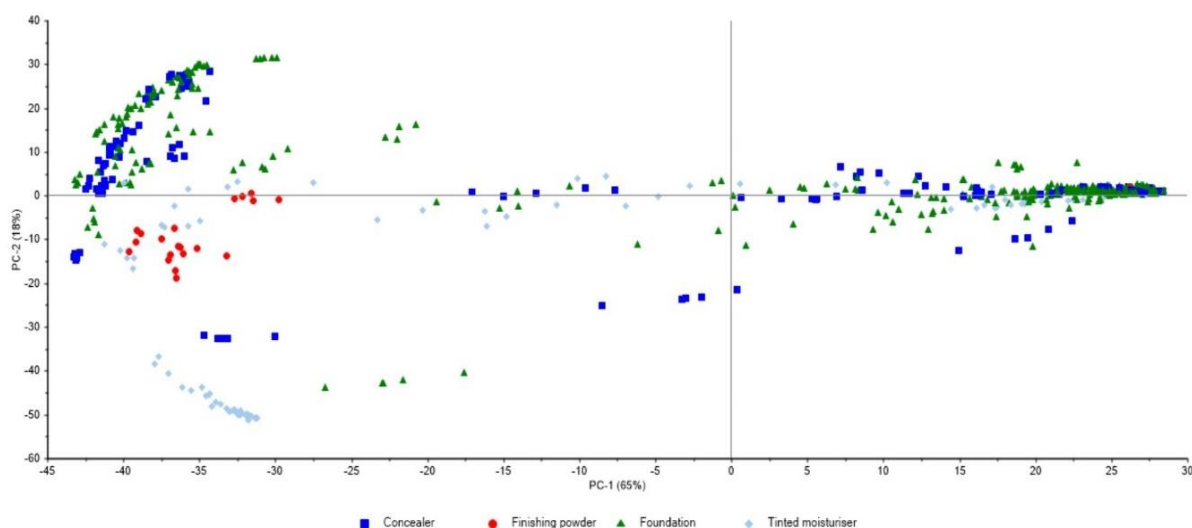


Figure 3.55: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous samples, grouped by product type.

As predicted, the corresponding influence plot for product type (Figure 3.56) shows that the tinted moisturisers and finishing powders are the main outlying types. This is expected given their increased chemical complexity and variability; as such, these products were not removed

from the data matrix. Foundations tend to be far more predictable and well modelled, while concealers tend to be highly descriptive and influential to the model.

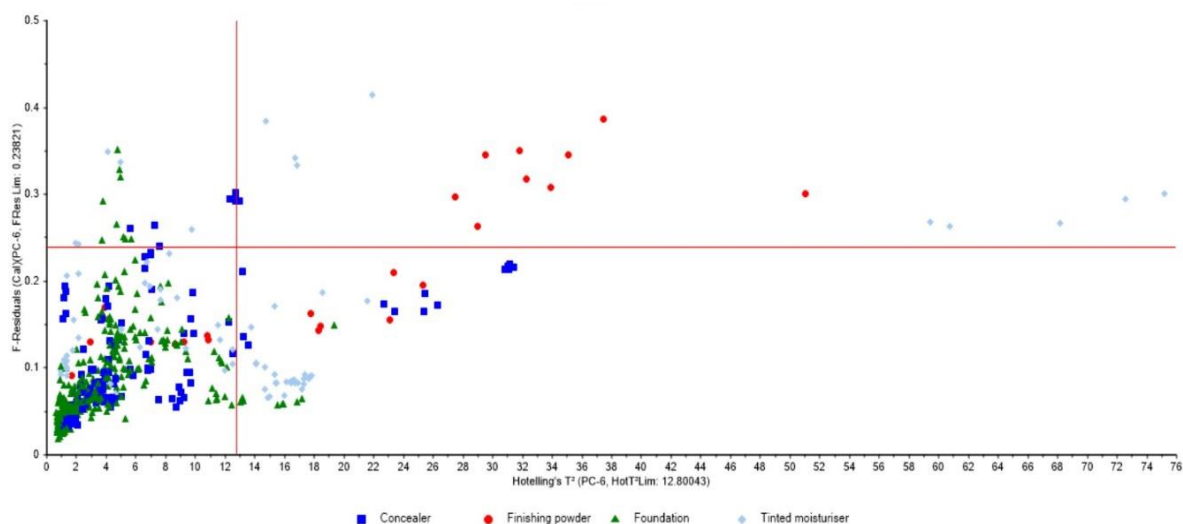


Figure 3.56: Influence plot (PC6) of all the spectrally homogeneous samples, grouped by product type.

It was debated whether a reduced sample set might help with sample discrimination, hence the PCA model was recalculated to only include products from the brand, BareMinerals, across the dataset. Gratifyingly, trends could now be observed with all the tinted moisturiser samples forming a tight cluster at the extreme negative end of PC1 and along positive PC2, in the top left section of the scores plot (Figure 3.57). Despite some overlap between the two anatase-containing finishing powders and the concealers in the top right section of the scores plot, good separation could be seen across all the other samples.

Whilst this is encouraging to see from a data analysis perspective, it should be borne in mind that there are several caveats to this approach from a casework perspective. Firstly, any cosmetic trace retrieved from a scene is highly unlikely to reliably compare with its original product, due to skin absorption of components, conversion of organics into other species, and many other variables. Secondly, these samples were analysed in bulk on clean non-porous (glass) slides, whereas casework samples may only be present as microscopic traces and will inevitably be on supporting substrates, further complicating spectral interpretation. If a questioned versus known comparison is being undertaken this should be fairly straightforward, but in the event an unknown sample is projected onto a representative population of target products, then it must be remembered that cosmetic companies change their formulations regularly and/or cease manufacturing certain products. Perhaps the most important factor to consider is that product layering (as discussed in Section 1.3) leads to complex product

mixtures that have not yet been investigated. The potential chemical diversity of these cosmetic mixtures is huge but it is not yet known whether all cosmetic products would yield Raman spectra. In the event they did, it is still not understood how these products would contribute to a Raman spectrum, thereby inhibiting efforts at spectral deconvolution.

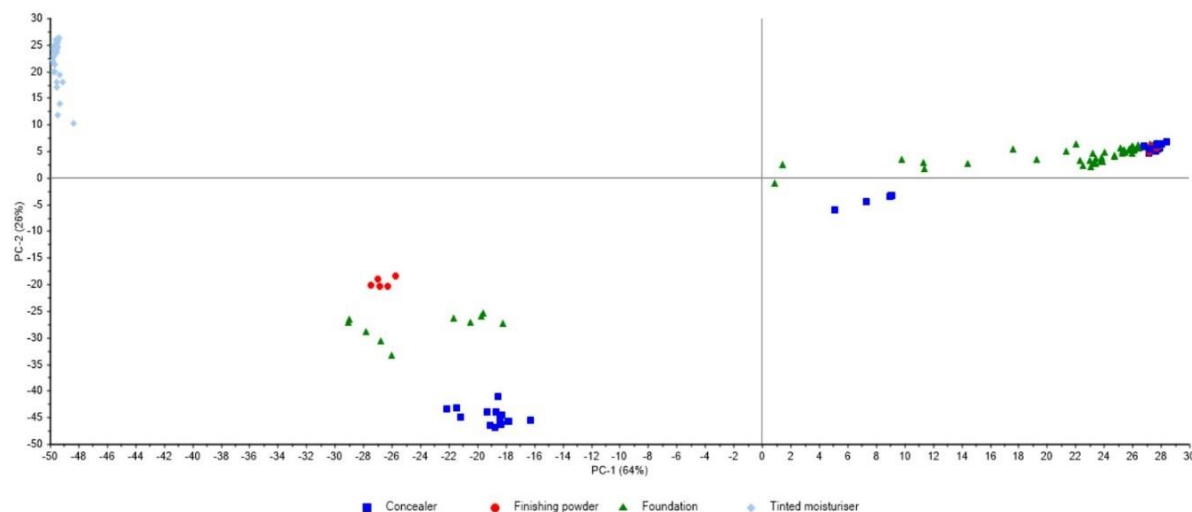


Figure 3.57: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous BareMinerals samples, grouped by product type.

Thereafter, the PCA model was recalculated again to plot products from another brand, Collection. This time the brand only contained foundation and concealer products but a stark contrast was observed in the resultant scores plot (Figure 3.58). In this case, the manufacturer of Collection products clearly exclusively uses anatase in their foundations but rutile in their concealers, as all the foundation products cluster at the extreme positive end of PC1, while the concealers cluster at the extreme negative end of PC1.

Whilst this was immediately apparent from simple visual comparisons of the spectra, the advantage of creating a PCA model to illustrate this, is that numbers can now be assigned to indicate how similar, or dissimilar a sample is from another. This would be extremely useful when used in forensic casework, as it would mean that subjective and unhelpful comparative statements could be replaced with actual measured values of similarity/dissimilarity^{43–45}.

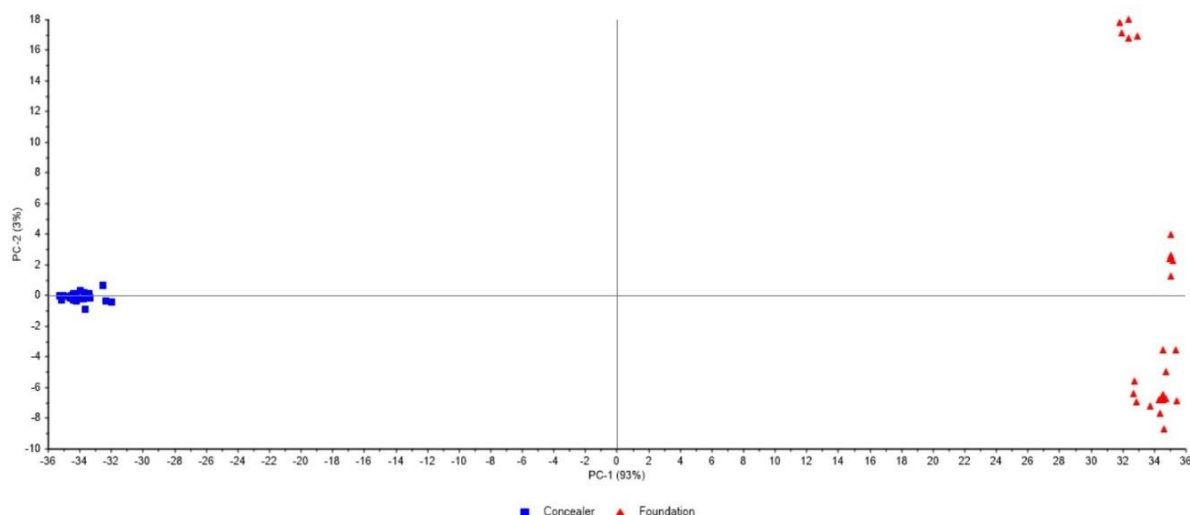


Figure 3.58: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous Collection samples, grouped by product type.

Interestingly, when PC1 is plotted against PC3 (Figure 3.59), the foundation samples that were divergent along PC2 in the previous plot, became tightly clustered along PC3. Conversely, the concealer samples were tightly clustered along PC2 but disparate across PC3. These spectral differences, unlike the titanium dioxide polymorph distinction, were **not** readily apparent during visual inspection, even when the “*lattice*” peak’s influence was negated, as is demonstrated in Figure 3.60. It also suggests that the pigments responsible for shade across the foundations are either chemically different to those used across the concealers, or that they appear spectroscopically different due to the chemistry of the respective matrices. This finding emphasises the value of using chemometric methods such as PCA, for both thorough interrogation of the data and for spotting trends that might otherwise have been easily missed.

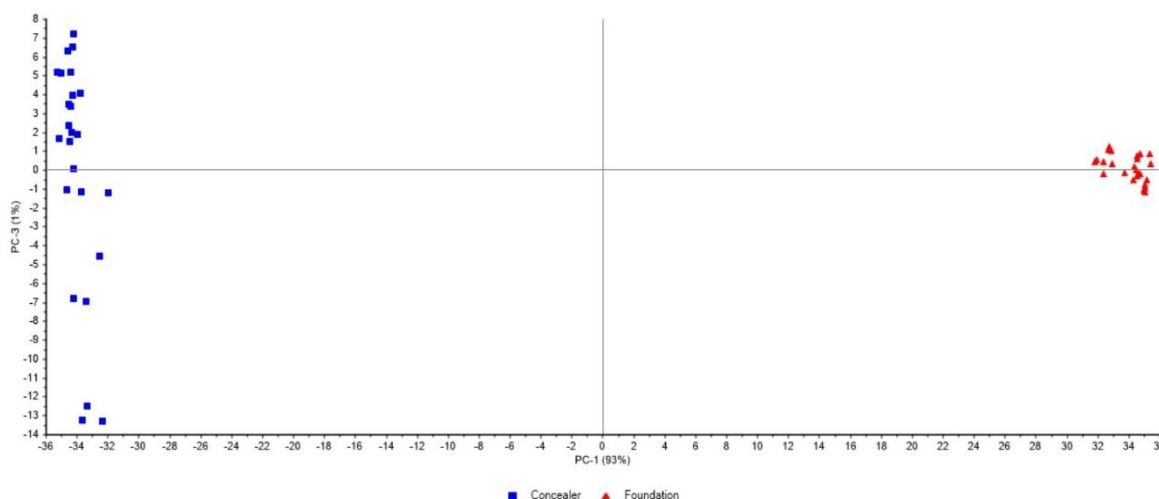


Figure 3.59: PCA scores plot (PC1 vs. PC3) of all the spectrally homogeneous Collection samples, grouped by product type.

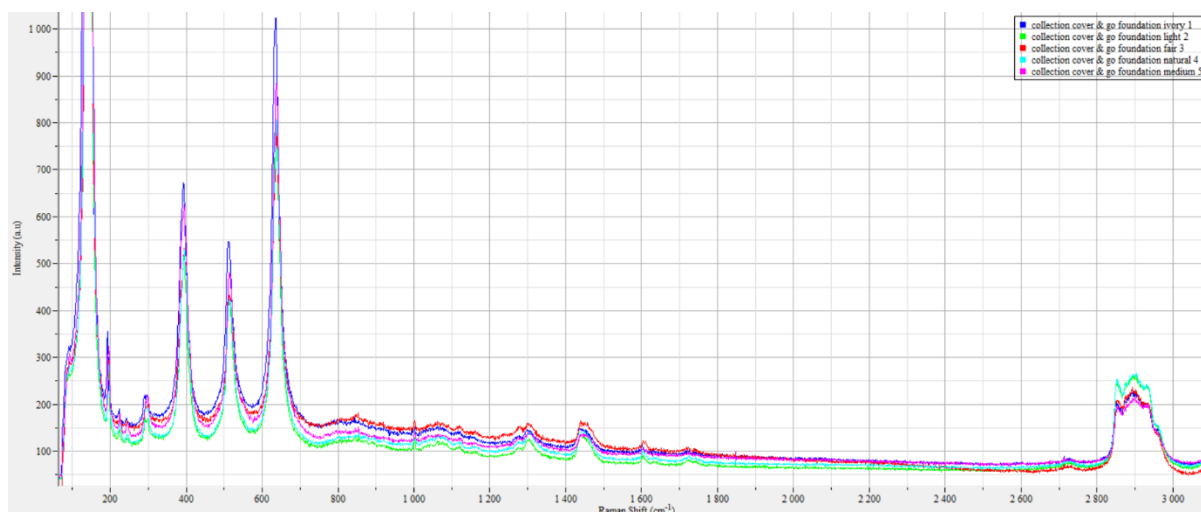


Figure 3.60: Raman spectra (unprocessed) of Collection Cover & Go Foundations in shades Ivory 1, Light 2, Fair 3, Natural 4, and Medium 5, demonstrating a high level of spectral similarity and difficulty in discriminating between the shades.

3.3.4.1 Foundations

The creation of row sets that defined each product by its type allowed for further PCA models to be investigated. The PC1 vs PC2 scores plot for all the spectrally homogeneous foundations (Figure 3.61) shows a very similar pattern to that of the overall model seen in Figure 3.31, indicating that these products were responsible for the general trend of the model. Again, we see that the primary cause of separation across PC1, and therefore the variable with the most weighting, is the titanium dioxide polymorph. We can also see that the anatase-containing foundations (found along the positive PC1 scores) do not exhibit much variation along PC2

(described by rutile and lecithin content), yet when PC1 is plotted against PC3 (Figure 3.62), far more separation and variation among the anatase containing foundations is evident.

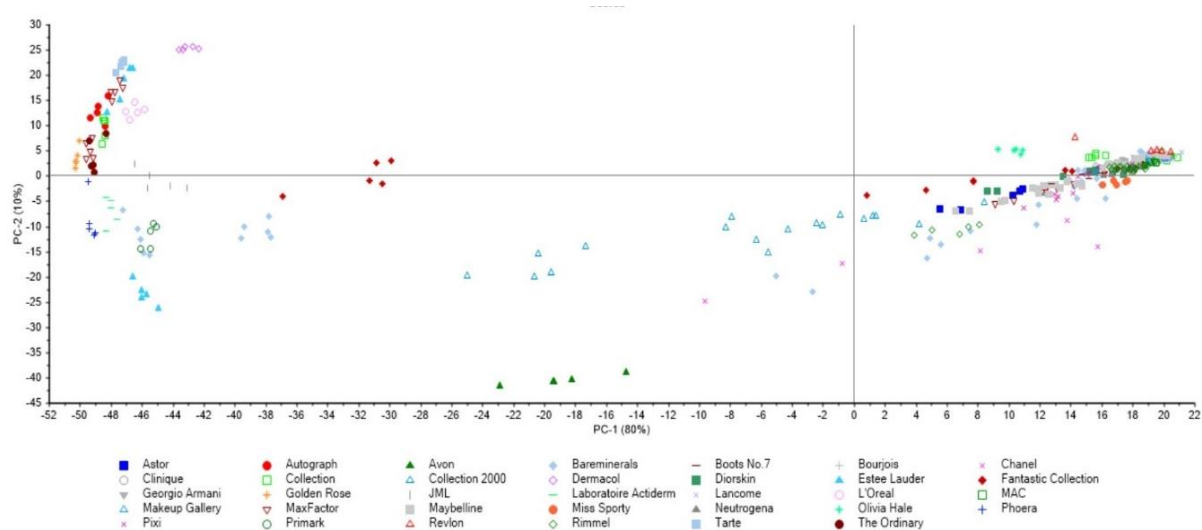


Figure 3.61: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous foundations, grouped by brand.

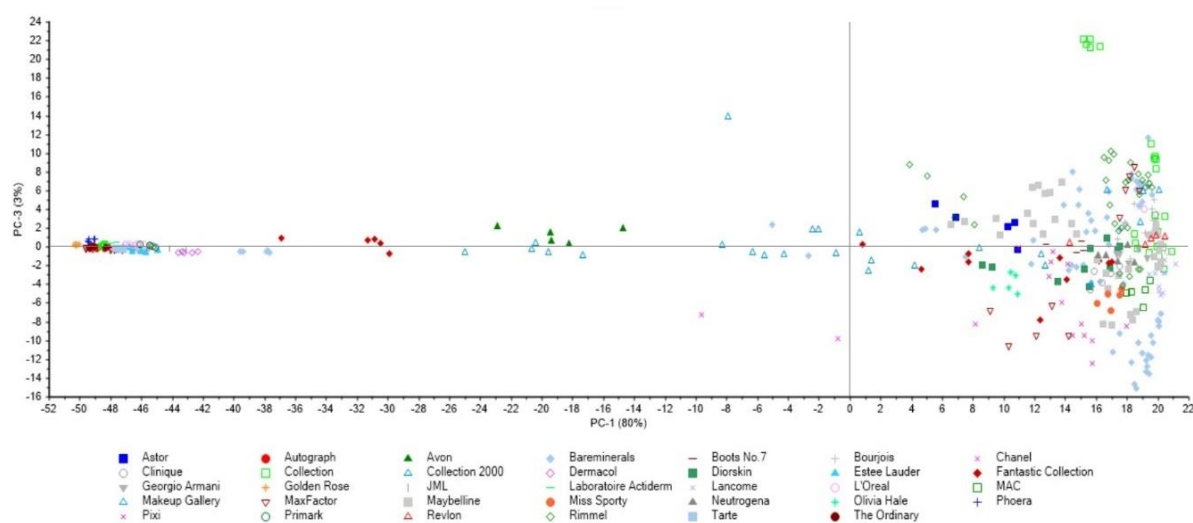


Figure 3.62: PCA scores plot (PC1 vs. PC3) of all the spectrally homogeneous foundations, grouped by brand.

Loadings for PC3 indicate a negative score for the “*lattice*” peak and positive scores for the CH region. This may suggest that anatase-based foundations are less likely to contain emollients that exhibit intense broad peaks around 1000 cm^{-1} , such as lecithin or castor oil (Figure 3.63), and more likely to contain other organic emollients such as polymers and siloxanes. Indeed, inspection of the loadings for PC3 (Figure 3.35) shows a distinctive pattern

in the CH region that is mirrored in the Raman spectrum for the acrylates copolymer reference standard (Figure 3.64).

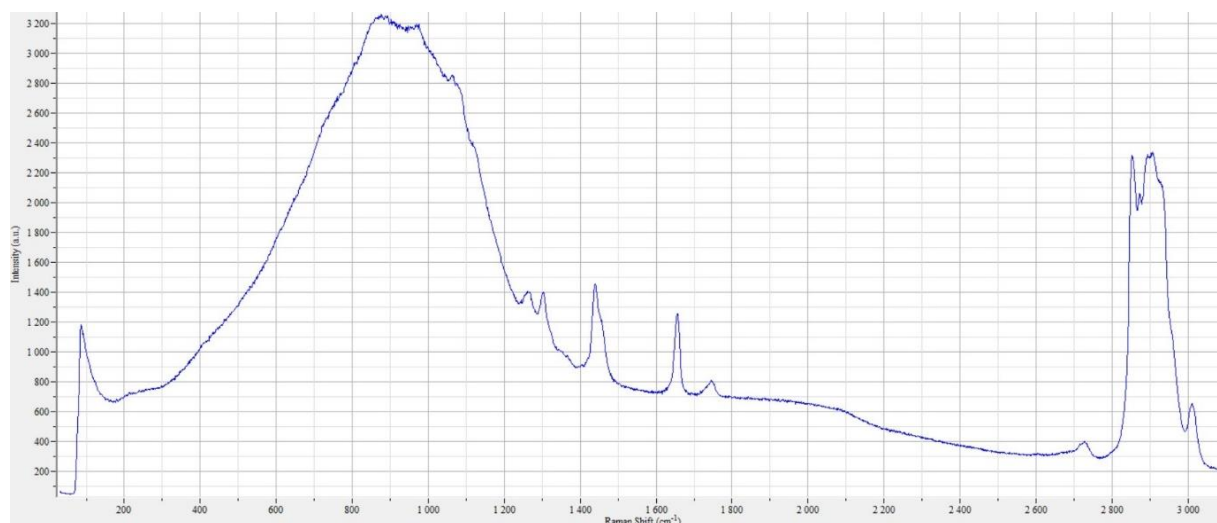


Figure 3.63: Raman spectrum (unprocessed) of the castor oil reference standard.

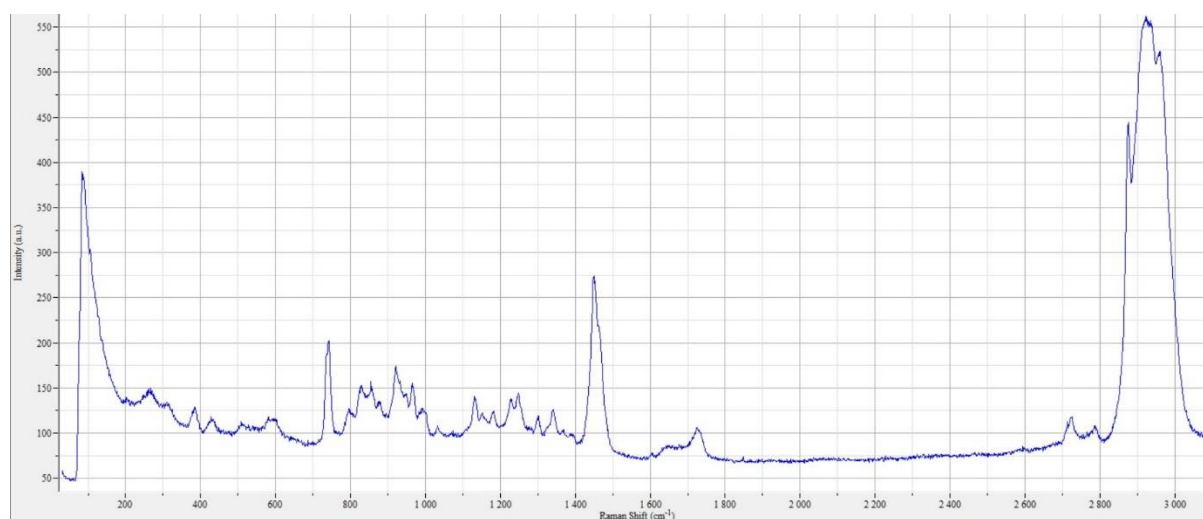


Figure 3.64: Raman spectrum (unprocessed) of the acrylates copolymer reference standard.

As was the case with the general PCA model, sample grouping all the foundations by formulation revealed no further trends, although it did highlight sample clustering (Figure 3.65). Sample grouping by shade also failed to reveal meaningful trends.

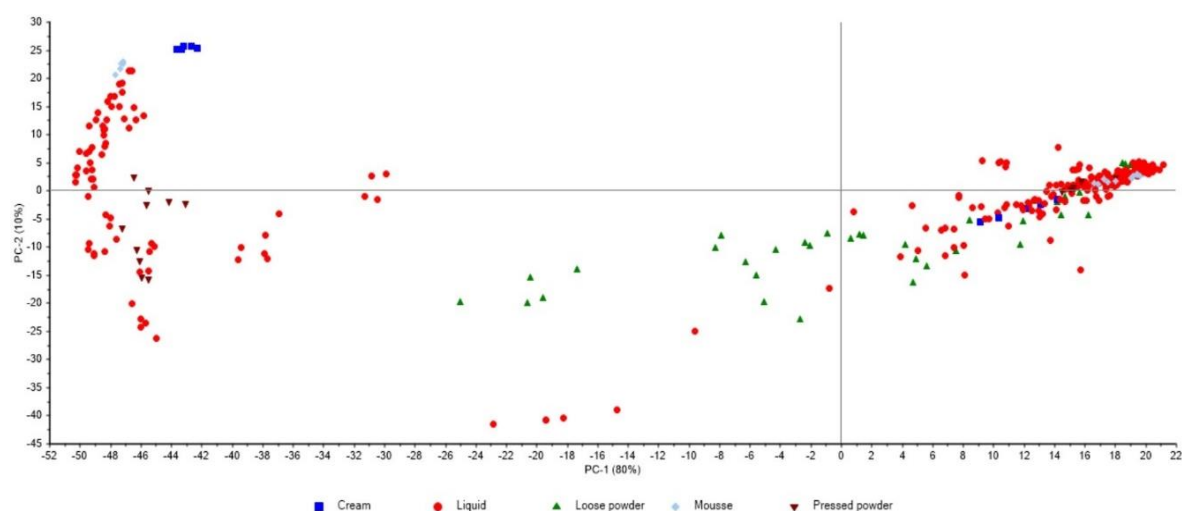


Figure 3.65: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous foundations, grouped by formulation.

3.3.4.2 Concealers

The PCA model based only on concealers allowed for better visualisation of individual sample points due to the lower number of products in this dataset. As can be seen in Figure 3.66, the scores plot shows some clear separation of products along PCs 1 and 2, but some overlapping of the Maybelline and BareMinerals concealers at the positive extreme of PC1 and the Barry M and Collection concealers at the negative extreme of PC1. Nonetheless, plotting PC1 against PC5 causes separation of these specific products, despite PC5 only accounting for 1% of the explained variance (Figure 3.67). Once again, this demonstrates the value of retaining PCs that contribute structure to the model, particularly so for complex datasets such as this, and shows why using the arbitrary cut-off figure of 1% explained variance to determine the optimum number of PCs is not always helpful⁴⁰.

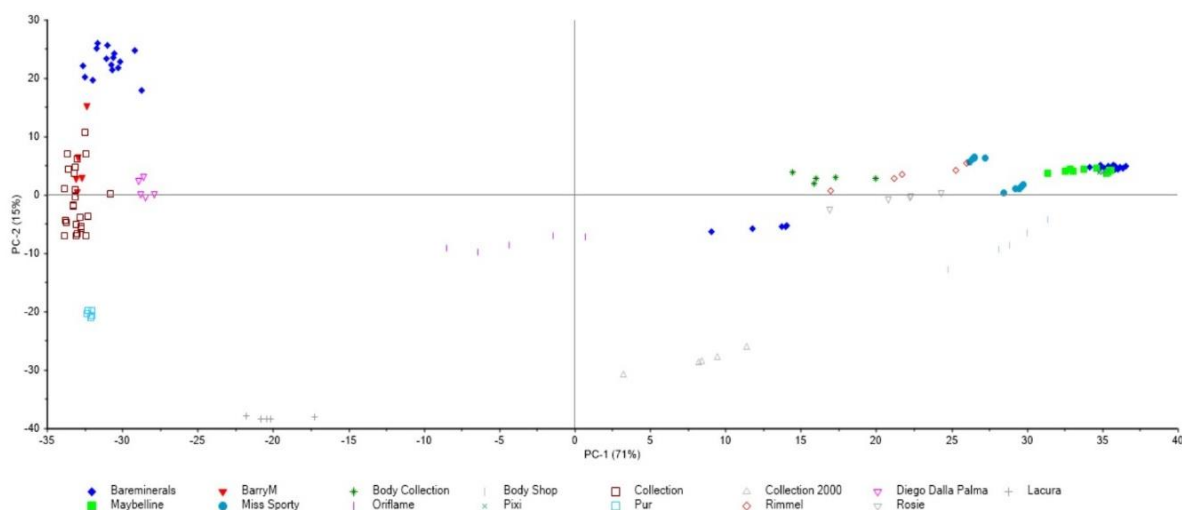


Figure 3.66: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous concealers, grouped by brand.

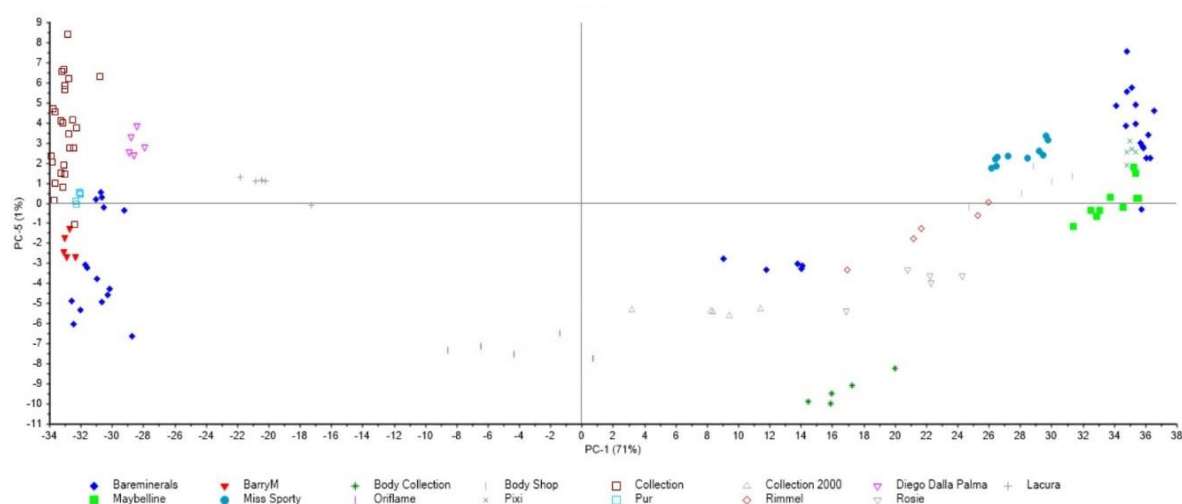


Figure 3.67: PCA scores plot (PC1 vs. PC5) of all the spectrally homogeneous concealers, grouped by brand.

Sample grouping by formulation only revealed that the loose powdered concealers clustered tightly at the extreme positive values of PC1 (Figure 3.68), which was to be expected from their anatase dominant spectral profiles. However, it should be mentioned that the loose powders only represented three out of the total 27 concealers in the dataset, they were all from the brand, BareMinerals, and they were all similar shades, so this is likely to have biased the results. No trends could be identified across the cream or liquid formulations, nor were any identified when sample grouping by product shade.

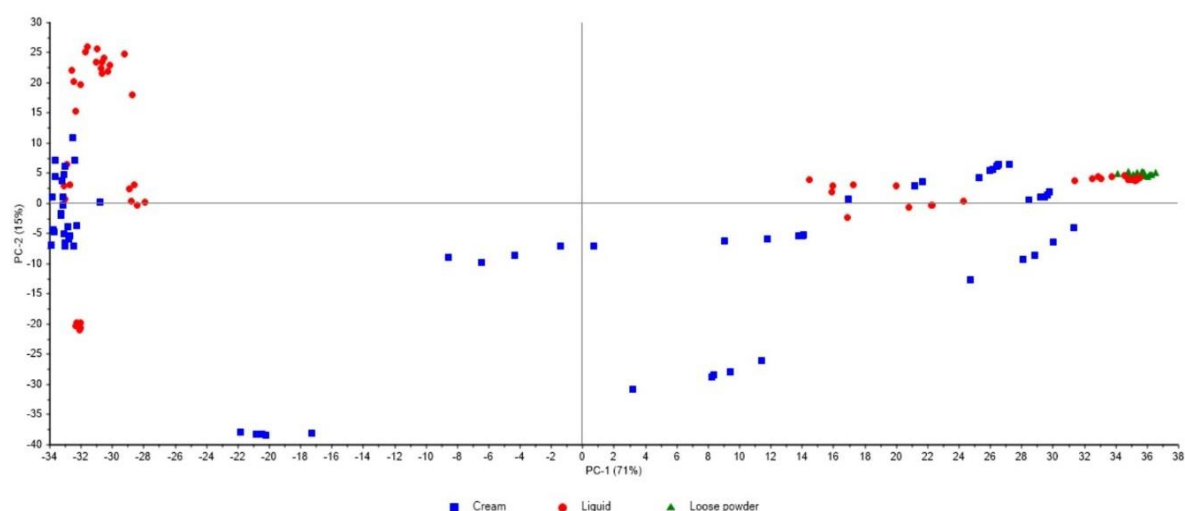


Figure 3.68: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous concealers, grouped by formulation.

3.3.4.3 Tinted moisturisers

Inspection of the PC1 vs PC2 scores plot for the tinted moisturisers revealed clear groupings of both the BareMinerals products and the Olay products, and a clear distinction of those products from the remainder of the samples (Figure 3.69). Nonetheless, it also showed that the two Boots No.7 products were not closely associated, reinforcing the lack of brand discrimination, due to the differing types of products within a particular brand. All six of the BareMinerals products contained within this sample cluster were from the same product line and differed only by shade, whereas the Boots No.7 products were totally different: Beautiful Skin BB Cream in Normal/Oily and Triple Protection Tinted Moisturiser in Fair. Their respective spectra were also noticeably different, so this separation along PC1 is wholly expected.

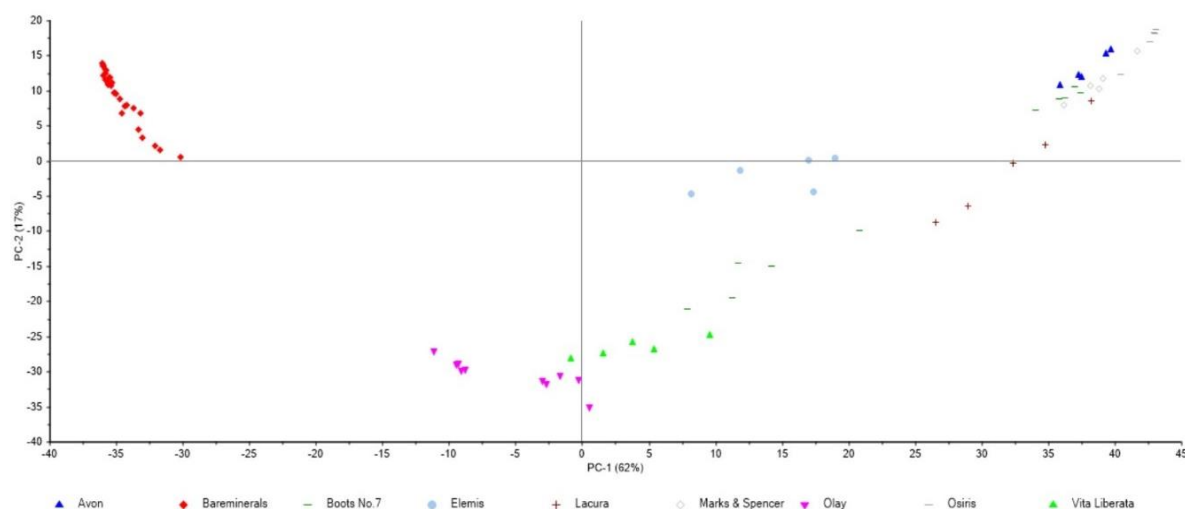


Figure 3.69: PCA scores plot (PC1 vs. PC2) of all the spectrally homogeneous tinted moisturisers, grouped by brand.

In Figure 3.69, most samples occupy their own space within the plot; the exception to this is the M&S tinted moisturiser. However, when plotting PC1 against PC4 (Figure 3.70) the M&S product becomes separated from the neighbouring samples. As discussed in Section 3.3.3.3, this product has a highly featured mid-spectral range (in part due to the chemical sunscreen contained in the formulation), which is driving the separation seen along PC4 (the loadings plot for PC4 can be referred back to in Figure 3.37). Both Olay tinted moisturisers also display an intense peak at 1605 cm^{-1} , hence their extreme negative values along PC4.

It should also be noted here that because these plots only contain a subset of the spectrally homogeneous samples, it is much easier to visualise each of the product's five replicates. This allows for an assessment of experimental repeatability and/or sample heterogeneity. Figure 3.70 shows that the five replicate spectra taken of the Avon product cluster far more closely together than those of the Vita Liberata and Elemis products. As discussed in Section 3.3.2.3 and illustrated in Figures 3.13 and 3.14, there were marked visual differences in the matrices of these products and the relative distributions of mica particles, so these chemometric distinctions are unsurprising.

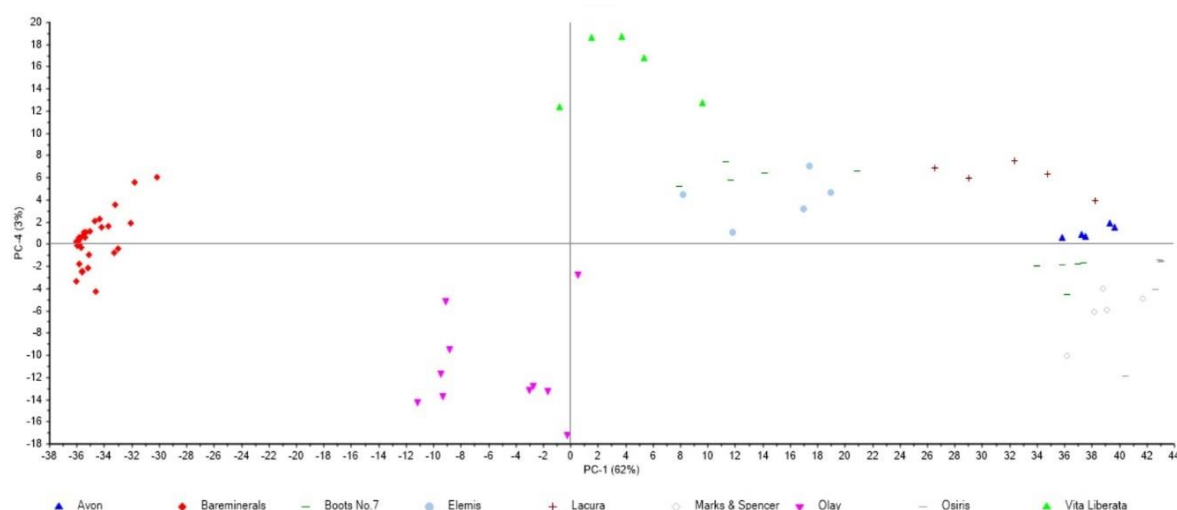


Figure 3.70: PCA scores plot (PC1 vs. PC4) of all the spectrally homogeneous tinted moisturisers, grouped by brand.

There was no value in sample grouping the tinted moisturisers by formulation as all were liquids. Likewise, all samples were of a similar shade so this grouping did not reveal any further trends.

3.3.4.4 Finishing powders

As there were only six finishing powders in the sample set, it was decided that this would be insufficient data to create a meaningful PCA model.

3.3.5 Classification

Various classifications were attempted using the linear (L), Mahalanobis, and quadratic (Q) variants of discriminant analysis (DA), using the PCs derived from the PCA in place of the original variables. DA models were created based on different classifiers: brand, product formulation, and product type, to assess the utility of this method. Unfortunately, the lack of defined classes in the PCA models, signified by sample overlaps in the scores plots, also transpired to be problematic for DA.

The first DA model created was a LDA classification of all the spectrally homogeneous samples, using the first five PCs from the PCA model, and classified according to brand. Overall, this demonstrated a poor classification accuracy of 37%, meaning it was less likely to correctly predict a sample to its class, than via the flip of a coin. The classification accuracy is calculated as the total number of correct classifications as a percentage of the total number of classifications (misclassifications included). It was thought that this low classification accuracy may be due to the linear distance measure used, and so, the Mahalanobis and quadratic distance measures were also trialled.

The classification accuracies when using the Mahalanobis and quadratic distance DA methods both gave better classification accuracies of 55% and 77% respectively, and indicated that the linear measure was not well suited to the chemical variability of the samples. Increasing the number of PCs used in the creation of the DA models from five to seven did not appreciably improve accuracies. However, these values still indicate that any classifications could not be made with confidence. These low classification accuracies are highlighting poor separation between classes (in this case, by brand) and are due a high ratio of *intra*-sample to *inter*-sample variability⁴². This ratio is often termed the Fisher ratio, or more simply F-ratio, named after the mathematician Sir Ronald Fisher who created the notion.

Just as PCA assigns a “score” to each sample, DA assigns a “*discriminant value*” (DV) to each sample. The DVs are a measure of the distance between a projected sample and the centroid of a particular class. DA will always assign an unknown sample to the class with the smallest DV as the closest match, but if the DVs of many samples are very similar this will result in misclassifications such as those we see here. The reason why the linear distance measure performs the worst out of the three methods trialled here is because it does not allow for small differences between adjacent classes. It is often referred to as a “*hard*” classification technique, unlike methods such as soft independent modelling of class analogy (SIMCA)⁴². LDA, as the name suggests, effectively draws a straight line between samples to categorise them³⁷, but due to small instrumental variations, inconsistent sampling methods, or sample heterogeneities, this can cause differences between replicate measurements and result in incorrect classifications.

The extreme diversity of the sample set will have made correct classifications less likely, but this is a good test of its true ability, rather than “*cherry picking*” data to present favourable statistics. The classification of this dataset will be more difficult because there are multiple samples represented by each brand, e.g. Rimmel, but not all of those samples will be represented by products of the same type, e.g. concealer, or the same formulation, e.g. liquid. Where samples are distinctly different, LDA will classify them to the most chemically similar sample, even if this means they do not derive from the same brand⁴².

It is hypothesised that the LDA and QDA classification accuracies would have been significantly improved had each sample spectrum been classified according to the actual product (variant and colour option) rather than the overall brand. Excellent sample clustering was observed across many of the sample replicates in the PCA scores plots, i.e. demonstrating a favourable Fisher ratio, and therefore correct classification by product may have been more successful. Unfortunately, access to the Unscrambler software expired

before this recalculation could be carried out but presents an opportunity for future work, perhaps via a freely available chemometric software package such as R.

It is worth mentioning that despite the poor classification accuracies reported here, they are likely to be even worse than predicted. This is because the accuracies are based upon the internal validation computed by the software, which uses the same data that defined the model (calibration data) as that to evaluate the model (validation data). Therefore, the classification accuracies stated are overoptimistic and it is for this reason that it is preferable to use different data for creating the model and testing the model: training and test sets⁴⁶.

Further DA models were created to classify by formulation and product type, using all three distance measures. The Mahalanobis method gave better accuracies than LDA but did not outperform QDA in either of the classifications. LDA classification of samples according to formulation, created using the first seven PCs from the PCA model revealed a better result than the brand classifier of 48% accuracy. This indicates that product formulation is a more predictable property than brand, however, given that there were 48 brands represented and only five different formulations, the within-group to between-group difference will be far more pronounced for the formulation classifier.

A QDA model was created to classify by formulation with the expectation that it would give a vastly improved accuracy, as was the case when classifying by brand. That was not the case; the QDA model only increased the accuracy given by the LDA model by 6% to 54% and suggests that a product's chemistry is not a reliable indication of its formulation and should not be used as a classifier.

Finally, DA models were constructed to classify by product type using the first seven PCs from the PCA model. The LDA classification accuracy for all the spectrally homogeneous samples was given as 68%, whilst the QDA classification accuracy was slightly improved at 74%. Yet again, the quadratic distance measure only affords a 6% increase in accuracy over the linear distance measure, suggesting that sample chemistry should also not be used as a reliable indicator of product type.

The low classification accuracies seen may be attributable to highly influential samples that distort the model⁴⁷, such as those identified in the influence plots as having high leverage values or high residuals. Thus, if these samples are removed, the remaining samples will not cluster so closely and classification may be more effective. It is also important to note that 126 samples (represented by 630 discrete spectra) were simultaneously analysed in these DA models, yet in casework it is far more likely that a questioned versus known comparison (Q vs. K) would be undertaken, i.e. a pairwise comparison. In this case it would be far easier to discriminate between the samples and classify accordingly. Nevertheless, the chemometric

analysis of datasets with larger sample sizes better define the data structure and add confidence to results^{39,41}. Furthermore, even in cases such as this, where DA classifications have failed to prove useful, they may help link an unknown sample to a manufacturer or distributor (e.g. L'Oreal) and yield investigative leads, even if they cannot correctly identify a particular sample. These models may also provide the means to exclude a sample from an investigation via elimination.

Given that the primary differentiation between all samples analysed in this Chapter (as confirmed via the PC1 loadings) was the titanium dioxide polymorph used, this could also be used as a binary classifier. If all anatase-containing products had their own PCA model and all rutile-containing samples had a separate PCA, this would result in a higher *inter*-sample differentiation, because PC1 would no longer be accounting for the polymorph distinction. Consequently, samples would show a lower *intra*-sample variation, i.e. a better Fisher ratio, and a subsequent DA would be more effective⁴⁸.

3.4 Conclusions

This Chapter has examined 126 different facial cosmetic products purchased from outlets in the UK via optical microscopy and confocal Raman spectroscopy. The products covered 48 different brands and within those brands, 93 different product lines were represented. The samples derived from four product types: foundations, concealers, tinted moisturisers, and finishing powders, and covered a range of different shades. The samples exhibited different formulations and spanned various pricing brackets, from budget ranges through to the premium market. In an attempt to profile some of the samples' components, 65 raw cosmetic ingredients were purchased and subjected to microscopic and Raman analysis.

Visual microscopic analysis of the samples revealed differences between their colour matrices and with regard to their pigment particles. Here, differences in the colour, size, morphology and distribution of pigment particles could be ascertained. Mousse, pressed powder and loose powdered formulations gave rise to varying surface topologies, which made focusing challenging. The presence of spherical particles could be observed in some of the samples; these varied in their function. Some spherical particles were found to be vesicles used for component encapsulation while others were solid particles designed to impart smooth application or even out an irregular complexion, i.e. a wrinkle filler.

Visual comparisons across all the products revealed that concealers and tinted moisturisers exhibited less variation than foundations, and that a distinction between corn starch and mica-based finishing powders could be made prior to Raman analysis. Nonetheless, it was discovered that the apparent **visual** homogeneity did not always correspond to a **spectral**

homogeneity and therefore predictions as to sample behaviour under Raman, could not be made based upon visual observations. This Chapter details the analysis of only the spectrally homogeneous samples; the spectrally heterogeneous samples are dealt with in the following Chapter.

The application of Raman spectroscopy to these types of samples was shown to be effective. Not only was it possible to obtain good quality spectra for every one of the 126 samples, i.e. acceptable reproducibility and repeatability, coupled with minimal baseline fluorescence and well defined peaks, but it also demonstrated a huge amount of chemical variability among the samples. The main spectral differences between samples transpired to be the titanium dioxide polymorph used, the iron oxide pigment intensities, and the presence of various silicones and other organic components.

The foundation products showed a higher reliance on the anatase polymorph, while the concealers and tinted moisturisers exhibited an equal split between anatase and rutile usage. Comparisons between sample spectra and raw cosmetic component spectra revealed that certain ingredients were not always apparent. The type of product packaging was seen to affect resultant sample spectra, as was the case with the BareMinerals Bareskin Foundations, but the cause could not be confirmed. As such, further analyses will be conducted in future Chapters to ascertain the origin of these spectral changes.

Chemometric analysis of the entire dataset was carried out, as the literature suggests that MVA of large datasets better defines structure and adds confidence to the results. This Chapter not only describes the first Raman spectroscopic investigation of facial cosmetic products in a forensic context, but also comprises a much larger and more varied dataset than is contained within the current literature.

PCA confirmed the visual assessment of Raman spectra: a huge range of chemical variability that required the first seven PCs in order to describe, that was dominated by the spread of the foundation products. The close proximity of the calibration and validation variances indicated a robust model despite the level of chemical variability. PCA was able to reveal trends that were not immediately apparent from visual inspection of the spectra, such as the reliance on lecithin or castor oil content according to PC2, and the covariance of mid-spectral peaks to those in the CH region. No trends by brand or formulation could be discerned but for the fact that all formulations except mousses could be regarded as “*extreme*”. When assessed by product type, tinted moisturisers and finishing powders were found to be the only outliers to the model, while concealers were found to be influential to the model. The importance of pigment content was seen in the influence plot grouped by shade, with light-coloured samples being “*typical*” of the model, medium-coloured samples being highly influential (denoted by

high leverages), dark-shaded samples being poorly described by the model (denoted by high residuals), and translucent samples being the main outliers. A separate PCA model for the BareMinerals branded products showed good sample clustering and trends across product type. Visual discrimination between samples of the same product line that differed only by shade was extremely difficult, but PCA modelling allowed for the quick and facile separation of these samples.

The discrimination potential amongst anatase-containing products was lower overall due to the overwhelming “*lattice*” peak that dominated the spectra and indicated that a PCA model of anatase-containing products exclusively might be a better method of sample discrimination. However, it also showed that the “*lattice*” peak was not solely an indicator of anatase content and that other cosmetic components such as bismuth oxychloride also contributed to peak intensity in the 143 cm⁻¹ region.

DA was found to be ineffective using the classifiers chosen but that an improvement could be made by selecting different distance measures. QDA was found to be a better technique than LDA for the classification of these samples, in particular when classifying by brand. This is due to LDA being a “*hard*” classification technique that is not well suited to the discrimination of overlapping sample clusters.

The classification of samples via product type and/or formulation was found to be unreliable and is not recommended. However, the brand classifier showed promise as there was a significant increase in classification accuracy when changing from a linear distance measure to a quadratic method. A specific product classifier rather than a brand classifier was proposed to be a better solution, as it would utilise the fundamental premise of the Fisher ratio. Alternatively, a separate PCA-DA model could be used for the discrimination and classification of anatase-containing products, for the same reasons set out above, or the samples designated as “*extreme*” could be removed from the model to increase the separation (and therefore distinction) between the remaining samples. Despite the lack of DA utility presented, the data contained within these models could be used for elimination purposes, to provide investigative leads, or in Q vs. K scenarios.

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Chapter Four: Determination of the spectral heterogeneity amongst facial cosmetics using Raman microspectroscopy and chemometrics

4.1 Introduction

The previous Chapter detailed the assessment of those cosmetics that were deemed to be spectrally homogeneous. However as stated in Section 3.3.1, some of the facial cosmetics analysed during the course of this research revealed a lack of spectral repeatability and were identified as being spectrally heterogeneous. The aim of this Chapter, is to determine the level of spectral heterogeneity amongst these samples and assess whether or not this has a substantial effect on sample discrimination. The models created in Chapter 3 will be used as a basis onto which these heterogeneous samples can be projected. This will not only allow comparisons to be made between the spectrally homogeneous and heterogeneous samples, but will also indicate the extent of the heterogeneity and whether an alternative analytical method, such as Raman mapping, might be more appropriate for these samples.

4.2 Experimental

There are no specific protocols for the collection of cosmetic samples within a forensic context, nor any for the subsequent analytical workflow. This lack of practical guidance is further exacerbated by these problematic samples, so the experimental procedure was kept exactly the same as for the spectrally homogeneous samples. Despite the ability to fine tune the Raman instrumental parameters and the benefit it would have brought for some of the samples, the decision was made to strictly apply the same experimental conditions across the entire sample set. In this way, any differences arising between sample spectra could be attributed to differences between the samples rather than variations due to differing acquisition parameters. Full experimental conditions are summarised in Chapter 2.

4.2.1 Samples

Of the total 170 new samples analysed during the project, 126 were identified as being spectrally homogeneous and are discussed in the previous Chapter. This Chapter discusses the remaining 44 samples that were assessed as being spectrally heterogeneous.

Cosmetic samples were purchased from a variety of UK outlets over the course of the project; a list of samples is given in the appendix. The spectrally heterogeneous sample population consisted of 44 new products, which comprised five foundations, four concealers, one tinted moisturiser, 13 finishing powders, four bronzers, 16 highlighters and one primer.

The decision to include these seven product types, rather than foundation alone, was based on three observations. Firstly, that cosmetic wearers tend to layer their products, and hence any traces transferred to a substrate are likely to contain a mixture of those products. Secondly, it is logical that the final products applied to the skin would be the first to be removed and transferred to any substrate. Products such as bronzers, highlighters and finishing powders (as the name suggests) are designed to be applied over base products – such as primer, foundation and concealer – and are therefore most likely to be transferred during criminal activity. Lastly, and as mentioned in the previous Chapter, consumers may not use a product in the manner that the manufacturer intended. For example, someone with a darker skin tone may prefer to use a bronzer in place of a foundation if the shades offered cause the skin to look too red, too black, or too ashen¹, whereas someone with a dull, sallow complexion may choose to wear a highlighter in place of a foundation, in order to achieve a brighter, glowing skin. It should be noted that all these products were chosen from the outset of the project, for the reasons stated above, but it was not apparent until the Raman spectra were assessed that all the bronzers and highlighters analysed, would yield heterogeneous spectra, and are therefore exclusively limited to this Chapter.

The sample population was deliberately chosen to represent as wide a range of facial cosmetic types as possible given budget constraints. The sample population spanned 32 different product ranges across eight different brands, ranging from the budget market (average product cost £1.00), through to the mainstream market (average product cost £12.00) and up to the premium market (average product cost £34.00).

The samples encompassed a range of different formulations: seven liquids (including gel consistencies), 20 loose powders (predominantly mineral based), 16 pressed powders (compact style), and one cream (wind-up stick style). There were no mousse formulations in this sample set.

All samples were prepared, analysed, and subjected to chemometrics as detailed in the experimental section of Chapter 2.

4.3 Results and discussion

4.3.1 Preliminary considerations

It should be reiterated here that the assessment of spectral heterogeneity was carried out in a subjective manner and was only intended to serve as a tool to aid further analysis. It was neither definitive nor exhaustive, but merely an indication of the sample's composition. Unlike the sampling guidelines provided by the Scientific Working Groups for the analysis of fibres (SWG-MAT)² no comparable guidance could be found for cosmetic residues.

As can be seen in the list of samples, there are additional cosmetic types not seen in the spectrally homogeneous samples in the previous Chapter. Whilst it cannot be proven due to the limited sample size, it can be assumed that these additional categories – namely, bronzers, highlighters, and primers – are intrinsically heterogeneous in nature and hence, did not feature in the spectrally homogeneous category. This heterogeneity could relate to larger suspended particle sizes, agglomeration of particles, or a lack of miscibility of various liquid phases.

It should also be noted that the vast majority of the samples included in this Chapter are from a single brand, BareMinerals, comprising 35 out of the total 44 products. As a result, this brand is overly represented in the sample set and this must be borne in mind when carrying out chemometric techniques that use the “*brand*” category variable.

It is apparent that these spectrally heterogeneous samples disproportionately comprise powdered formulations (36 of the total 44 samples). Again, it cannot be definitively stated, but it is assumed that loose and pressed powder formulations are more likely to result in heterogeneous replicate spectra, due to the microscopic sampling difficulties discussed in Section 3.3.1 of the previous Chapter. In the same vein, none of the samples were mousse formulation and only one was a cream formulation, suggesting that these are more likely to give repeatable spectra and thus be considered as spectrally homogenous. Furthermore, all seven liquid formulations in this category were found to be visually heterogeneous, as well as spectrally heterogeneous. This was often due to the presence of large pigment particles (relative to the incident laser beam diameter) suspended within the matrix and was again an indicator that the sample would struggle to yield repeatable spectral results.

Nearly a third of all the spectrally homogeneous samples seen in Chapter 3 were also assessed as being visually homogeneous at the x50 magnification. Conversely, only a single sample from this set of 44 spectrally heterogeneous samples was identified as being visually homogeneous. Surprisingly, this single sample was the only cream formulation in the sample set. Obviously, no firm conclusions can be drawn from the analysis of a single sample, but it is interesting to note that a homogeneous visual appearance can result in heterogeneous replicate spectra.

4.3.2 Characterisation using optical microscopy

As was seen in Chapter 3, visual inspection of the samples gave an initial level of discrimination. Most notable differences were the colours of the sample matrices and the colour, size, morphology and distribution of pigment particles. However, the addition of highlighter, bronzer and primer products in this sample set varied the visual appearances considerably, as will be discussed herewith. The pressed powder and loose powder samples

corroborated what was seen amongst the spectrally homogeneous samples in that there were differences in surface topologies that gave rise to focusing difficulties. All photomicrograph images in this Chapter were obtained at x50 magnification.

4.3.2.1 Foundations

The foundations only consisted of five samples; this represents a small proportion of the total number of spectrally heterogeneous samples analysed (contrary to what was seen in the spectrally homogeneous foundations) and suggests that foundations, in general, tend to have a more uniform consistency. These five samples comprised two pressed powders, two loose powders and one liquid formulation. All five foundations exhibited reflective pigment particles, to a greater or lesser degree, as is illustrated by the mirror-like flecks in the images below (Figure 4.1).

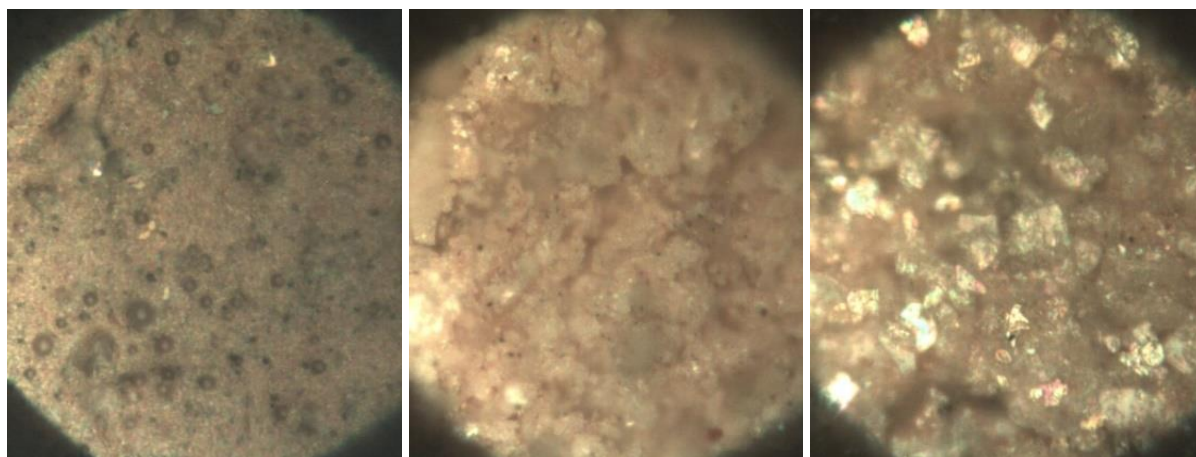


Figure 4.1: Colour photomicrograph images of spectrally heterogeneous foundations in three different formulations, exhibiting varying amounts of reflective pigment particles, (L-R) Avon Ideal Flawless Foundation in shade Nutmeg (liquid), BareMinerals Ready Foundation in shade R210 (pressed powder), BareMinerals Pure Transformation Day Treatment in shade Medium (loose powder).

4.3.2.2 Concealers

Similar to the foundation sample set, the concealers also made up only a small proportion of the spectrally heterogeneous samples – four in total – and suggest that concealers too, tend to have consistent mixtures. These four samples comprised two liquids, one loose powder and one cream, examples of which are given in Figure 4.2. Once more, they all contained reflective pigment particles to a greater or lesser extent, but prior to Raman analysis it was not clear whether these signified the presence of pearlescent pigments such as bismuth oxychloride, guanine or talc, or the presence of interference pigments such as metal oxide coated mica, coated borosilicate glass, or nacre³.

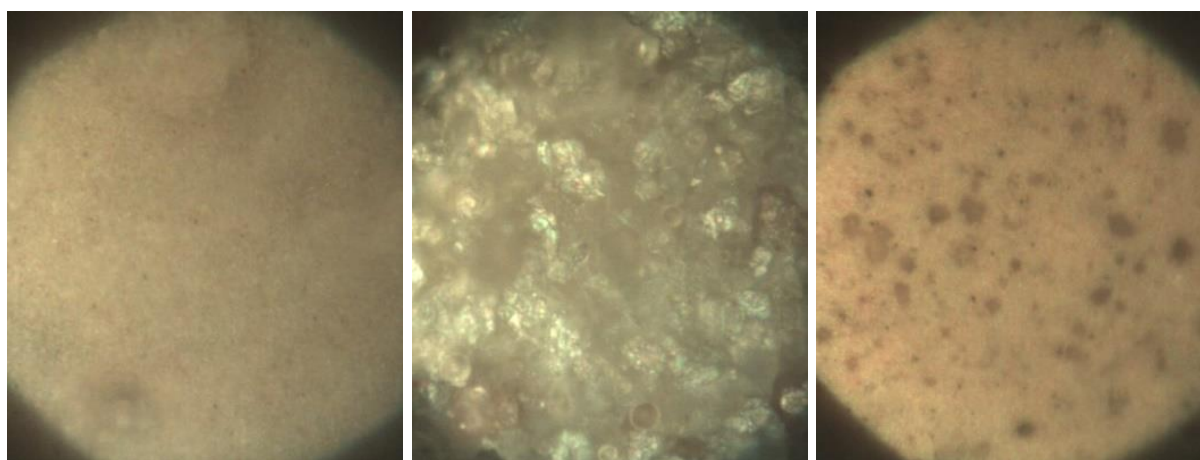


Figure 4.2: Colour photomicrograph images of spectrally heterogeneous concealers in three different formulations, (L-R) BareMinerals Blemish Remedy Concealer in shade Light (cream), BareMinerals Blemish Remedy Treatment Concealer in shade Original (loose powder), Rimmel Match Perfection Concealer in shade 010 Ivory (liquid).

4.3.2.3 Tinted moisturisers

Only a single tinted moisturiser was found to be spectrally heterogeneous. Despite this sample being a liquid formulation, the consistency was rather thick and cream-like. This caused focusing difficulty as can be seen in Figure 4.3. A few reflective pigment particles were evident, as were scattered red and black iron oxide pigments, presumably giving rise to the spectral heterogeneity seen.

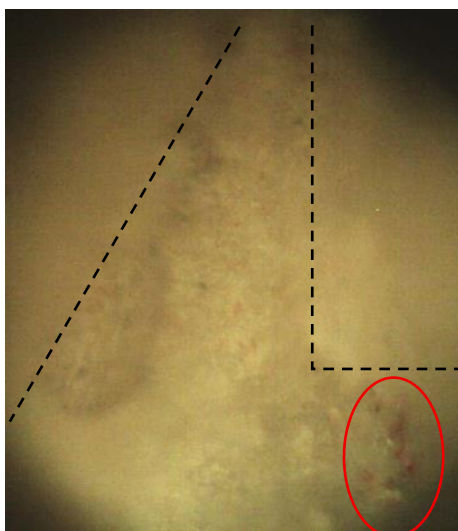


Figure 4.3: Colour photomicrograph image of the only spectrally heterogeneous tinted moisturiser, *Autograph Daily Defence Tinted Moisturiser in shade Medium Nude (liquid)*, showing red iron oxide pigments in the bottom right corner. Blurred areas denoting focusing difficulties marked with black dashes; iron oxide pigments highlighted with red ellipse.

4.3.2.4 Finishing powders

There were 13 finishing powders in this sample set, representing approximately 30% of the total number of spectrally heterogeneous samples. This contrasts dramatically with what was seen in the spectrally homogeneous samples (Chapter 3) in that finishing powders made up less than 5% of that total sample set. Clearly, this indicates that this type of facial cosmetic is prone to sampling inconsistencies at the microscopic level, and this would have serious implications for sample analysis in casework. Given that finishing powder tends to be the last layer of facial makeup applied by cosmetic users, it is likely to be one of, if not the first layer to be transferred in the event of a crime. Therefore, this product type is more likely to be encountered as a trace than base-layer products such as foundation, and this requires careful consideration as to the analytical workflow.

Unsurprisingly, all 13 samples were powders, with four being of the pressed variety and nine being loose powders. Figure 4.4 shows the visual differences between three of the finishing powder samples. The BareMinerals 5 in 1 BB Advanced Performance Mineral Veil loose powder displays a blurry appearance consistent with focusing difficulty on an uneven surface and paler spherical areas that correlate with the silica microbead components. The BareMinerals BareSkin Perfecting Veil has a more reflective appearance than the other two samples in this figure, highlighting its zinc stearate and magnesium stearate content, whereas the Fashionista Catwalk 360 Pressed Powder exhibits a less reflective and more consistent appearance, which is congruent with its predominant ingredient, talc.

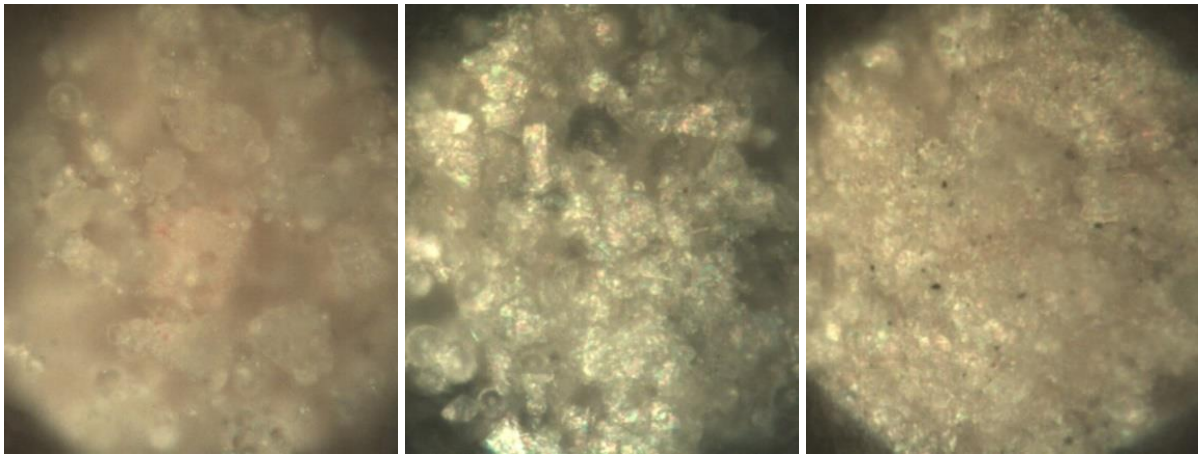


Figure 4.4: Colour photomicrograph images of the spectrally heterogeneous finishing powders in two different formulations, (L-R) BareMinerals 5 in 1 BB Advanced Performance Mineral Veil in shade Light (loose powder), BareMinerals BareSkin Perfecting Veil in shade Light-Medium (pressed powder), Fashionista Catwalk 360 Pressed Powder in shade Taupe (pressed powder).

All but one of these samples came from the BareMinerals brand, meaning that performing sample grouping by category variable “*brand*”, would be of little use statistically. Nonetheless, of these 12 BareMinerals finishing powders, 11 were from different product lines demonstrating a good range of available products. Over half of the BareMinerals finishing powders were found to contain spherical particles of varying sizes across the different product lines, as can be seen in Figure 4.5. When used in foundation and concealer products, these spherical particles are often used to encapsulate pigment and/or active ingredients in a vesicle-like manner⁴. However, in finishing powders the most likely function of these particles is to blur the appearance of pores and fine lines on the skin and give the complexion an airbrushed finish⁴.

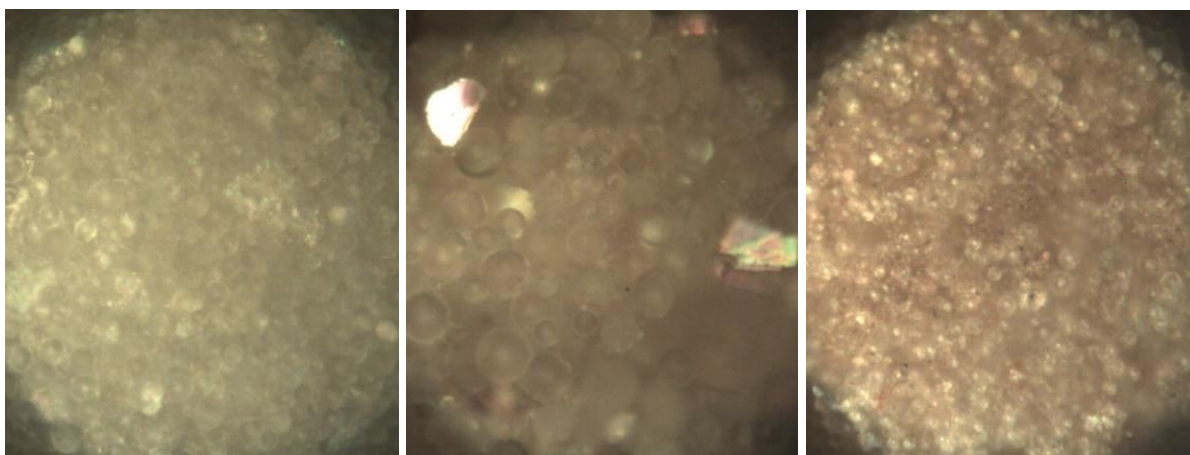


Figure 4.5: Colour photomicrograph images of the spectrally heterogeneous finishing powders indicating a high level of variously sized spherical particles, (L-R) BareMinerals Complexion Rescue Mineral Veil (no shade specified), BareMinerals Brightening Pearl Natural Light Mineral Veil (no shade specified), BareMinerals Ready Touch Up Veil in shade Tinted.

Many of the finishing powders contained reflective pigment particles of varying sizes, shapes and distributions. Figure 4.6 shows two different views of the BareMinerals Illuminating Mineral Veil and illustrates the extent to which the particle sizes could differ in a single sample. Inspection of the ingredients list confirms that the reflective particles in this product are titanium dioxide and/or iron oxide coated mica flakes, but not all the reflective particles seen across the range of finishing powder samples could be attributed to mica, as not all samples contained mica in their ingredients lists. This emphasises the need for a vibrational spectroscopic approach after visual microscopic examination.

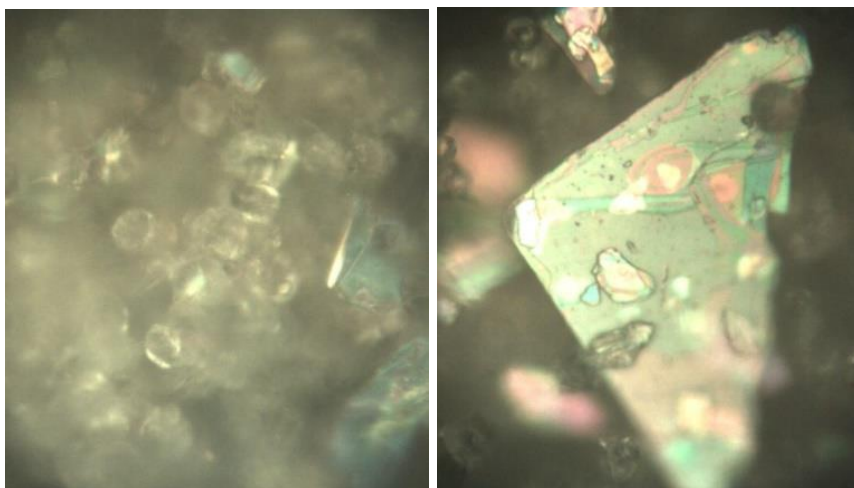


Figure 4.6: Colour photomicrograph images of BareMinerals Illuminating Mineral Veil, (L) general appearance, (R) focused on a large titanium dioxide coated mica flake. Note: both images taken at x50 magnification.

4.3.2.5 Bronzers

This sample set comprised only four bronzers, all of which were from the BareMinerals brand. Three of the bronzers were pressed powder formulations and one was liquid. All three pressed powder bronzers demonstrated a high concentration of reflective pigments, yet the pigments in the liquid formulation were more sparsely distributed as can be seen in Figure 4.7.

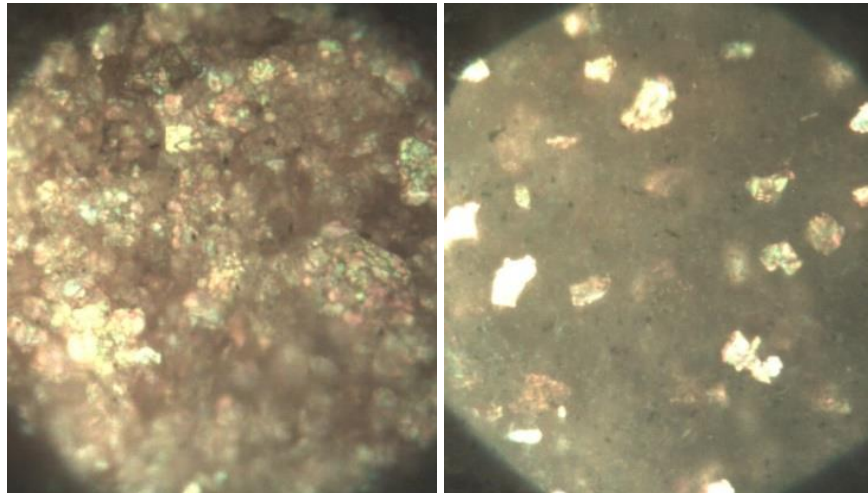


Figure 4.7: Colour photomicrograph images of the spectrally heterogeneous bronzers in two different formulations, demonstrating differences in reflective pigment concentrations, (L-R) BareMinerals Ready Bronzer in shade The Skinny Dip (pressed powder), BareMinerals BareSkin Serum Bronzer in shade Bare Glow (liquid).

4.3.2.6 Highlighters

The highlighters comprised the largest sample set within the spectrally heterogeneous category, with 16 products represented by three different brands. The formulation split for the highlighters was eight loose powders, seven pressed powders and one liquid, visual examples of which are given in Figure 4.8.

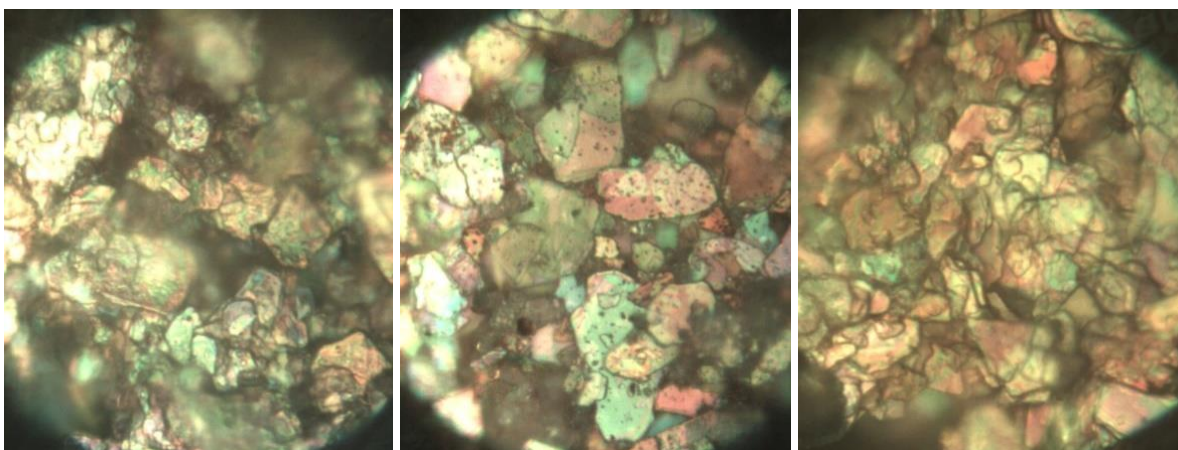


Figure 4.8: Colour photomicrograph images of the spectrally heterogeneous highlighters, in the three different formulations, (L-R) BareMinerals Stroke of Light Face Highlighter (shade not specified) (liquid), BareMinerals All Over Face Colour in shade Pure Radiance (loose powder), Pixi Glow Gossamer Duo in shade Subtle Sunrise (gold side) (pressed powder).

As was seen in Section 4.3.2.4 with the BareMinerals Illuminating Mineral Veil finishing powder, the BareMinerals highlighter, All Over Face Colour in shade Clear Radiance, also contains large titanium dioxide coated mica flakes (Figure 4.9). However, the large mica flake shown in Figure 4.9 (R) has an obvious demarcation line across its middle. This indicates that the mica flake is coated with either two differing thicknesses of titanium dioxide, and therefore producing different interference effects, or is coated with two different pigments. In addition to mica and titanium dioxide, the ingredients list for this product includes bismuth oxychloride, the three iron oxides, and carmine⁵. Given the red/pink hue of the bottom half of the flake it is most likely the pigment responsible is either red iron oxide (CI 77491) or carmine (CI 75470).

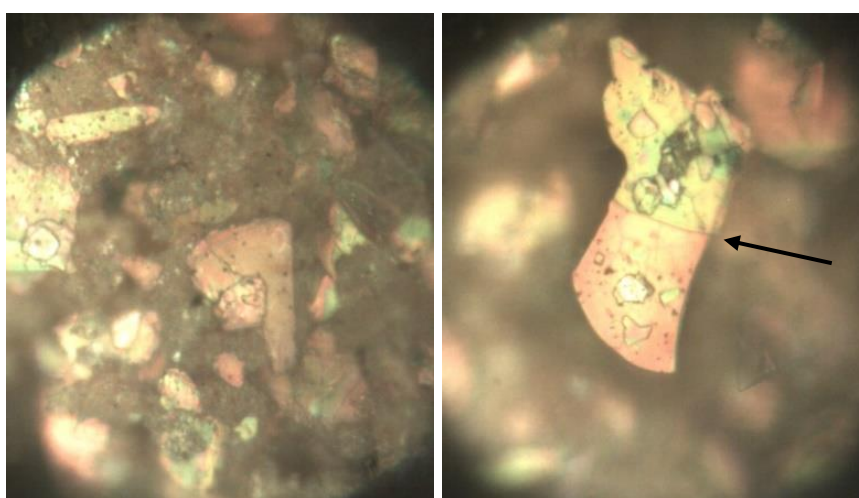


Figure 4.9: Colour photomicrograph images of BareMinerals All Over Face Colour in shade Clear Radiance, (L) general appearance, (R) focused on a large titanium dioxide coated mica flake. Black arrow denotes demarcation line. Note: both images taken at x50 magnification.

As previously stated in Chapter 3, a visual distinction can be made between mica flakes and borosilicate glass flakes due to their differing morphologies. Mica flakes appear in a variety of shapes and sizes but are comparatively much smaller overall than borosilicate glass flakes. Mica flakes also tend to display rounded, irregular edges, whereas borosilicate glass flakes have smooth, sharp, well-defined edges⁶. According to the ingredients list, the MAC Mineralise highlighter shown in Figure 4.10 contains both calcium sodium borosilicate and calcium aluminium borosilicate glass particles, in addition to both natural mica and synthetic fluorphlogopite⁷. In spite of this complex chemical composition, the visual appearance is very similar to other, more simple highlighters analysed in this sample set (Figure 4.10).

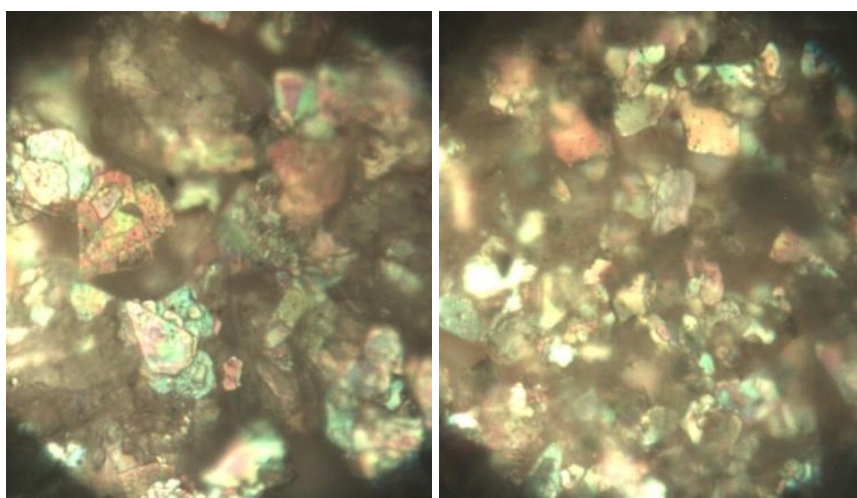


Figure 4.10: Colour photomicrograph images of two spectrally heterogeneous highlighters, (L-R) MAC Mineralise Skin Finish in shade Soft & Gentle, and BareMinerals All Over Face Colour in Turn On Gold. Despite the presence of two forms of borosilicate glass pigments in the MAC sample, the overall appearance is consistent with the BareMinerals sample that exclusively contains mica flakes.

4.3.2.7 Primers

This sample set included only one primer product: Rimmel Fix & Protect Primer. This primer was a liquid formulation and translucent white in colour. The decision to include a primer within the dataset was not only based upon the fact that makeup wearers use products in a layered combination, and therefore any makeup traces recovered from a scene may constitute a mixture of various products, but also to see how its visual appearance (and Raman spectra) differed from those of foundations that claim to have a “*built-in*” primer in their formulation.

Having viewed the ingredients list for this product it was immediately apparent that its composition was very similar to foundation but for the obvious exclusion of the three iron oxide pigments. This product not only contains titanium dioxide, mica, and ultramarines as pigment,

but also a further 53 ingredients that are commonly found in foundations, concealers and tinted moisturisers. These include compounds such as glycerin, acrylates crosspolymer, various siloxanes, silica, boron nitride, aluminium oxide, plant oils, vitamins, humectants, clays, and preservatives. Furthermore, this product contains both chemical (octyl methoxycinnamate) and physical sunscreens (SPF 25), and claims to be mattifying, brightening, smoothing, resurfacing and protective against environmental aggressors, so it is conceivable that this product could be used in isolation.

As is evident from the photomicrograph in Figure 4.11, the mica flakes are fairly uniformly distributed and relatively large compared to other mica containing samples within this set. It is therefore likely that the characteristic peaks for mica, titanium dioxide, and/or ultramarine will be seen in the Raman spectra.

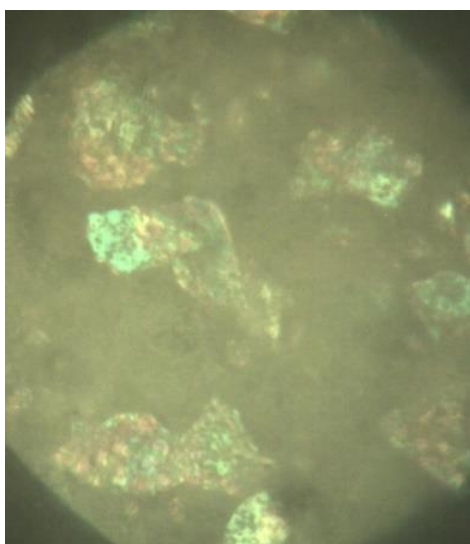


Figure 4.11: Colour photomicrograph image of the only spectrally heterogeneous primer, *Rimmel Fix & Protect Makeup Primer* (no shade specified), showing large mica flakes within its composition.

4.3.3 Characterisation using Raman spectroscopy

Chapter 3 dealt with the subjective determination of each sample's spectral homogeneity or heterogeneity, and as such will not be repeated here. However, amongst the spectrally heterogeneous samples there was also considerable variation of the degree of heterogeneity. Some samples were found to be only slightly heterogeneous, i.e., differing only by the presence or absence of a single peak, or differences in relative intensities of the same peaks, as can be seen in Figure 4.15; whereas other samples were significantly heterogeneous and could display totally different spectra across their surface, despite only moving the focal point slightly (sometimes in the order of ~100 microns), as is demonstrated in Figure 4.16.

Unlike the spectrally homogeneous samples seen in Chapter 3, the Raman spectra of the heterogeneous samples exhibited far more variation in the mid-range and CH regions, and did not demonstrate such a reliance on titanium dioxide polymorph for discrimination. This suggests that these samples comprise a higher proportion of organic-based components and/or silicones, and a lesser need for hiding white pigment and/or physical sunscreen respectively. Indeed, across the set of 44 samples, nine products appeared to use a mixture of both anatase and rutile, and eight products contained neither anatase nor rutile. This is likely due to the greater number of different product types, reliance on other inorganic pigments and potentially a higher usage of chemical-based sunscreens. Nonetheless, amongst those products that contained a single polymorph, anatase-containing products outweighed rutile-containing products in a 3:1 ratio. This suggests that spectrally heterogeneous samples might benefit from a more thorough analytical technique such as Raman mapping and presents an opportunity for future work.

4.3.3.1 Foundations

Interestingly, whereas the majority of the spectrally homogeneous foundations explored in Chapter 3 were found to contain the anatase polymorph, the opposite was true when considering the spectrally heterogeneous foundations. Four of the five heterogeneous foundations analysed were found to contain the rutile polymorph, but this was not the main contributory factor to the heterogeneity seen. The main areas of heterogeneity were found to be differences in iron oxide and aluminium oxide (alumina, Al_2O_3) content between replicate spectra, as is demonstrated by the Avon Ideal Flawless Foundation in Figure 4.12. The reference spectrum for aluminium oxide (Al_2O_3) is given in Figure 4.13 for comparison and shows the large spectral peaks at 1370 cm^{-1} and 1400 cm^{-1} . Given that “*alumina*” is the fourth ingredient on this product’s list of 36 total ingredients, it is reasonable to assume that it comprises a large portion of this product’s composition.

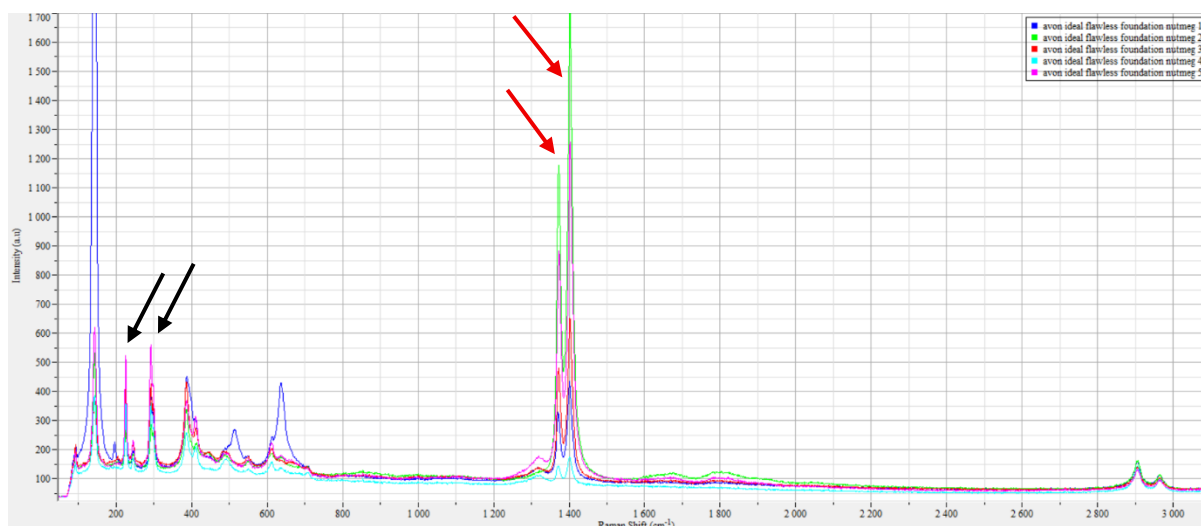


Figure 4.12: Raman spectra (unprocessed) of Avon Ideal Flawless Foundation in shade Nutmeg, showing differences in the iron oxide (marked with black arrows) and aluminium oxide (marked with red arrows) peak intensities between the five replicates.

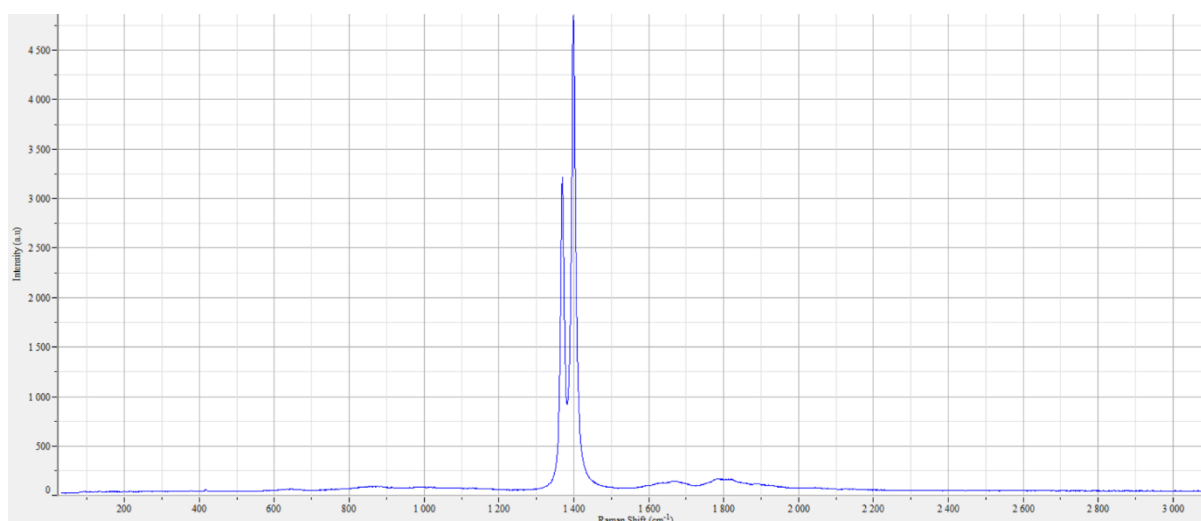


Figure 4.13: Raman spectrum (unprocessed) of the aluminium oxide reference standard.

It is also worth noting that this Avon foundation was a much darker shade (Nutmeg) than the majority of the foundations analysed during the course of this research. Whether or not this results in the heterogeneity seen cannot be determined with just one sample, hence, it would be beneficial to analyse a much larger range of different shades in this particular product line in order to establish this for sure. The increase in iron oxide pigment particles necessary to provide darker cosmetic shades could be responsible for the discrepancies seen between these spectra. However, the principal difference between these spectra is the intensity of the aluminium oxide peaks⁸. Aluminium oxide (Al_2O_3), despite being a fine white powder, is not regarded as a cosmetic pigment, rather a thickening agent or absorbent. As such, it has a high

viscosity and a sticky consistency, meaning the particles are likely to aggregate, which could also cause the spectral heterogeneity seen.

Research conducted by Skobeeva *et al.*⁹ concluded that the NIR spectra of darker-shaded foundation samples were less accurately classified using a combination of PCA-LDA, suggesting that heterogeneity may in fact be a function of shade. However, the authors suggest that the black iron oxide (Fe_3O_4) pigments seen across the darker shades are interacting with the NIR light in a different manner to the titanium dioxide pigment particles within the lighter shades and this distinction does not directly apply to the Raman scattering potential of these pigments.

It was surprising to see the BareMinerals Original Foundation in shade “*Fairly Medium*” appear in the spectrally heterogeneous sample set, as the other four shades from this range were determined to be spectrally homogeneous and therefore included in Chapter 3. What was even more surprising was the realisation that the “*Fairly Medium*” shade was found to contain the rutile titanium dioxide polymorph, yet all the other shades definitively contained the anatase polymorph. Upon closer inspection of the product packaging, there were subtle differences between this shade and the other four shades, such as the type font, the batch code format and the type of plastic used to construct the container. It was therefore deduced that this product was in fact a counterfeit; this conclusion seemed even more likely given that the product was purchased from Amazon for approximately 60% of the recommended retail price.

Figure 4.14 illustrates just how different the replicate spectra for the Fairly Medium shade are, compared with those of the four other shades (Medium Beige, Fair Ivory, Fairly Light, and Light) yet there are two distinctions to be made here. Firstly, the spectra across the genuine products, as previously mentioned, are spectrally homogeneous and exhibit good repeatability across replicates, whereas the spectra for the counterfeit product are inconsistent and spectrally heterogeneous; and secondly, the overall spectral shapes between the genuine and counterfeit products are markedly different. The genuine foundations contain the anatase polymorph of titanium dioxide and bismuth oxychloride, the counterfeit foundation contains neither. The 3-dimensional PCA scores plot for these data is displayed in Figure 2.4 and further demonstrates how disparate the products are.

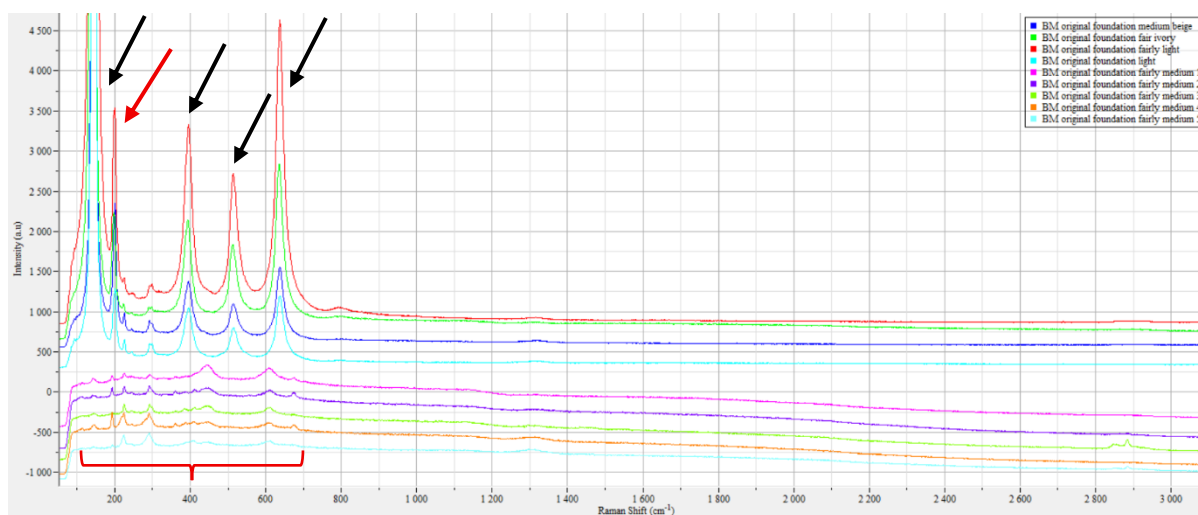


Figure 4.14: Raman spectra (unprocessed but stacked for clarity) of BareMinerals Original Foundation in shades Medium Beige, Fair Ivory, Fairly Light, Light, and Fairly Medium, highlighting the obvious differences between the genuine and counterfeit (Fairly Medium) products. Black arrows denote anatase titanium dioxide content and red arrow denotes bismuth oxychloride content in genuine foundations; red brace denotes absence of these components in the counterfeit foundation.

4.3.3.2 Concealers

Despite this sample set only comprising four concealers, there was a marked difference between the spectra and, consequently the samples' respective chemistries. Two of the concealers were found to contain anatase, one contained rutile and the last did not appear to contain either titanium dioxide polymorph. The rutile containing concealer was also found to be only very slightly spectrally heterogeneous as is evident in Figure 4.15, with the presence or absence of the peak at 1365 cm^{-1} being the main source of discrepancy between replicates.

This peak at 1365 cm^{-1} could be linked to the presence of an O-H bond in-plane deformation vibration or the CH_2 wagging deformation found in primary alcohols¹⁰ that might have arisen from the glycerin (glycerol), dimethiconol, phenoxyethanol, caprylyl glycol, hexylene glycol or dipropylene glycol in the formulation¹¹. Alternatively, this peak could signify the presence of the CH_3 functional group, particularly the symmetric bending vibration¹⁰ often seen at 1365 cm^{-1} . This could derive from the terminal CH_3 group of the alkyl benzoate molecule, which is included in this product's formulation as an emollient ester, or the terminal CH_3 of the tocopherol (vitamin E) molecule. Nevertheless, it is unlikely that the peak at 1365 cm^{-1} relates to the CH_3 groups associated with the cyclopentasiloxane or the various forms of dimethicone contained in the formulation, as both the “end group” and cyclic methyl groups in siloxanes display Raman peaks in the $780\text{--}850\text{ cm}^{-1}$ region. A time-of-flight mass spectrometry (ToF-MS) analysis could be used to determine the specific environment for the CH_3 functional group.

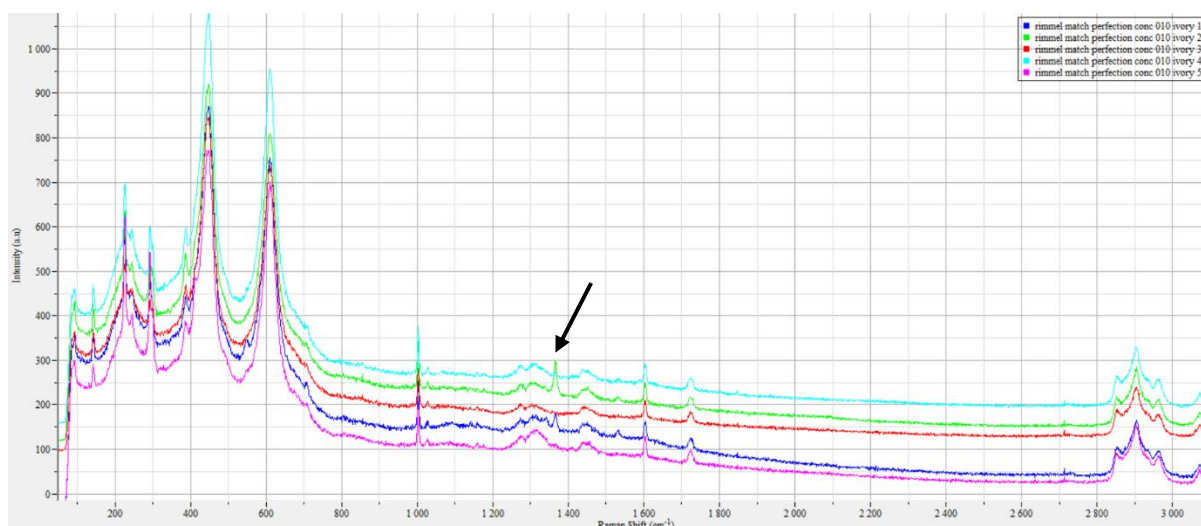


Figure 4.15: Raman spectra (unprocessed) of a rutile containing concealer, Rimmel Match Perfection Concealer in shade 010 Ivory, that was found to be only minimally spectrally heterogeneous, as indicated by the black arrow.

Conversely, the concealer that contained no discernible titanium dioxide was found to be highly heterogeneous (as is illustrated in Figure 4.16), displaying extremely variable spectra across replicates. Yet microscopically there were no obvious particles within the matrix, suggesting the components responsible for the differing spectra must have appeared visually consistent. This raises a further concern for casework; it may not always be visibly obvious that the analytical focal point is different until the spectrum is taken, and even then, the level of heterogeneity cannot be reliably assessed unless a series of spectra are acquired.

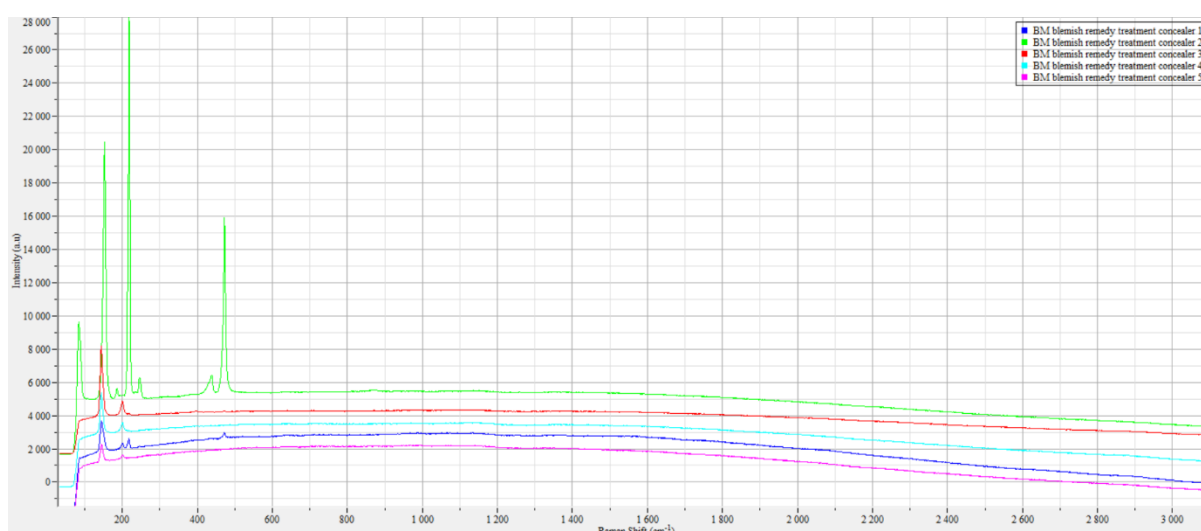


Figure 4.16: Raman spectra (unprocessed but stacked for clarity) of an extremely spectrally heterogeneous concealer, BareMinerals Blemish Remedy Treatment Concealer in shade Original.

Three of the concealers analysed in this sample set were blemish specific treatments, whereas the fourth was designed to conceal darkness under the eyes. As a result, the chemical compositions were quite different, with strong sulfur peaks evident in two of the blemish specific concealers (Figure 4.17). Sulfur is widely used in anti-blemish cosmetic preparations as it inhibits sebum production, dries out and exfoliates the skin, and kills acne-causing bacteria¹².

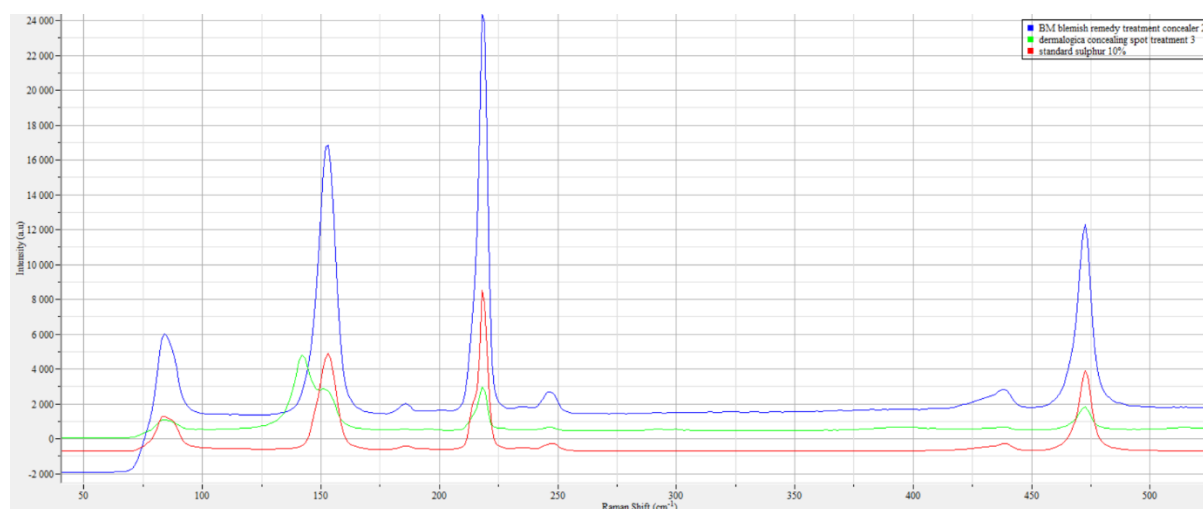


Figure 4.17: Raman spectra (unprocessed but stacked for clarity) of BareMinerals Blemish Remedy Treatment Concealer and Dermalogica Concealing Spot Treatment, with the reference standard for sulfur.

4.3.3.3 Tinted moisturisers

One can assume that as only a single tinted moisturiser from the total sample set appeared to be spectrally heterogeneous, that these products are either better mixed or contain smaller particle sizes than the other types of facial cosmetics. As can be seen in Figure 4.18, the spectral discrepancies are the titanium dioxide polymorph and also the yellow iron oxide content indicated by the peaks at 229 cm^{-1} and 292 cm^{-1} . What is surprising here is usually when a mixture of anatase and rutile is seen, such as those seen in Figure 3.19, they tend to appear together in the same spectrum as a true mixture. What can be seen in this case, however, is either one polymorph or the other is observed but that they are seldom both seen simultaneously. Further research would need to be carried out here, in order to adapt the Raman microscopic method for spectrally heterogeneous formulations.

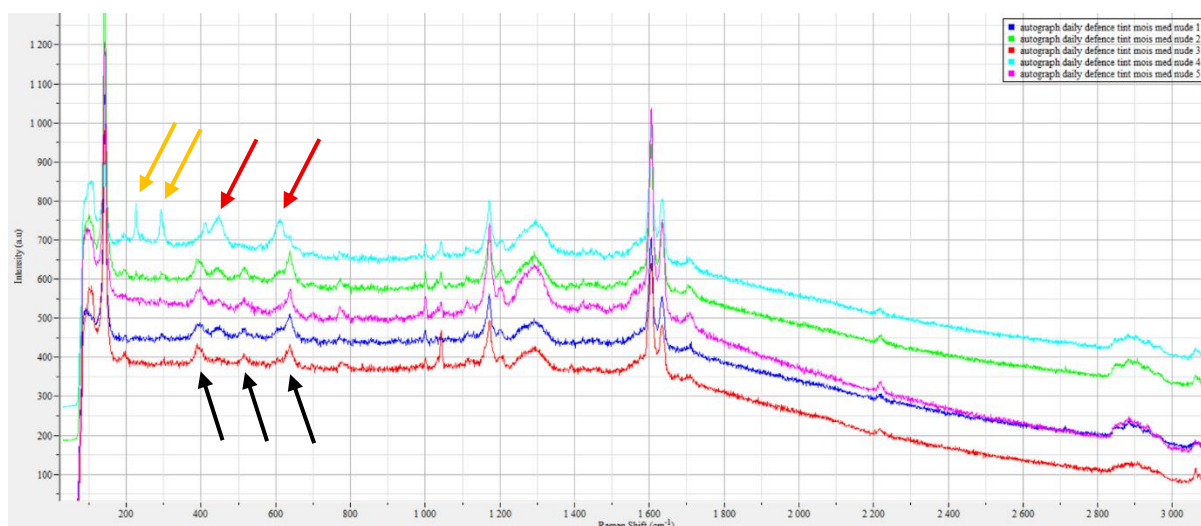


Figure 4.18: Raman spectra (unprocessed but stacked for clarity) of Autograph Daily Defence Tinted Moisturiser in shade Medium Nude, an example of a spectrally heterogeneous product that contains both titanium dioxide polymorphs. Orange arrows denote iron oxide content; red arrows denote rutile content; and black arrows denote anatase content.

4.3.3.4 Finishing powders

Contrary to what was seen amongst the spectrally homogeneous finishing powders, the spectrally heterogeneous finishing powders relied more heavily upon titanium dioxide and less upon rice starch. Nevertheless, this was not often the cause of the heterogeneity seen. Figure 4.19 (top) shows the replicate spectra obtained for the BareMinerals Original Mineral Veil in the SPF 25 variant. No differences can initially be identified other than the anatase peak intensities, however, once zoomed into the baseline multiple differences are apparent, as is displayed in Figure 4.19 (bottom).

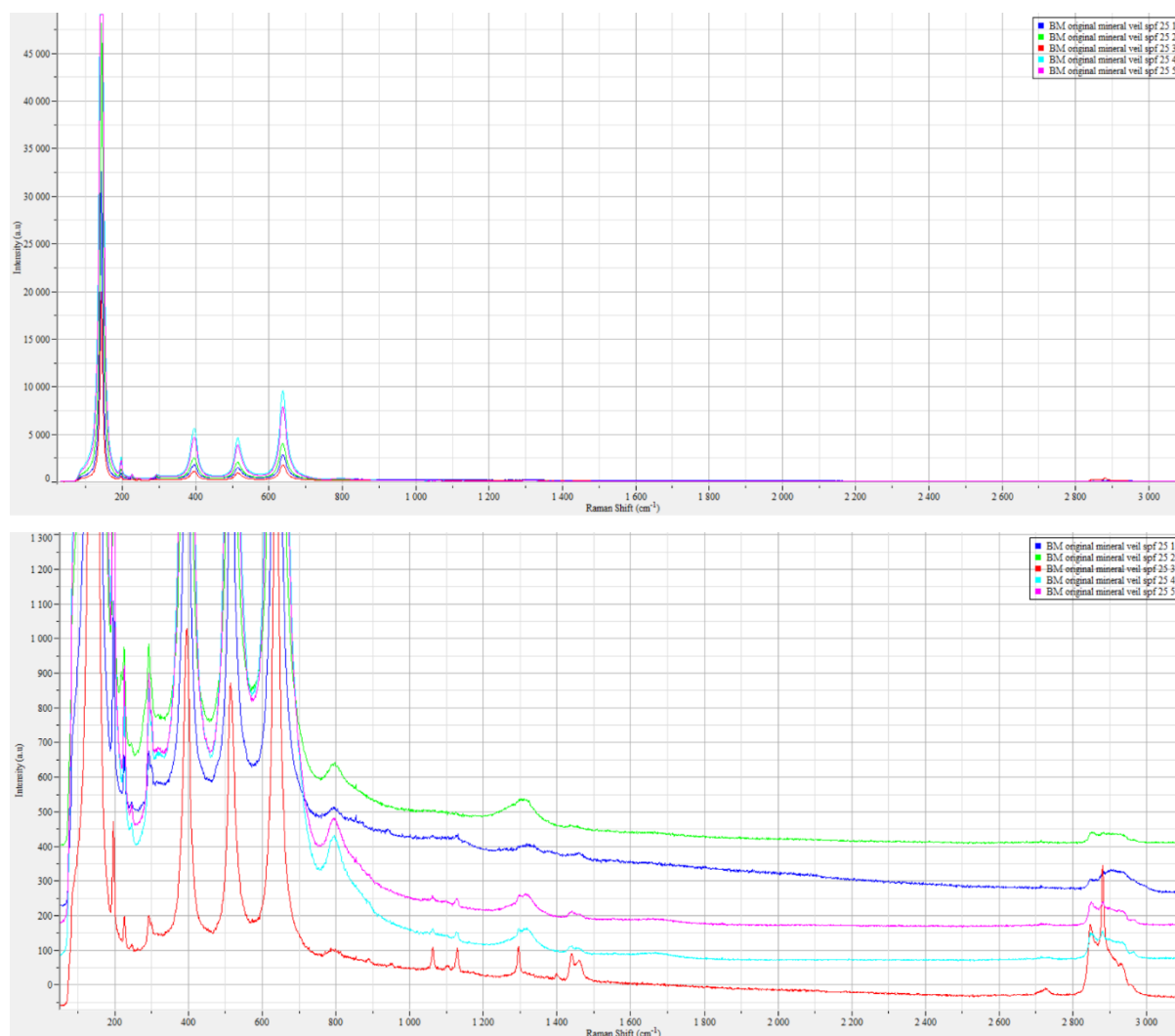


Figure 4.19: Raman spectra (unprocessed) of the spectrally heterogeneous finishing powder, BareMinerals Original Mineral Veil SPF 25 (top), and (unprocessed but stacked for clarity) magnified view showing multiple differences between replicates (bottom).

Inspection of the ingredients list revealed only six components: titanium dioxide, corn starch, zinc stearate, magnesium stearate, silica, and iron oxides. Hence, a reference standard sample of magnesium stearate was analysed for comparison and aligns very closely with that of one of the BareMinerals sample's replicate spectra Figure 4.20. This demonstrates that due to the heterogeneous mixture of solid components within this product, the location of microscopic analysis is critical. For samples such as these it may be preferable to use a microscope objective with a lower magnification than x50 in order to mitigate sampling differences.

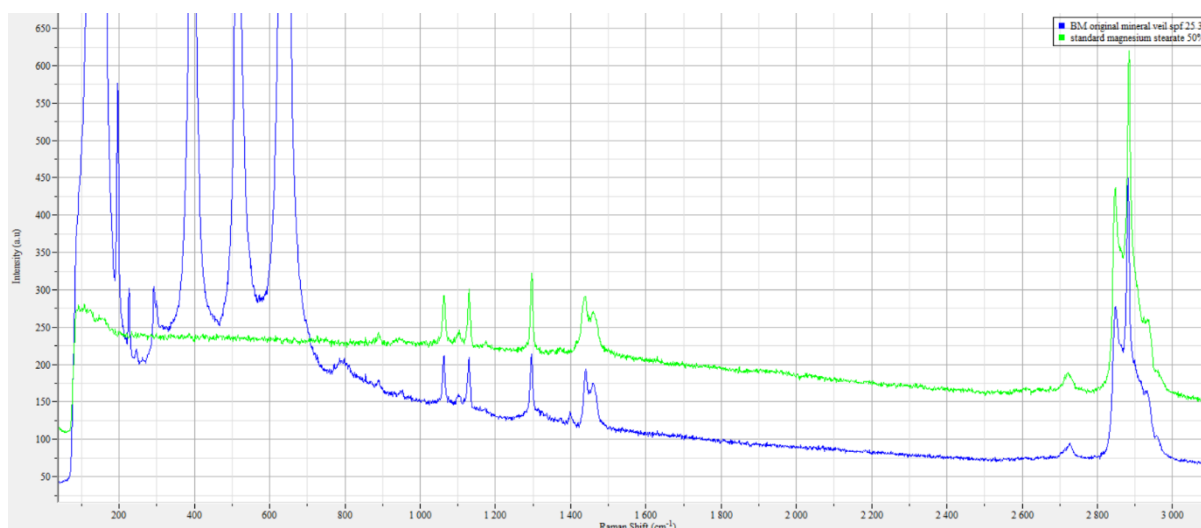


Figure 4.20: Raman spectra (unprocessed but stacked for clarity) of BareMinerals Original Mineral Veil SPF 25 and the reference standard for magnesium stearate, showing multiple similarities between the two.

4.3.3.5 Bronzers

The most notable spectral difference between replicates taken from the BareMinerals Ready Bronzer in shade “*The High Dive*”, is the peak centred at 1365 cm^{-1} (Figure 4.21). As previously discussed in Section 4.3.3.2, the CH_3 symmetrical bending vibration¹⁰ often occurs at $\sim 1365\text{ cm}^{-1}$, which correlates well with the capric/caprylic triglyceride, the lauroyl lysine, and the camellia oleifera seed (tea seed) oil components of this pressed powder. The ingredients list also features boron nitride, a component widely used in cosmetic formulations as an adherent, binder, opacifying agent and texture modifier. Boron nitride is not listed in Socrates Group Frequencies, however, a search of the literature revealed that the hexagonal ordered polymorph exhibits a strong peak at 1366 cm^{-1} , also making it a candidate for the peak seen in this region¹³.

Besides this peak intensity difference, replicate number 3 shows an additional peak at 200 cm^{-1} . Together with the “*lattice*” peak at 143 cm^{-1} – which is also shared with many other inorganic minerals such as anatase – this suggests the presence of bismuth oxychloride.

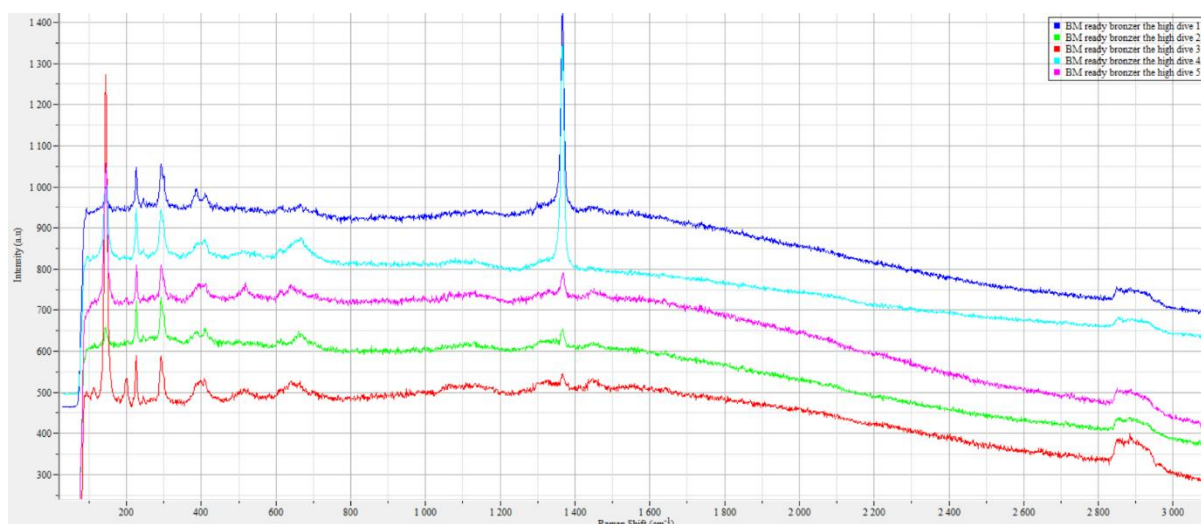


Figure 4.21: Raman spectra (unprocessed but stacked for clarity) of the spectrally heterogeneous bronzer, BareMinerals Ready Bronzer in shade The High Dive.

4.3.3.6 Highlighters

The highlighters not only represented the largest product sample set, but also the most spectrally diverse. Of the 16 highlighters, seven were found to contain anatase, six contained an anatase/rutile mixture, one contained rutile, and two contained no discernible titanium dioxide. In addition to spectral heterogeneity, all the highlighters exhibited visual heterogeneity. Unsurprisingly and consistent with the product's purpose, this was due to the presence of randomly sized, shaped, and distributed reflective metal oxide coated mica flakes.

One of the highlighters found to contain both titanium dioxide polymorphs was MAC Mineralise Skin Finish. Figure 4.22 shows the extent of the spectral heterogeneity across five replicates, and as can be seen, the titanium dioxide polymorph is not the only source of discrepancy across the series. The most significant spectral difference here is seen in the uppermost spectrum (in light blue) and was immediately recognised as being one of the iron oxide minerals, goethite. Goethite, otherwise known as iron oxyhydroxide, is the main component of the natural pigment, brown ochre but when used in its hydrated state ($\text{FeO}(\text{OH}) \cdot n\text{H}_2\text{O}$) becomes yellow (or gold) ochre. Figure 4.23 illustrates how closely the reference spectrum obtained for goethite aligns with that of one of the replicate spectra obtained for the MAC product.

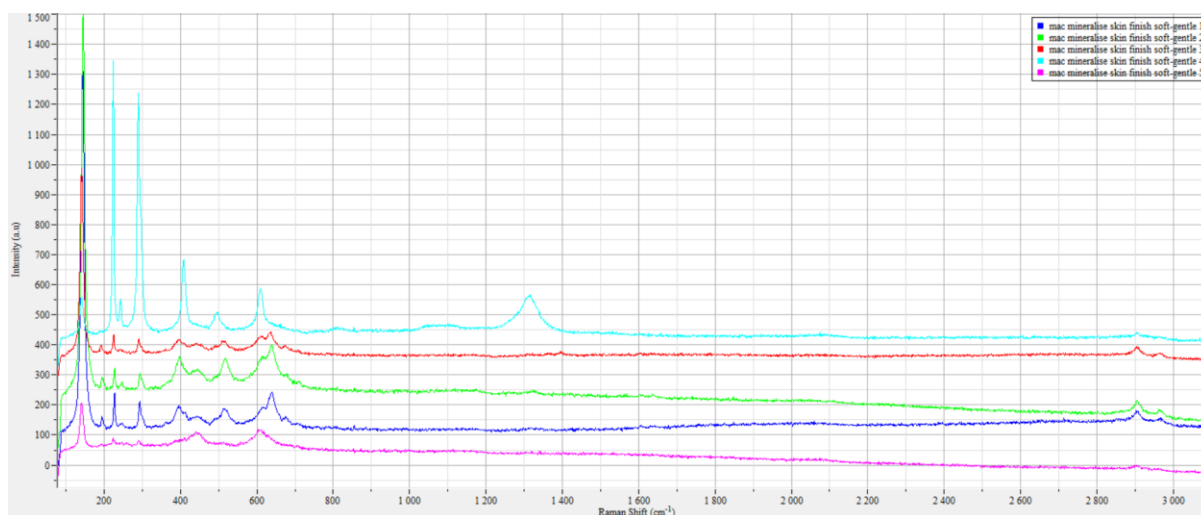


Figure 4.22: Raman spectra (unprocessed but stacked for clarity) of the spectrally heterogeneous highlighter, MAC Mineralise Skin Finish in shade Soft & Gentle, showing multiple differences between replicates.

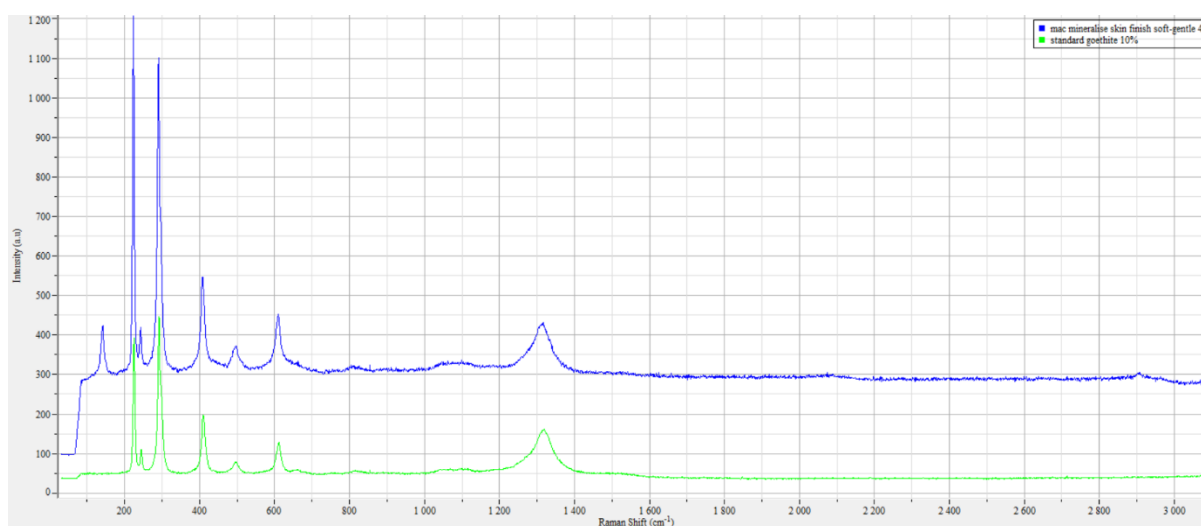


Figure 4.23: Raman spectra (unprocessed but stacked for clarity) of MAC Mineralise Skin Finish in shade Soft & Gentle with the reference standard for goethite.

Two of the highlighters analysed came from the same product: Pixi Glowly Gossamer Duo in Subtle Sunrise. One side of the compact was metallic gold and the other side was metallic rose coloured. It was expected that their pigment types and/or ratios would differ between spectra, however, what was not expected was that they would contain different titanium dioxide polymorphs. Both anatase and rutile are white in colour so the choice is not related to pigment, and it appears odd that a single product with one purpose, presumably made in the same factory at the same time, would be manufactured using different ingredients when this is seemingly unnecessary.

The top five spectra displayed in Figure 4.24 are those taken from the gold side of the compact, and the bottom five spectra are from the rose side. It is evident that whilst the gold highlighter uses a mixture of the two polymorphs, the rose highlighter has no anatase in its formulation. This is confirmed by the absence of the intense “*lattice*” peak at 143 cm^{-1} . These two series of replicate spectra also indicate the extent to which the spectral heterogeneity amongst product types could vary; the rose highlighter replicates are very similar with the exception of some minor peak intensities, whereas the gold highlighter exhibits some obvious peak differences, mainly owing to the titanium dioxide polymorph used.

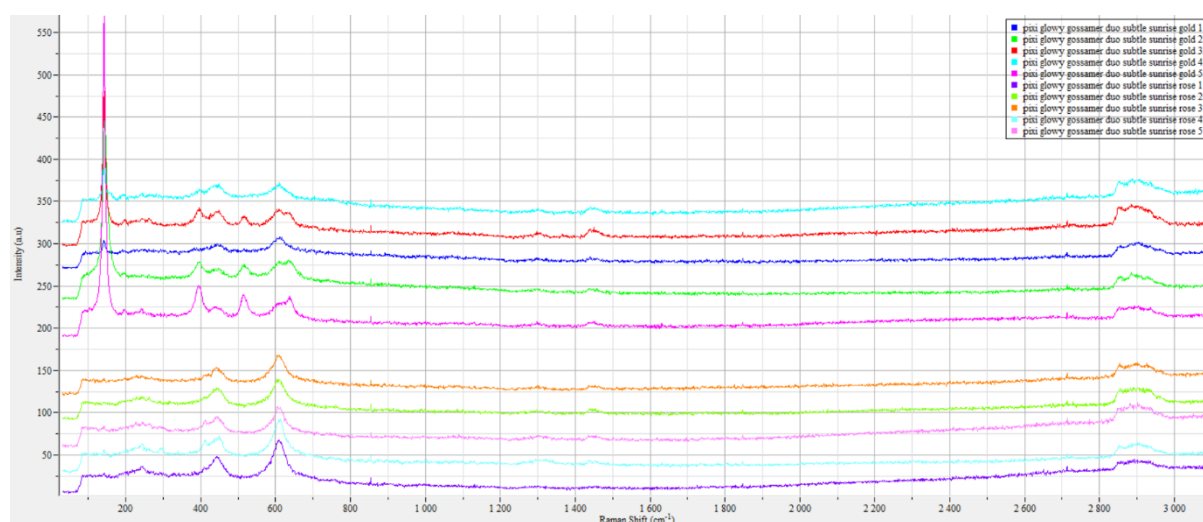


Figure 4.24: Raman spectra (unprocessed but stacked for clarity) of Pixi Glowly Gossamer Duo in shade Subtle Sunrise, the top five replicate spectra relate to the gold-coloured variant and the bottom five replicates relate to the rose-coloured variant.

4.3.3.7 Primers

The Rimmel Fix and Protect Primer exhibited excellent homogeneity across four of the five replicates, both in terms of peak intensities and also the overall background shape. However, as can be seen in Figure 4.25, one of the replicate spectra shows an additional peak at 549 cm^{-1} , which could be attributed to yellow iron oxide pigment¹⁰, and a change in the trajectory of the background. This sample has therefore been classified as spectrally heterogeneous.

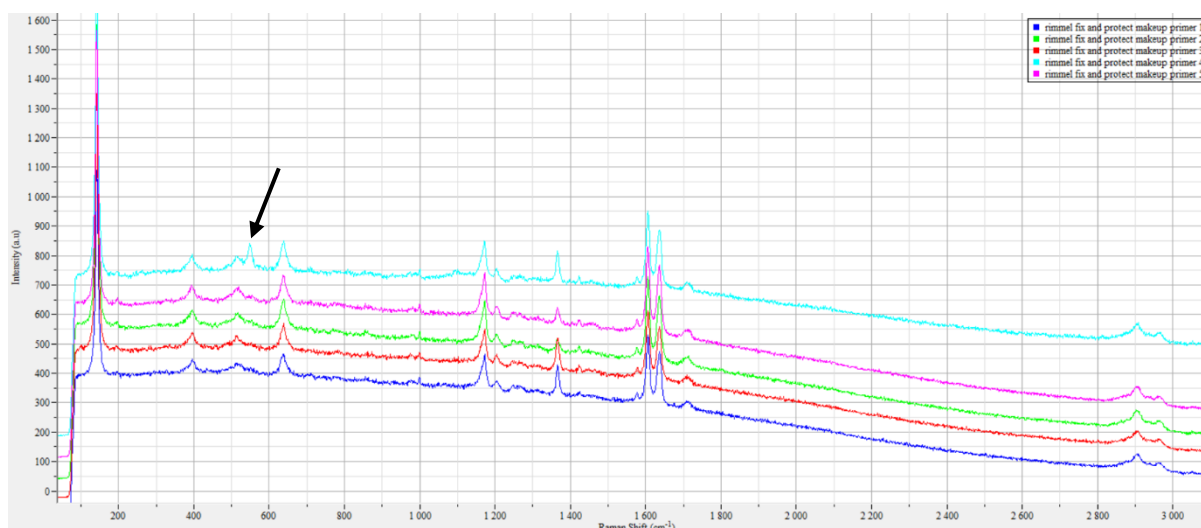


Figure 4.25: Raman spectra (unprocessed but stacked for clarity) of the spectrally heterogeneous Rimmel Fix and Protect Makeup Primer (no shade specified), showing a peak at 549 cm^{-1} (marked with the black arrow) in one of the replicates.

4.3.4 Exploratory data analysis

Notwithstanding the utility of visual spectral comparisons, the ultimate goal across this thesis was the chemometric differentiation of samples. The application of chemometric methods to the analysis of highly variable and quantitative data is superlative to subjective visual comparisons for the many reasons discussed in Chapter 2.

The five replicate Raman spectra for each sample were imported into the Unscrambler software as described in Chapter 2. Inspection of the explained variance plot was used to assess the optimum number of principal components (PCs) (Figure 4.26). Typically, between 3-5 PCs is reported in the literature as suitable for ensuring the data structure is modelled sufficiently, without introducing error due to noise^{14,15}. Nevertheless, for highly variable samples or a large number of samples, it may be suitable to include more PCs than this¹⁶. As predicted, the explained variance plot showed that the samples used in this PCA were not as well described by the model as the spectrally homogeneous ones (Chapter 3). The “elbow” in the trendline (Figure 4.26) indicated that three PCs would be sufficient to model the data structure, as this accounted for 86% of the total variance. However, upon viewing the loadings plots, it became clear that the first seven PCs (95% of the total variance) all carried useful chemical meaning (as evidenced by clear, well defined spectral peaks rather than the onset of random noise) and were therefore valuable to the model. As was the case in Chapter 3, this complex variance correlates well with the intricacies of the sample set, although not to quite the same extent as with the homogeneous samples. Furthermore, the range of score values across all samples indicated that there was a lot of variability throughout the data.

Nonetheless, the close proximity of the validation variances to the calibration variances is indicative of a robust model.

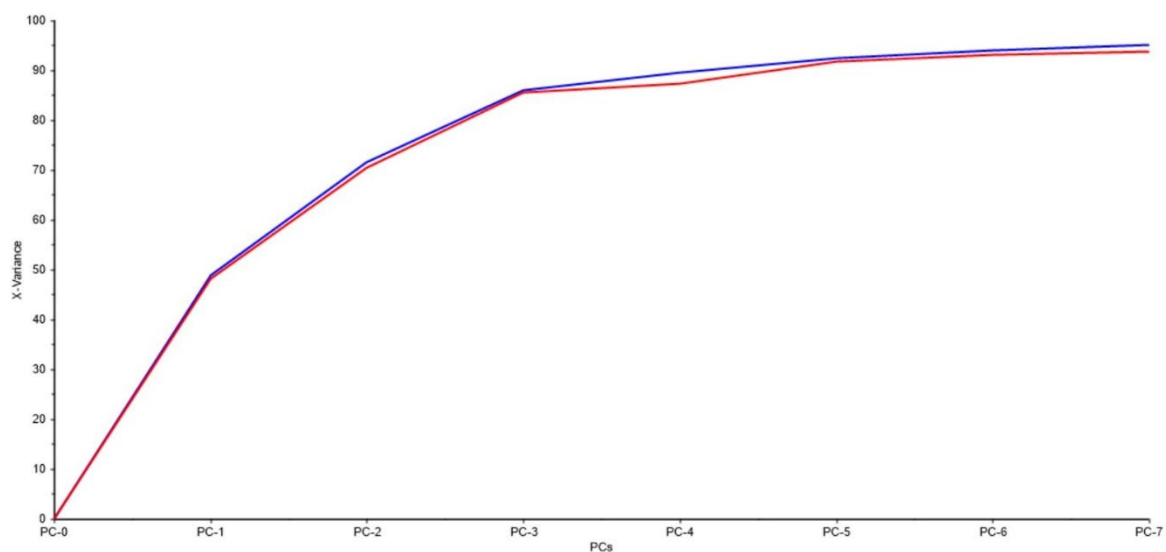


Figure 4.26: Explained variance plot for the spectrally heterogeneous sample set ($n=44$), showing 95% of the model variance accounted for in the first seven PCs, the blue trace denotes the calibration variance, the red trace denotes the validation variance.

Despite there being fewer samples in this data set than in the spectrally homogeneous set, visualisation of trends using a 3-dimensional scores plot was still difficult, so 2-dimensional scores were used. Sample grouping was applied to the scores plot, in the same way as before, according to the category variables: brand, product type, formulation, and shade. Figure 4.27 shows the first scores output from the PCA, with the five replicates across 44 samples representing 220 data points.

What is immediately apparent is that the sample set disproportionately comprises BareMinerals products and that the overall shape of the data swarm is very similar to that of the same plot for the homogeneous samples. This is reassuring and confirms that the chemistry of these samples is also similar. However, the purpose of separating these samples from their spectrally homogeneous counterparts was to assess the level of heterogeneity and, furthermore, compare the subjective selection based on spectral differences, with the chemometric separation seen via PCA.

Sample clustering is evident, for example among the Autograph, Fashionista, and Pixi samples, yet the MAC sample shows obviously disparate scores. The five replicates of this sample appear in all four quadrants of the scores plot indicating that this sample is highly variable and unpredictable. When considering the sample's ingredients, this is to be expected; this product contains several variants of borosilicate glass interference pigments, natural mica

and synthetic mica (fluorophlogopite), both polymorphs of titanium dioxide, and large particles of all three iron oxide pigments. The pressed powder formulation of this product will also contribute to the spectral heterogeneity seen, because the particles tend to be larger in powders than those suspended within a liquid matrix.

Interestingly, the Dermalogica sample's scores are not as disparate as predicted. The replicate spectra were highly variable yet the five replicates are plotted in close proximity in the scores plot (Figure 4.27). This suggests that once dimensionality reduction has been applied, the spectral differences that were thought to be important in terms of repeatability and reproducibility, do not pose so much of an issue. It may be that the chemometric algorithm is assigning less weight to the spectral differences we see, and more to the spectral areas that we assume are unimportant. In other words, PCA may be a workaround technique for the analysis of spectrally heterogeneous samples.

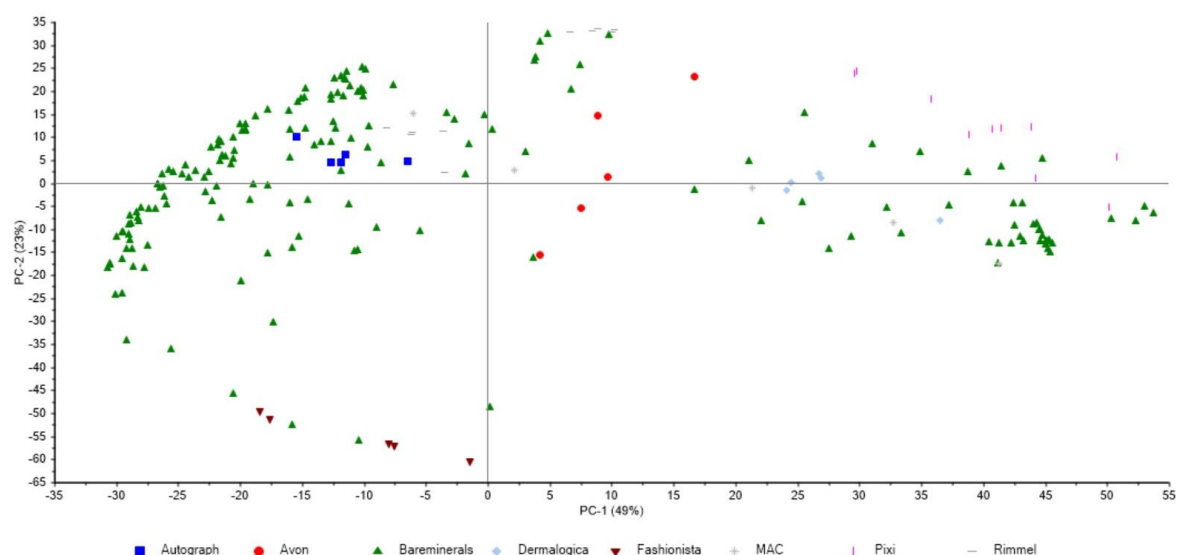


Figure 4.27: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous samples, grouped by brand.

From the loadings plot for PC1 (Figure 4.28) we can see that there is less emphasis on anatase content among this sample set, as the typical triplet pattern seen at 395 cm^{-1} , 515 cm^{-1} , and 637 cm^{-1} is less pronounced. Instead, the loadings plot for PC1 appears to comprise a mixture of anatase and rutile. Again, this is unsurprising as the distribution split between the titanium dioxide polymorphs was approximately half and half. Figure 4.29 clearly shows that samples with a positive score along PC2 can be expected to contain the rutile titanium dioxide polymorph and a higher concentration of iron oxide pigment. Samples with a negative score along PC2 will display a characteristic peak in the 1400 cm^{-1} wavenumber region.

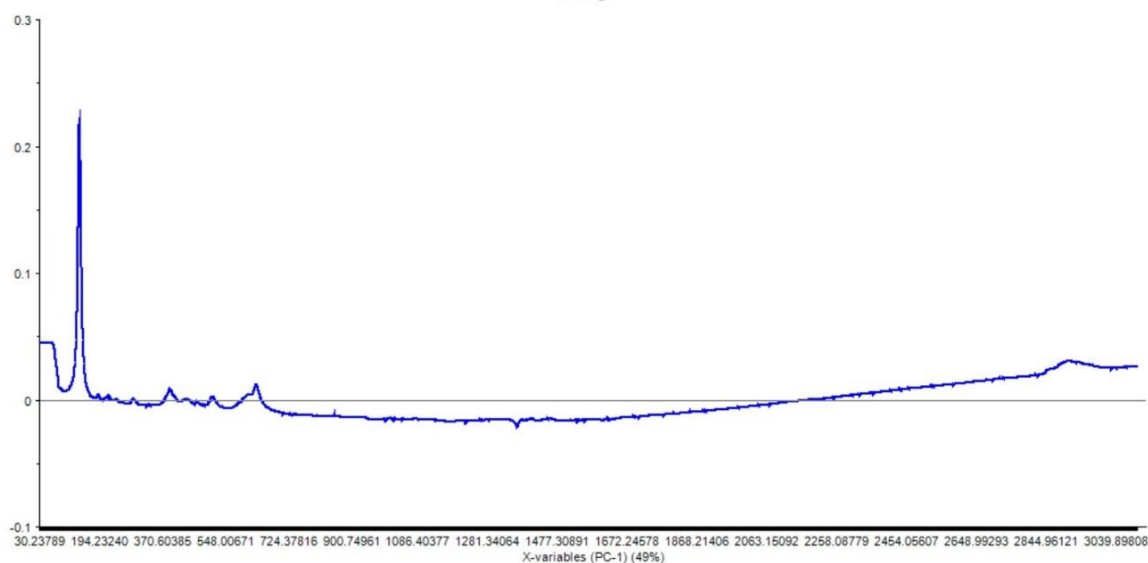


Figure 4.28: PCA loadings plot for PC1 of all the spectrally heterogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading.

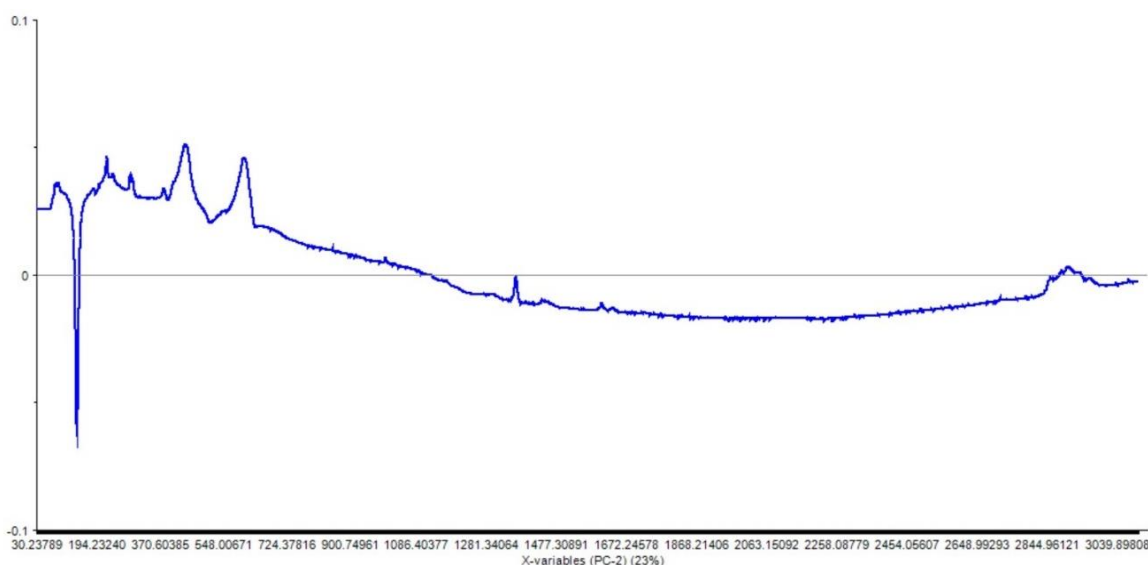


Figure 4.29: PCA loadings plot for PC2 of all the spectrally heterogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading.

When plotting PC1 against PC3 we see different sample separation (Figure 4.30). All but two of the Pixi samples now occupy their own space in the bottom right corner of the scores plot. The loadings for PC3 (Figure 4.31) indicate that samples with positive scores will have a “lattice” peak and likely contain anatase, while those with negative scores will have rutile, a characteristic doublet in the 1400 cm^{-1} region, and a response in the CH stretching region around 2900 cm^{-1} . A sample with a markedly low score on PC3, appearing around -40 in the scores plot (Figure 4.30) is the Fashionista finishing powder. Inspection of this sample’s

replicate spectra reveal it does indeed contain rutile and have both a response in the CH region and a characteristic doublet at 1367 and 1395 cm^{-1} , which is most likely attributable to aluminium oxide (Figure 4.32).

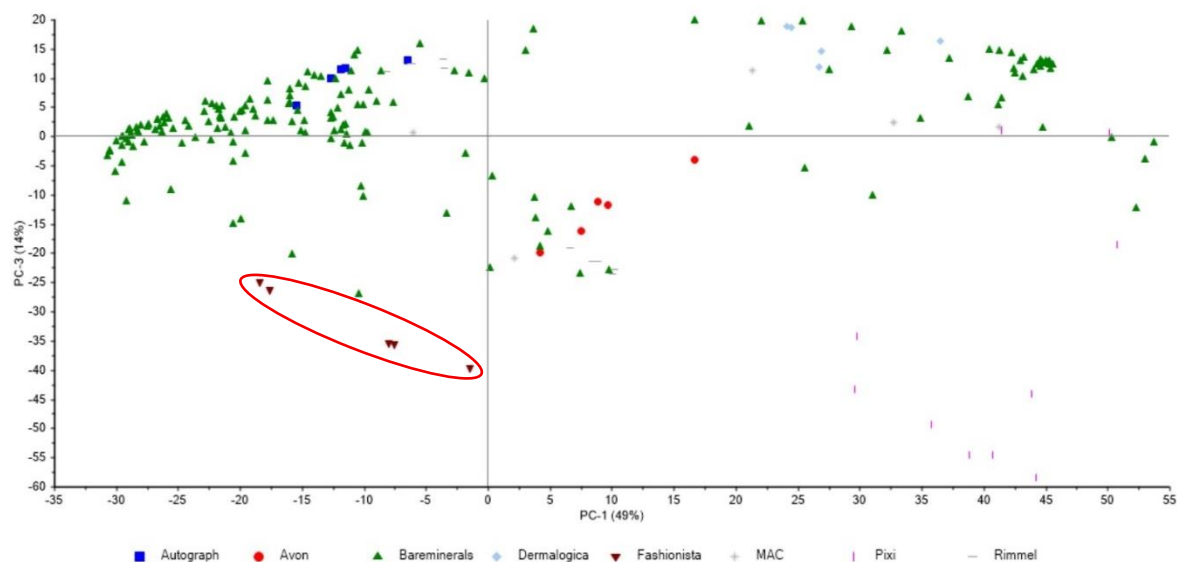


Figure 4.30: PCA scores plot (PC1 vs. PC3) of all the spectrally heterogeneous samples, grouped by brand. Replicate spectra for the Fashionista product highlighted with the red ellipse.

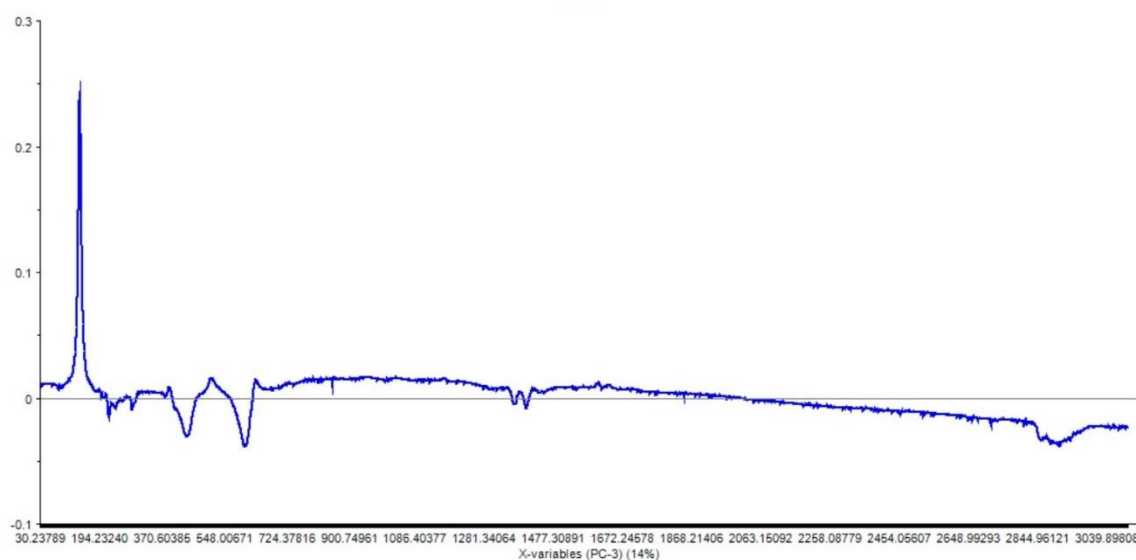


Figure 4.31: PCA loadings plot for PC3 of all the spectrally heterogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading.

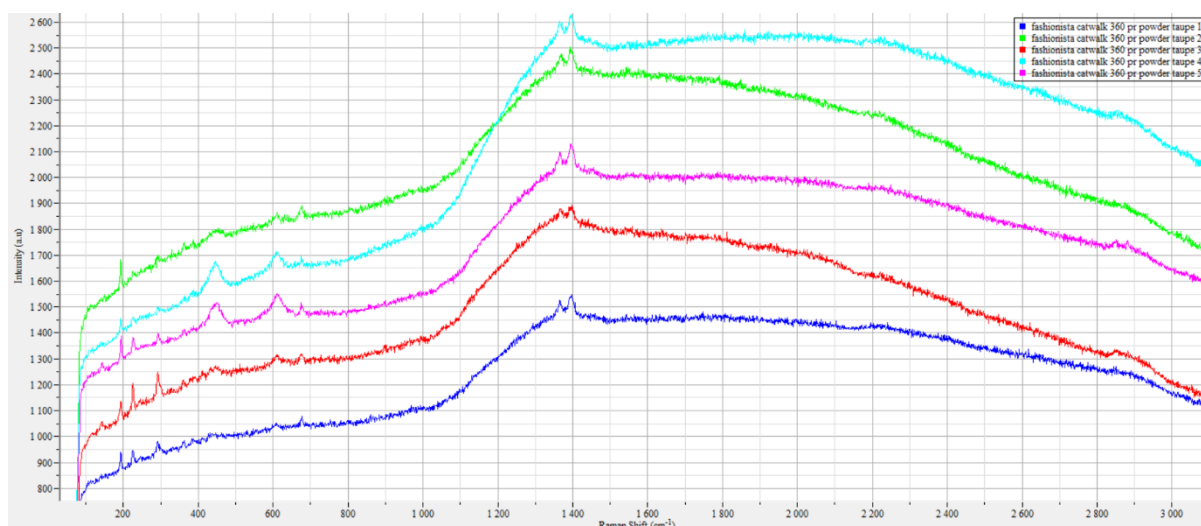


Figure 4.32: Raman spectra (unprocessed but stacked for clarity) of Fashionista Catwalk 360 Pressed Powder in shade Taupe, a spectrally heterogeneous finishing powder that contains rutile titanium dioxide, aluminium oxide, and an organic component in the CH stretching region at around 2900 cm^{-1} .

The scores plot for PC1 against PC4 noticeably separates the Avon sample out from the rest of the set, with a high positive score along PC4 (Figure 4.33). Referring to the loadings for PC4 (Figure 4.34), it can be seen that although it also displays a doublet in the 1400 cm^{-1} region (as previously seen in Figure 4.31), the peaks now extend into the positive region of the loadings, the baseline is much flatter and there is a higher proportion of iron oxide pigment compared to titanium dioxide pigment. Given that titanium dioxide is a white pigment, we would expect this product to be darker in colour. The Avon sample was Ideal Flawless Foundation in shade Nutmeg and was the darkest coloured product across the entire spectrally heterogeneous and homogeneous sample sets, consistent with this observation.

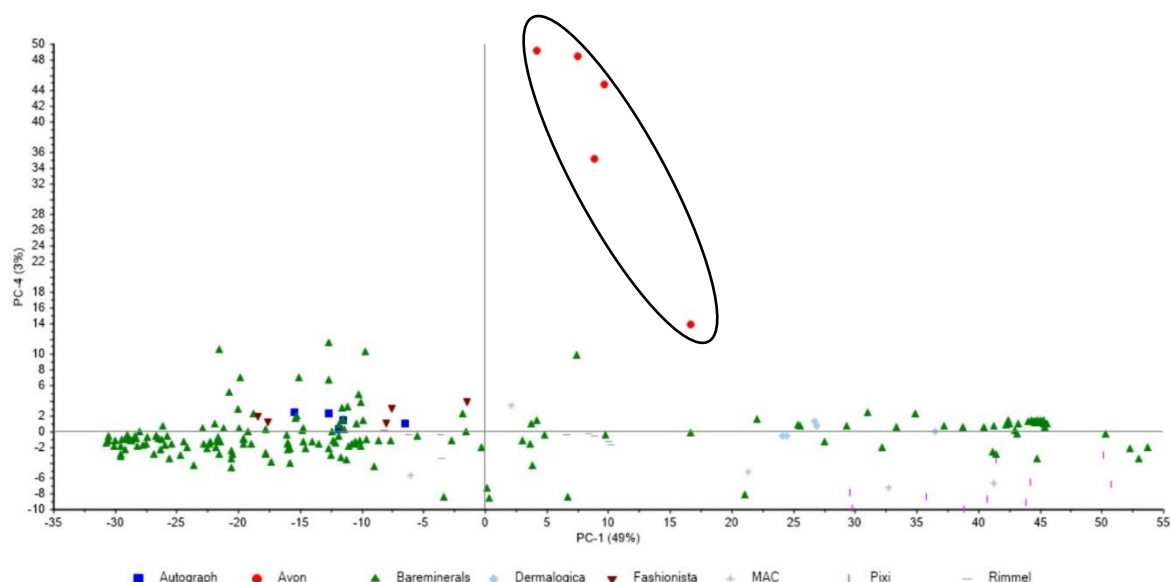


Figure 4.33: PCA scores plot (PC1 vs. PC4) of all the spectrally heterogeneous samples, grouped by brand. Replicate spectra for the Avon product highlighted with the black ellipse.

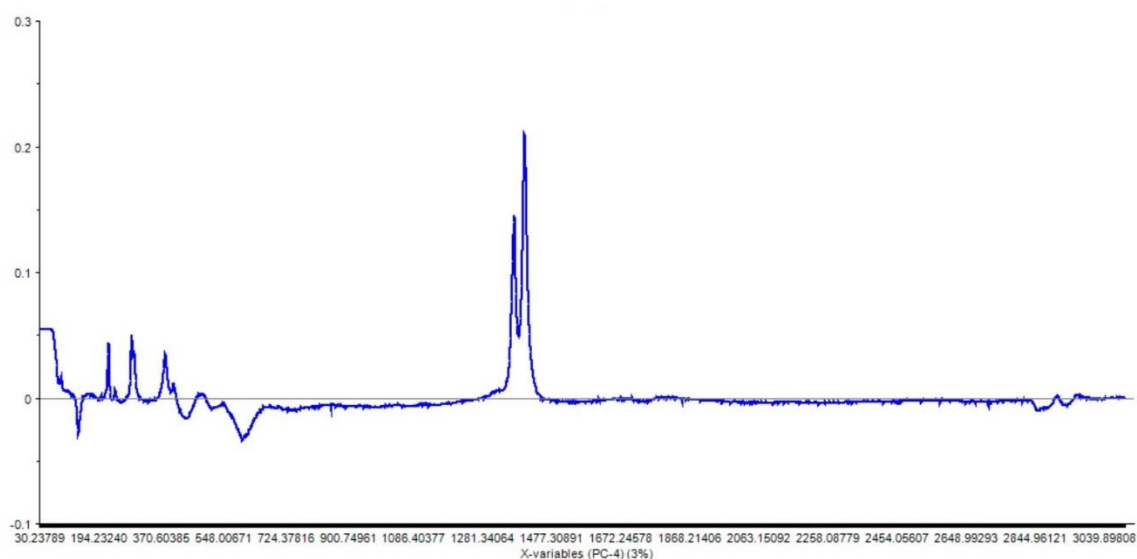


Figure 4.34: PCA loadings plot for PC4 of all the spectrally heterogeneous samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading.

In addition to inspection of the scores plots, the influence plots highlight which samples have high leverage, or are highly influential to the model. Inclusion of these “*extreme*” samples helps build a general model, whereas inclusion of only “*typical*” samples would build a robust model. The influence plot for all samples, grouped by brand, is given in Figure 4.35. In contrast to what was seen with the spectrally homogeneous samples, only a single replicate from one spectrally heterogeneous sample appears in the top right box (the area where samples are usually classified as outliers). It was assumed that due to the higher spectral variability and

lower explained variance scores that there would be many samples poorly described by the model, but this was not the case. This indicates that the model is performing better than expected given the lack of spectral repeatability, and suggests that spectral heterogeneity is not as much of an issue when chemometric techniques are used to analyse the data. As before, no samples were removed from the model as outliers; all spectra were verified as being accurate representations of the sample.

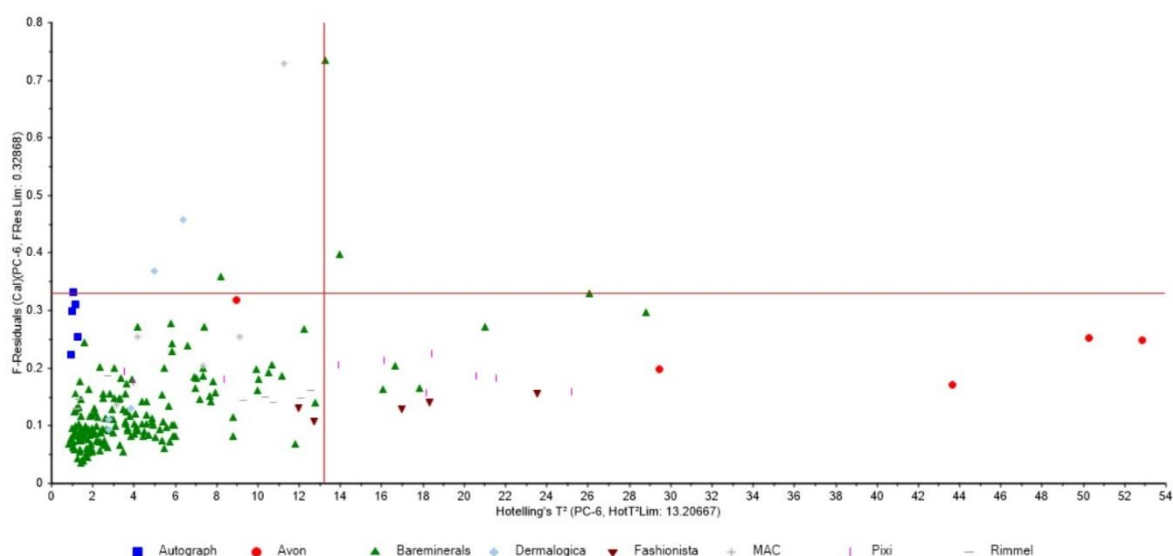


Figure 4.35: Influence plot (PC6) of all the spectrally heterogeneous samples, grouped by brand.

The PCA scores were then repeated to sample group by product type. This time, some tentative trends were observed in the scores plot: the foundations, concealers, primer, and tinted moisturiser were all positive or slightly negative along PC2, i.e. appeared in the top half of the plot, and all the bronzers had negative scores along PC1, i.e. they appeared in the left half of the plot (Figure 4.36). No obvious trends were identified when the samples were grouped by formulation, as is evident in Figure 4.37, and only a weak correlation was seen when samples were grouped by shade (Figure 4.38). In this case, the light coloured and translucent samples grouped at the top of the scores plot, i.e. positive PC2 values, which correlate with a higher titanium dioxide content. This finding reveals that the product type is the most important factor for sample clustering, rather than the product's brand, formulation, or shade.

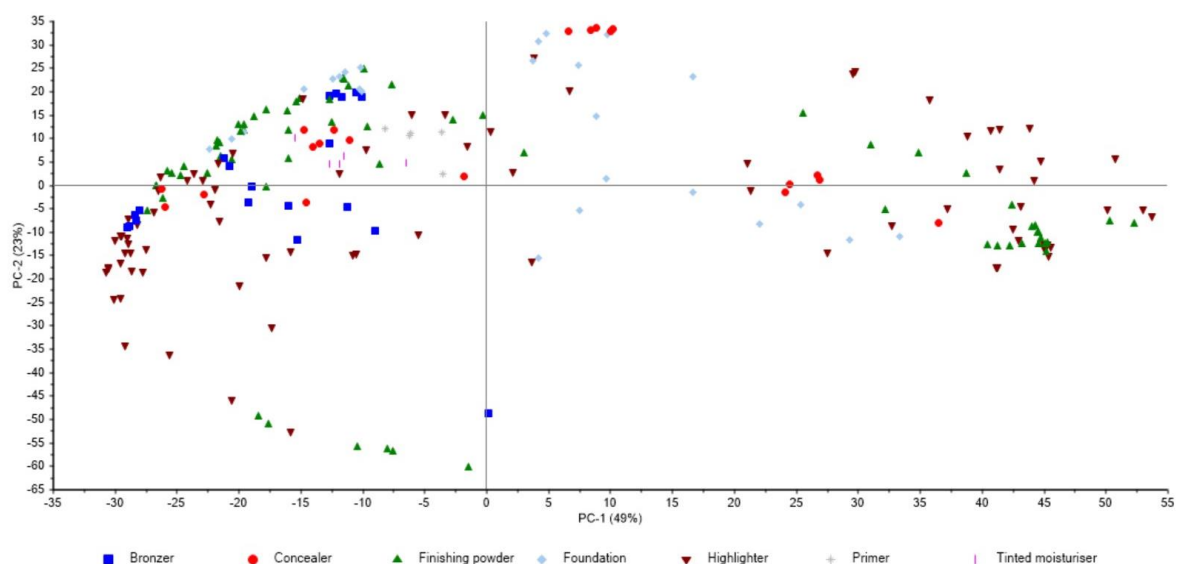


Figure 4.36: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous samples, grouped by product type.

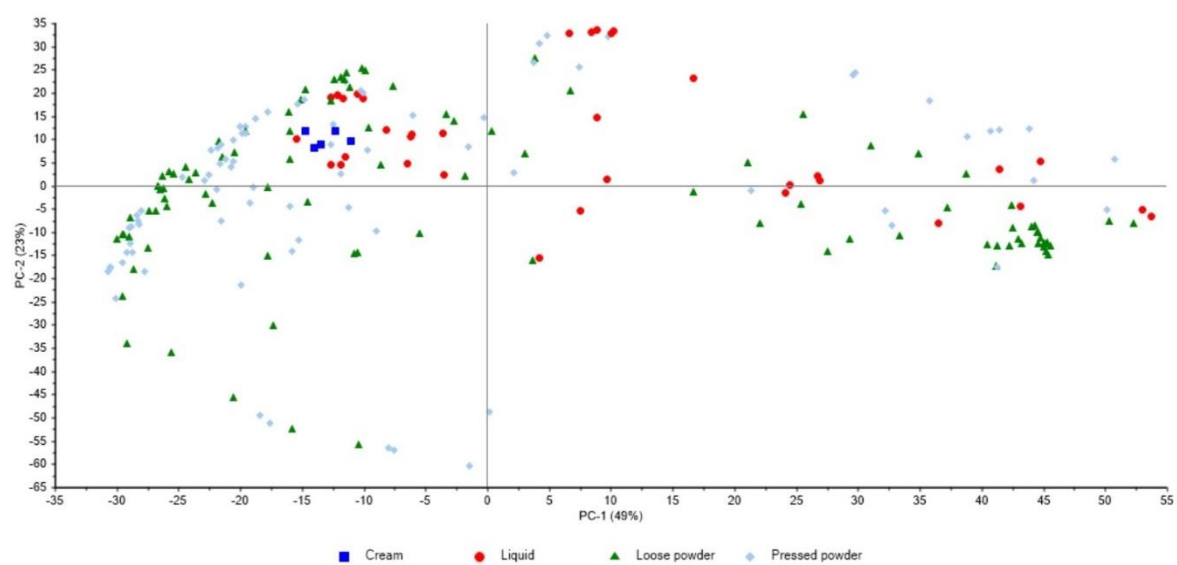


Figure 4.37: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous samples, grouped by formulation.

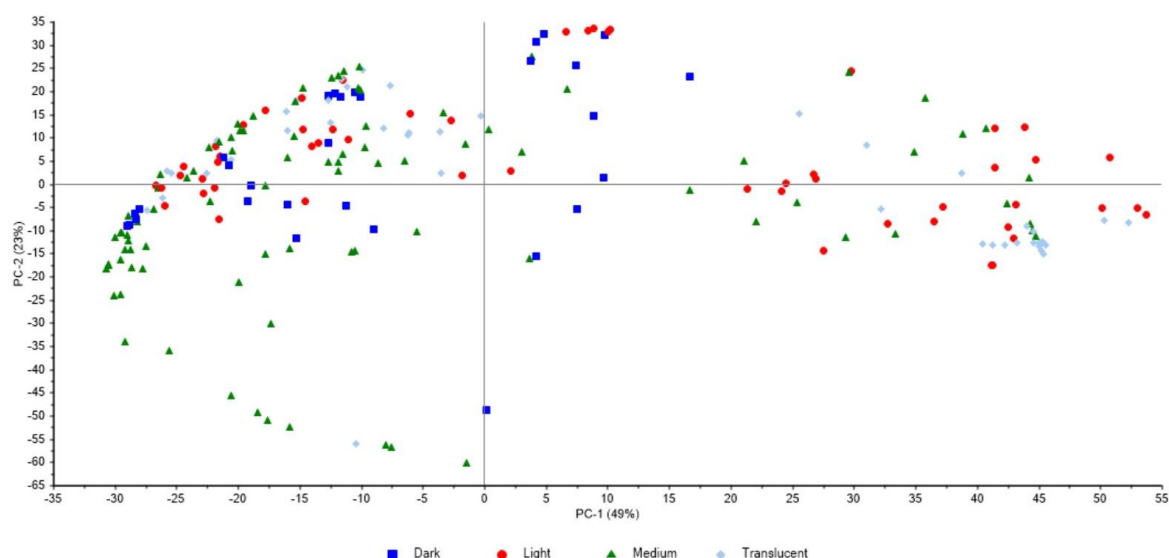


Figure 4.38: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous samples, grouped by shade.

It was decided that due to the limited number of samples in each product type category, the PCA would not be broken down further into a discussion of every type. However, given that the BareMinerals products made up 35 out of the 44 products in this sample set, a separate PCA was performed on this brand and revealed a better cumulative explained variance (Figure 4.39).

The scores were grouped by formulation and trends observed. In general, the cream and liquid formulations appeared in the top half of the plot, i.e. had positive scores along PC2, whereas the loose and pressed powders were scattered over the whole of the plot. Nonetheless, the majority of the pressed powder samples clustered in the left half of the plot, i.e. negative scores along PC1 and, with the exception of a single pressed powder sample, only loose powders appeared in the far right of the plot (positive PC1 scores).

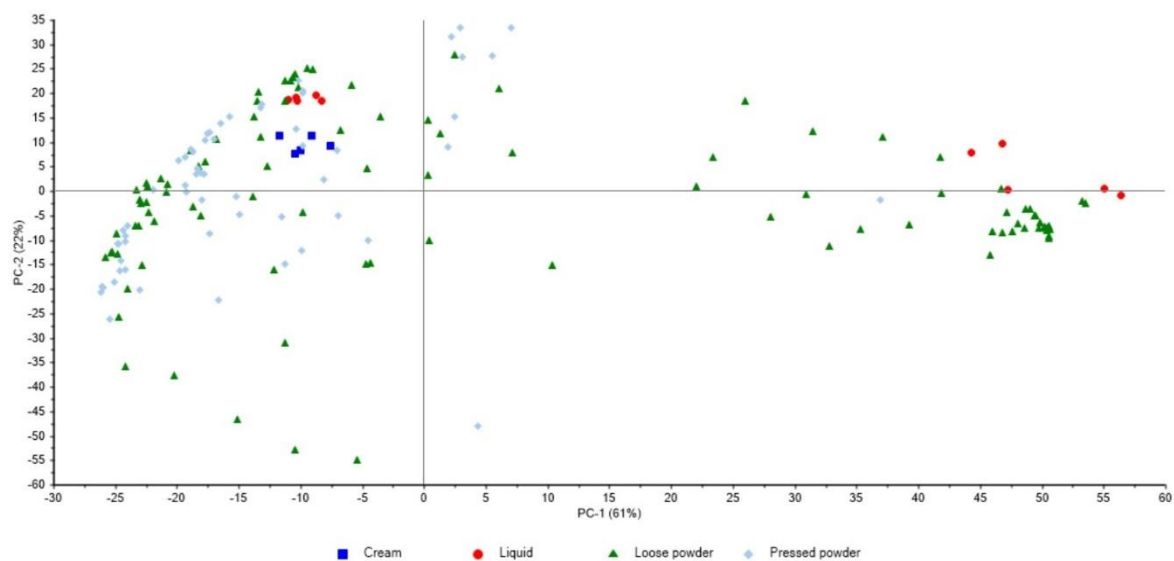


Figure 4.39: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous BareMinerals samples, grouped by formulation.

When grouping the BareMinerals samples by product type further clustering was identified, as can be seen in Figure 4.40. The concealers and bronzers tended to have a negative score along PC1 and the foundations had largely positive scores along PC2. The most “extreme” samples, unsurprisingly, were the highlighters. A similar trend was observed when the scores plot was sample grouped by shade, with the darker samples clustering in the same region as the bronzers. This is to be expected as the bronzers were all darker shades than the other products in this sample set.

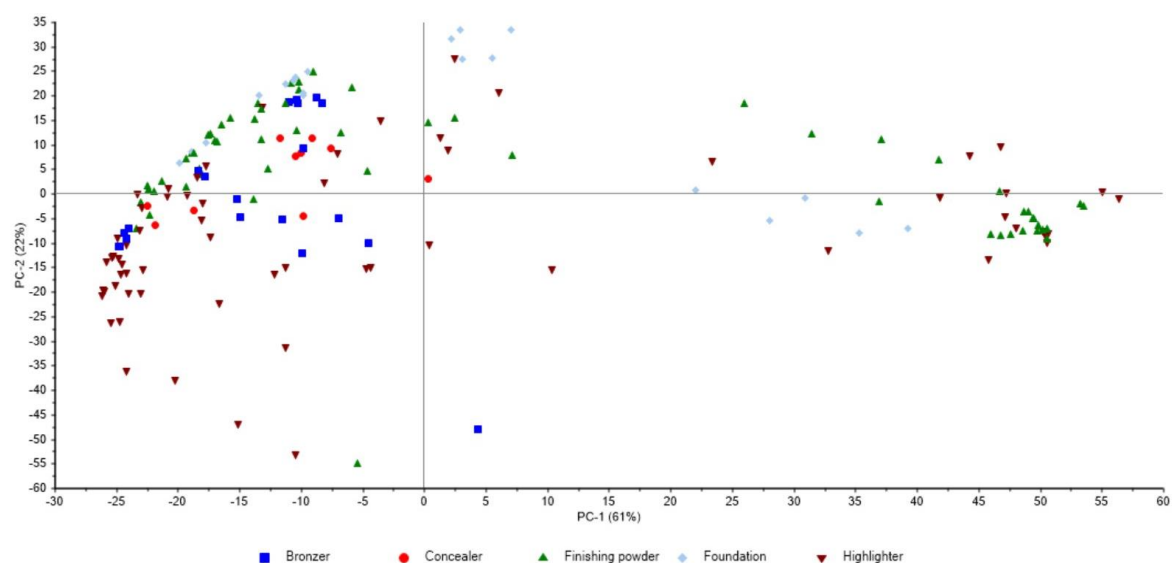


Figure 4.40: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous BareMinerals samples, grouped by product type.

Two of the seven product types included in this sample set, namely finishing powders and highlighters, contained more products than the other categories and in sufficient number to perform a PCA. Figure 4.41 shows the PCA scores plot for the 13 spectrally heterogeneous finishing powders with a sample grouping by brand. With the exception of a single outlying BareMinerals replicate at around -65 along PC2, it is clear there is a distinction between the two brands represented here. Nonetheless, when the same data was sample grouped by formulation (Figure 4.42), it confirmed that this separation seen was not due to the samples' formulations, as pressed powders feature across both brands. The explained variance for the first two PCs of this sample subset (90%) was slightly better than that of the main model (83%). This is to be expected as there is inherently less variability between products with a similar purpose and therefore, a similar chemical composition.

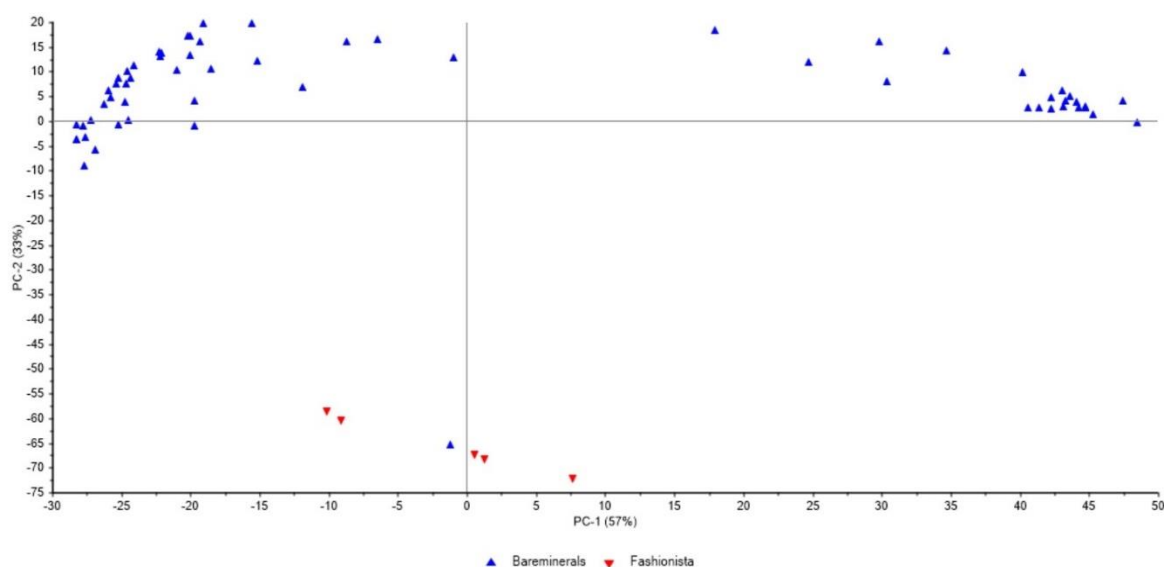


Figure 4.41: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous finishing powders, grouped by brand.

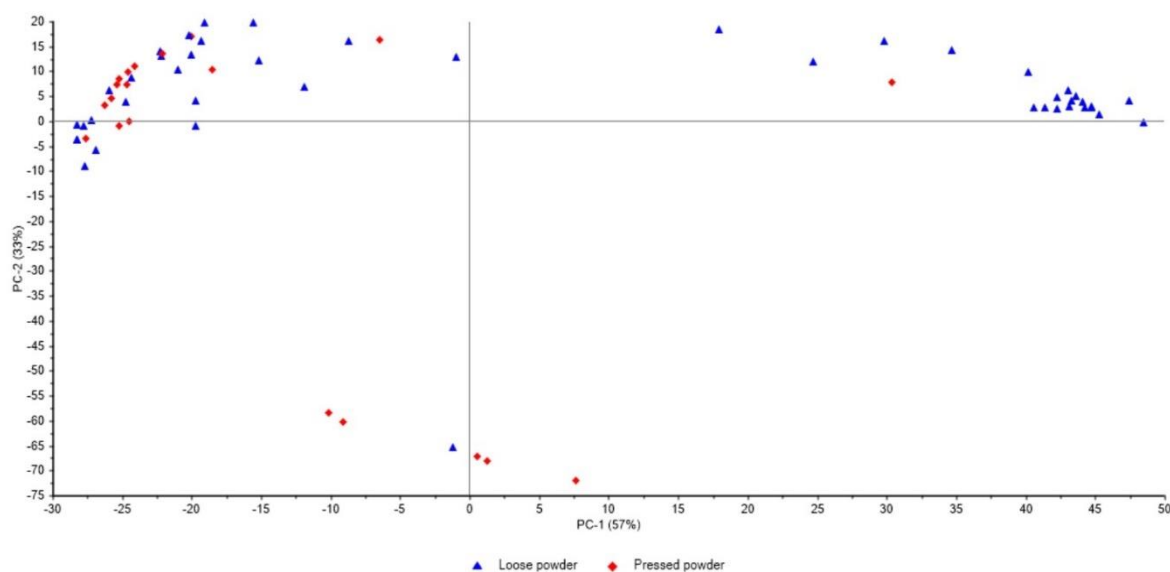


Figure 4.42: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous finishing powders, grouped by formulation.

The 16 spectrally heterogeneous highlighters were also subjected to their own PCA, as there were sufficient samples represented in this category for a PCA to be meaningful. Interestingly, the explained variance for the first two PCs (82%) was very slightly worse than that of the overall model. This reveals that there is more variability amongst these samples, which is not surprising given the lack of repeatability seen amongst the replicate spectra. Furthermore, the accuracies for the validation do not follow the calibration as closely as those of the other PCA models. This indicates that the model is not as robust because it is not able to predict an unknown sample as effectively.

The PCA scores were firstly sample grouped by brand (Figure 4.43). Whilst the Pixi highlighters were well discriminated from the BareMinerals highlighters, the MAC highlighter was too heterogeneous to permit separation. It is even more apparent in this subsample scores plot than the overall scores plot, just how diverse the repeat spectra were for this sample. No obvious trends were observed when this PCA was sample grouped by formulation, however, a trend can be seen when sample grouping by shade. Figure 4.44 demonstrates how the lighter coloured and translucent highlighters generally have positive scores along PC1, whereas the medium coloured (darker) highlighters tend to have negative scores along PC1.

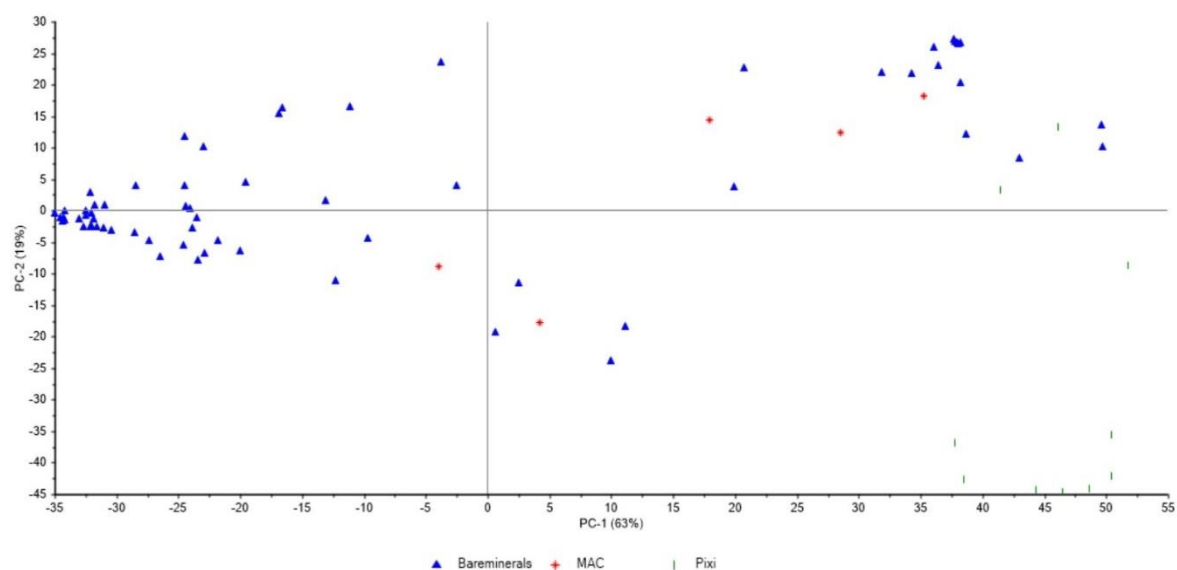


Figure 4.43: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous highlighters, grouped by brand.

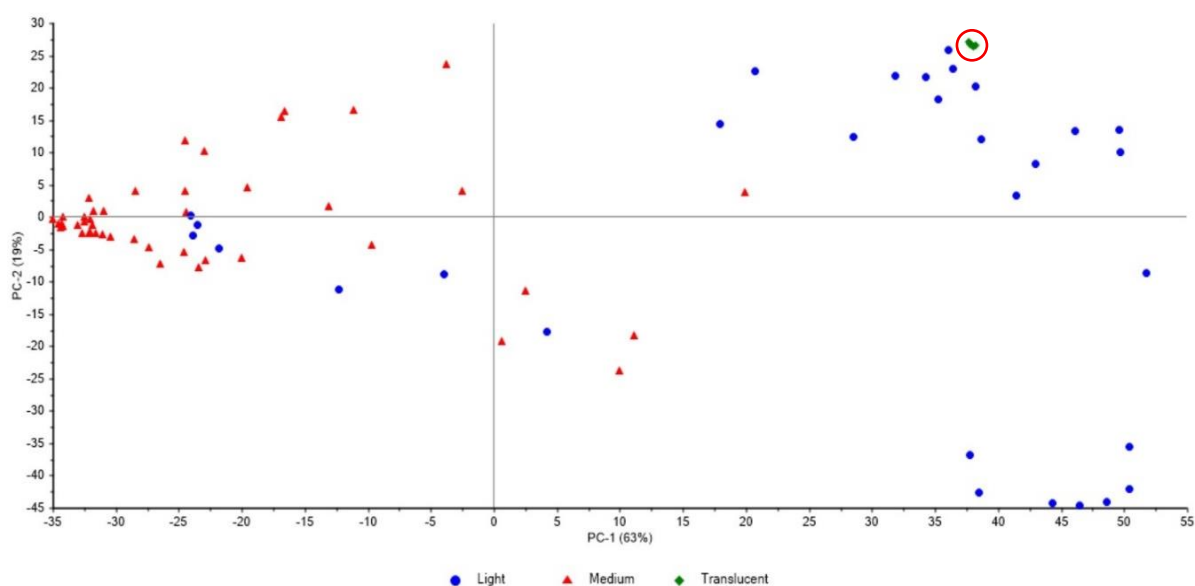


Figure 4.44: PCA scores plot (PC1 vs. PC2) of all the spectrally heterogeneous highlighters, grouped by shade. Replicate spectra for the BareMinerals translucent product highlighted with the red circle.

Also worthy of note is the translucent highlighter sample indicated by the green diamonds in Figure 4.44. These five replicates unexpectedly group together extremely closely. This sample was designated as heterogeneous due to the peak intensity disparities between replicate spectra, as can be seen in Figure 4.45. Not only are there differences in peak intensities, there are also some peaks which feature in certain replicates but not in others. For example, the peaks at 800 cm^{-1} and 1300 cm^{-1} are present in two of the spectra but absent in the other three.

These combined differences were sufficient for this sample to be classified as spectrally heterogeneous. However, as is evident from the subsequent PCA, these differences are clearly not as important to the final model.

It is assumed that this is due to the dimensionality reduction step of the PCA, which deduces the underlying directions of maximum variance^{14,17}. The creation of the PCs determines which variables are correlated, i.e. which variables are covariate, (something that is not readily assessed by eye) and hence reveals the hidden data structure. Therefore, the number and magnitude of the spectral differences discernible by eye do not necessarily correlate with how the PCs are calculated. In this case, visual inspection of the spectra in Figure 4.45 is accentuating the *intra*-sample variation without considering the *inter*-sample variation of the entire dataset, thus placing too much emphasis on spectral differences on a subjective level.

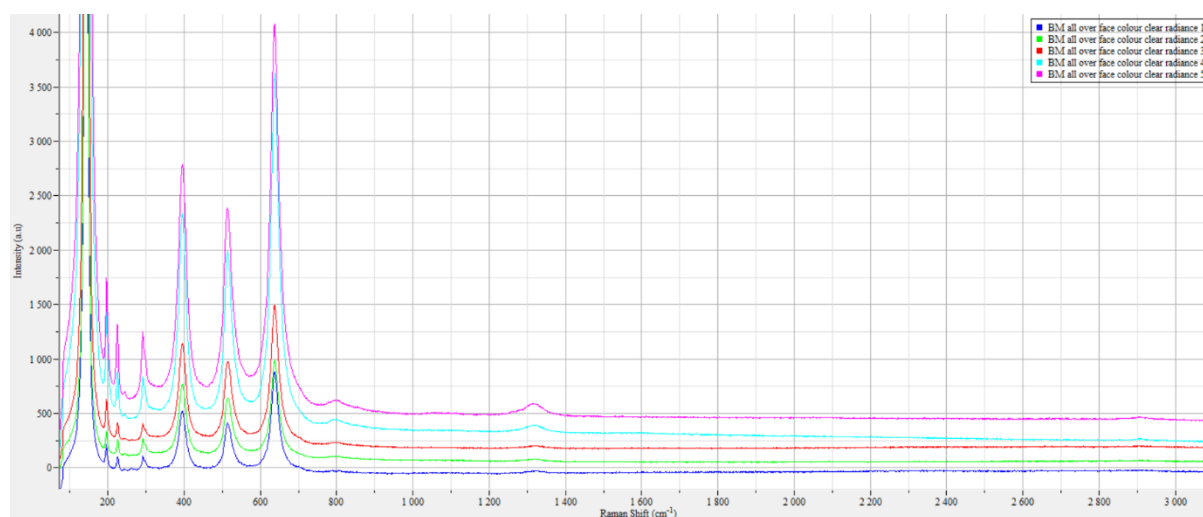


Figure 4.45: Raman spectra (unprocessed but stacked for clarity) of BareMinerals All Over Face Colour in shade Clear Radiance, showing differences in peak intensities between the five replicates.

4.3.5 PCA projection

In order to see how this set of 44 spectrally heterogeneous samples compared to the 126 spectrally homogeneous samples analysed in Chapter 3, a PCA projection was performed. This would not only assess the robustness of the Chapter 3 model and enable outlier detection, but also determine whether or not these spectrally heterogeneous samples could be considered part of the same sample population.

The PCA model created for the spectrally homogeneous samples from Chapter 3 was used as the basis upon which the spectrally heterogeneous samples were projected. The spectrally heterogeneous sample matrix was transformed using the loading vectors assigned to the original homogeneous model and a new set of scores were generated. Initially the PCA of all

the heterogeneous samples (Section 4.3.4) showed explained variance values of 49% for PC1 and 23% for PC2. However, once these samples were projected onto the homogeneous model, the explained variance values dropped to 44% for PC1 and 13% for PC2, indicating that this model did not describe the variance in the heterogeneous dataset as well. This was to be expected as bronzers, highlighters, and primers did not feature in the original homogeneous model. Nonetheless, as can be seen in Figure 4.46 the spectrally heterogeneous samples (green dots) fall in the same general space as the spectrally homogeneous samples (blue dots), with no obvious outliers. Therefore, it can be concluded that these projected samples are from the same population as the calibration samples.

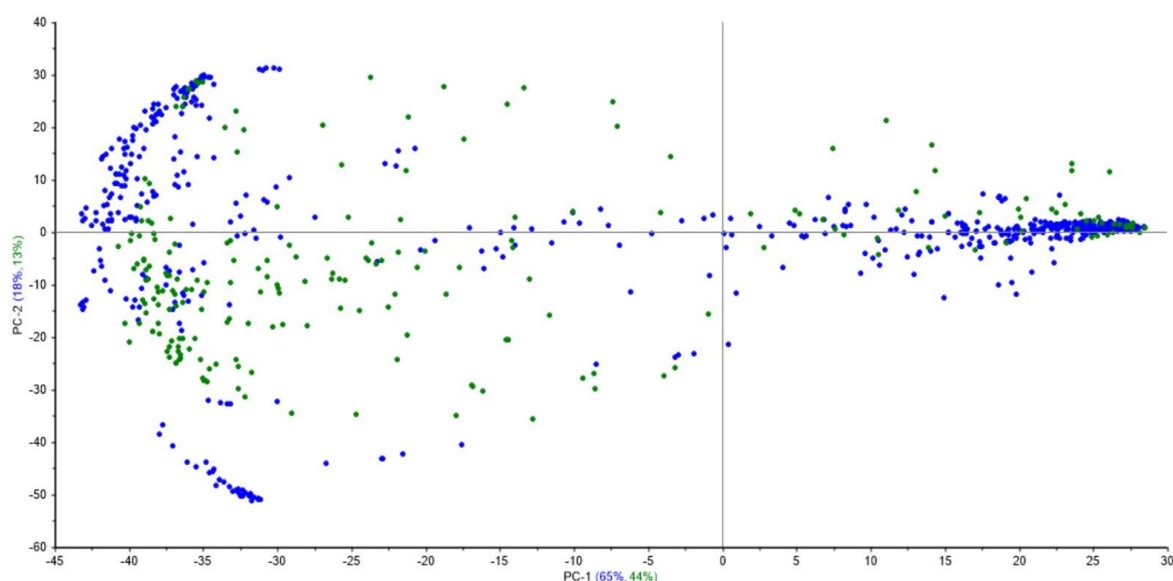


Figure 4.46: PCA projection scores plot (PC1 vs. PC2) of all the spectrally heterogeneous samples (green dots) projected onto the spectrally homogeneous sample model (blue dots).

Whilst it is apparent in Figure 4.46 how the newly projected samples compare to the original samples, it is not possible to visualise trends by brand without further data manipulation. In order to achieve this the data matrices for both sample sets must be amalgamated by exporting the scores values to MS Excel, combining within a single spreadsheet and reimporting back into the Unscrambler. Then a new scatter plot can be generated for the combined sample scores and the sample grouping by brand applied.

Figure 4.47 shows how all the spectrally homogeneous and heterogeneous samples lie in the sample space when grouped by brand. The sample points according to a particular brand that align most closely indicate good sample repeatability and, in general, these were categorised as spectrally homogeneous. However, as was discussed in the previous Section, some of the samples that were categorised as being spectrally heterogeneous also resulted in closely

aligned sample points in the scores plot. This disparity between visual assessment and chemometric analysis not only shows that subjective categorisation can be flawed, but also reveals that true sample variance does not always amount to simple spectral differences.

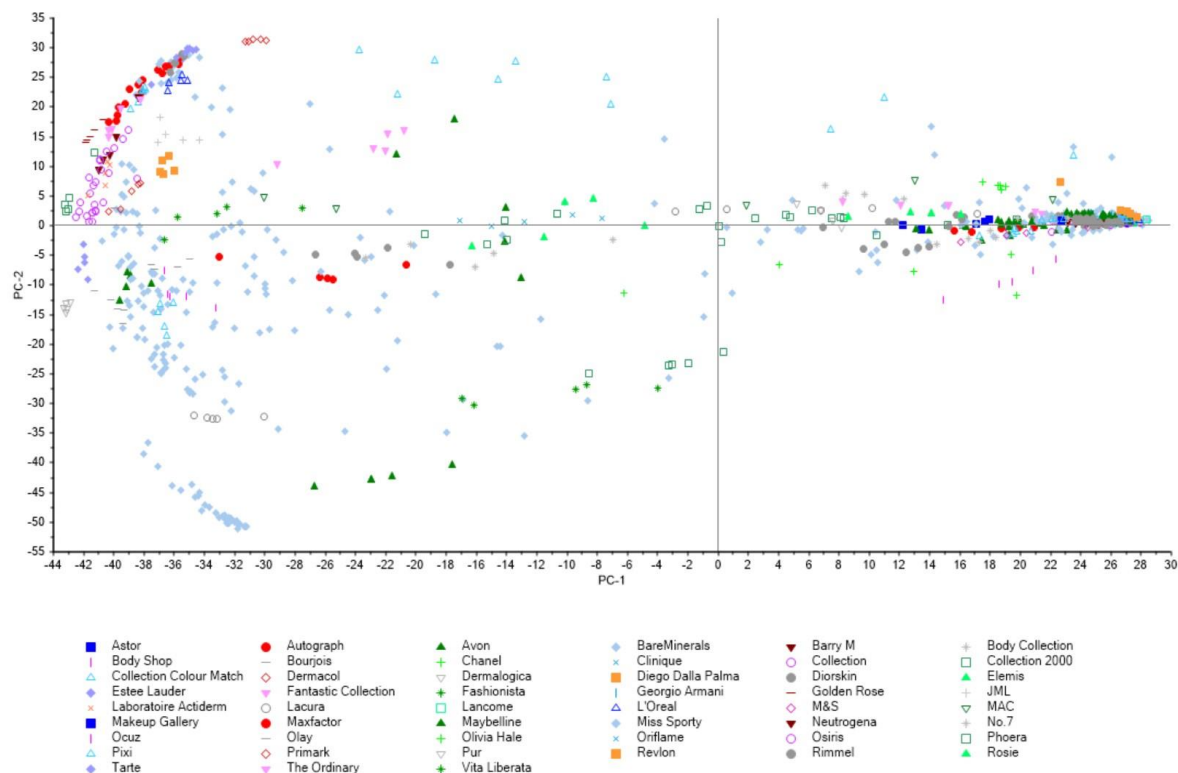


Figure 4.47: PCA projection scores plot (PC1 vs. PC2) of all the spectrally heterogeneous samples projected onto the spectrally homogeneous sample model, and grouped by brand.

4.4 Conclusions

This Chapter sought to characterise the chemical variability amongst a subgroup of the 170 new facial cosmetic samples purchased from the UK market. The 126 spectrally homogeneous samples were discussed in Chapter 3; this Chapter describes the analysis of the 44 spectrally heterogeneous samples.

The samples examined throughout this Chapter spanned 32 different product lines from eight different brands, across a variety of price brackets from budget ranges through to the premium market and a variety of shades. Unlike the samples analysed in Chapter 3 that all derived from four product types, the samples analysed in this Chapter spanned all seven product types, adding bronzers, highlighters and primers to the dataset. Interestingly, no mousse-based products were included in this sample set suggesting that these formulations are inherently spectrally homogeneous. Conversely, 36 of the total number of samples investigated were

pressed or loose powdered products, suggesting the opposite is true of powdered formulations.

Microscopic analysis of all samples revealed the same visual discriminating features as those seen across the samples in Chapter 3 but for the surprising finding that one of the spectrally heterogeneous samples had a visually homogeneous appearance. Again, this reinforces the finding from Chapter 3 that visual assessment of sample homogeneity is not a good indicator of its Raman spectral homogeneity. Visual analysis of the finishing powders in particular, showed a larger number of these to contain spherical particles than was seen across the finishing powders in Chapter 3, suggesting that these microspheres contribute to the spectral heterogeneity seen, presumably due to their light scattering properties.

Raman analysis of the samples revealed less reliance on titanium dioxide than was seen in Chapter 3, meaning the polymorph was not such a discriminating factor. Raman spectroscopy also allowed for the unexpected discovery that one of the five BareMinerals Original Foundation products was a counterfeit product. The degree of spectral heterogeneity among all 44 samples differed dramatically; some samples only exhibited a single extra peak while others displayed entirely different profiles. However, this subjective assessment proved to be unreliable when compared to the PCA results.

The creation of a PCA model revealed a data swarm similar in shape to that of the Chapter 3 model, with loadings for PC1 showing that the titanium dioxide polymorph used across the various samples was still the most discriminating factor, albeit with a lesser emphasis on anatase. Sample grouping within the scores plots affirmed the chemical complexity within the samples, with no clear trends arising for either brand or formulation, and only weak trends identifiable for product type and shade.

In an attempt to further discriminate between samples, PCA models were recalculated for subgroups of the dataset. A PCA model containing BareMinerals products exclusively, revealed that weak trends could be identified according to formulation and product type. A PCA model created for the finishing powders only showed a good brand separation but no formulation separation, while a PCA model for the highlighters showed no clustering by brand or formulation, but a tentative trend when grouped by shade.

A PCA projection model was created by projecting the 44 spectrally heterogeneous samples onto the model created for the 126 spectrally homogeneous samples (the calibration samples). The base model did not explain the variance among the projected samples well; nevertheless, all projected samples fell into the same space as the calibration samples with no outliers, indicating they derive from the same sample population. These findings once again confirmed that the subjective assessment of sample spectral homogeneity/heterogeneity is unhelpful,

and that chemometric techniques are superlative for the objective assessment of chemical variability within a dataset.

4.5 References

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Chapter Five: Investigation into ageing effects amongst facial foundations using optical microscopy, Raman spectroscopy and chemometrics

5.1 Introduction

The aim of this Chapter was to assess the effect of ageing on a specific subset of the spectrally homogeneous foundations, as defined in Chapter 3. The effects of ageing were investigated as both a sample deposited on a slide, and also from aliquots taken from the original packaging once opened. High-definition optical microscopy, Raman spectroscopy, and chemometric analysis were employed for the interrogation of the samples. Trends and sample groupings were explored using the category variables established in previous chapters so that comparisons could be drawn.

5.2 Experimental

The same Raman experimental method described in Chapter 2, and used for the preceding chapters was applied to this study, however, the acquisition of a new comparison microscope during the course of the project enabled far better photomicrograph images to be taken. Therefore, the images obtained for this section of the project utilised the Projectina Vision X comparison microscope rather than the Raman microscope imaging function of the Horiba LabRam.

5.2.1 Samples

Seven samples were chosen to complete the ageing study; these samples were deliberately chosen to fulfil specific criteria. It was decided that in order to keep inter-sample variability to a minimum that all samples should be foundations and not concealers, tinted moisturisers, or any of the other product types. For the same reason, it was decided that they should all exhibit the same formulation. Liquids were chosen as these are one of the most common formulations on the market¹ and because it was hypothesised that powders would not exhibit a marked change over time. It was also deemed important to minimise colour variation, given that earlier work (Chapters 3 and 4) had indicated that pigment content was influential to chemometric modelling. Therefore, samples were chosen from the light-medium colour range only.

With the exception of the BareMinerals foundation (a premium-priced product), all samples were purchased from the mainstream market. Nonetheless, samples were specifically chosen from different brands to identify possible manufacturer trends; two Rimmel products were

chosen due to product availability at the time of purchase, but were deliberately selected from different product lines.

Perhaps the most important factor to consider when selecting products for this study was the degree of heterogeneity seen via confocal Raman microscopy. Hence, all products were chosen based on their behaviour in Chapter 3. Furthermore, wherever possible the same shade of product was purchased to match those from Chapter 3, so that comparisons could be made between the samples purchased from different batches.

The samples included in this study were as follows:

1. BareMinerals BarePro Performance Wear Liquid Foundation in shade 02 Dawn
2. Bourjois Healthy Mix Anti-Fatigue Liquid Foundation in shade Vanilla 52W
3. L'Oreal Infallible 24H Matte Cover Liquid Foundation in shade 145 Rose Beige
4. Max Factor Miracle Pure Skin-Improving Liquid Foundation in shade 32 Light Beige
5. Maybelline Superstay 30H Long-Lasting Liquid Foundation in shade Light Beige 05
6. Rimmel Lasting Finish 25Hr Full Coverage Liquid Foundation in shade 200 Soft Beige
7. Rimmel Match Perfection Invisible Coverage Liquid Foundation in shade 200 Soft Beige

It was decided that sample ageing would be carried out in a passive manner, by allowing the samples to age via exposure to air and natural light. Additional factors such as active weathering, exposure to intense UV radiation, or deliberate transfer were not investigated. The reasoning behind this was that if all variables were investigated simultaneously, it would be very difficult to ascertain which variables had an effect on ageing, and by how much. Therefore, a conservative approach was adopted, which could later be extended to cover other elements of transfer and persistence.

All samples were prepared in the same way as detailed in Chapter 2 with the exception that they were not left for a minimum of 14 days to dry prior to analysis. This distinction was important as the degree of product drying or evaporation needed to be assessed as one of the key findings of this study. All spectral pretreatment and multivariate analyses (MVA) were carried out in the Unscrambler X version 11.0 as detailed in Chapter 2.

5.3 Results and discussion

5.3.1 Preliminary considerations

It was hypothesised that the most significant changes in the texture, composition and viscosity of the foundations would occur in the initial stages of ageing. Hence, it was decided that upon product opening, photomicrograph images and Raman spectra would be obtained for all of

the samples on microscope slides (hereafter referred to as “*from slide*” samples) on a weekly basis up to the first month, and then monthly thereafter. The monthly analyses would incorporate both the ageing samples “*from slide*”, and new aliquots taken from each product (hereafter referred to as “*from bottle*”), to check for ageing within the packaging.

5.3.2 Characterisation using optical microscopy

5.3.2.1 Foundations “*from bottle*”

Images were taken at varying magnifications but it was deemed that the x135 magnification was the most effective at showing the texture, composition, distribution of particles, and degree of drying of each sample. Figures 5.1 through to 5.7 show the appearance of each foundation when taken as an aliquot from its original container, at the following time intervals: newly opened, week 5, week 9, and month 15.

For some of the products, most notably the L’Oreal and Maybelline foundations, a significant difference in colour was observed for the week 9 images, which was not replicated in the month 15 images as is demonstrated in Figures 5.3 and 5.5. This transpired to be how the ambient lighting in the laboratory (very bright sunny days compared to overcast or rainy days) affected the auto contrast function on the microscope and highlights the need for a consistent lighting protocol (such as the use of a dark room environment) if using microscopy to differentiate colour comparatively. It also underlines the importance of using several techniques in an analytical workflow to fully investigate a sample of interest. For objective colour discrimination and to mitigate against variations in lighting, a microspectrophotometer (MSP) should be used. MSP yields spectra based on the absorption or emission of UV and/or visible light by the sample of interest. These spectra permit colour comparisons of samples that may appear the same colour via subjective visual assessment².

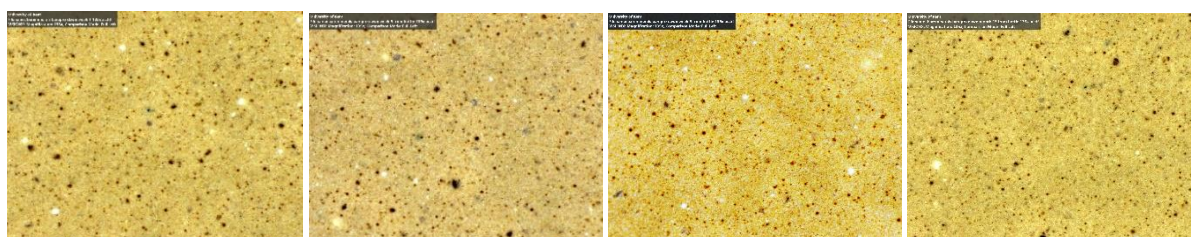


Figure 5.1: Colour photomicrograph images of BareMinerals BarePro Foundation (x135 magnification), taken directly from the bottle at various time intervals after product opening, (L-R) week 1 (newly opened), week 5, week 9, month 15.



Figure 5.2: Colour photomicrograph images of Bourjois Healthy Mix Foundation (x135 magnification), taken directly from the bottle at various time intervals after product opening, (L-R) week 1 (newly opened), week 5, week 9, month 15.

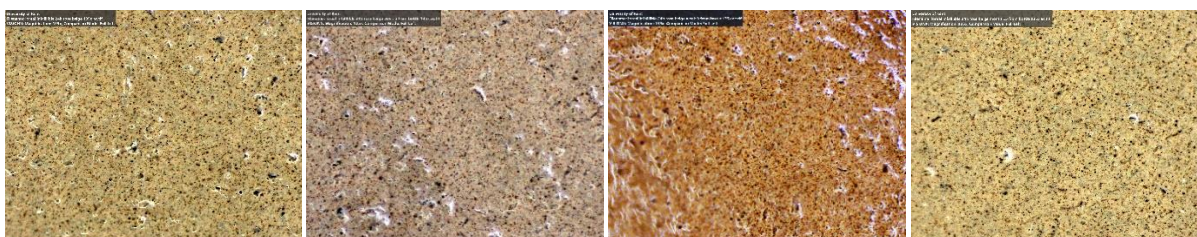


Figure 5.3: Colour photomicrograph images of L'Oreal Infallible Foundation (x135 magnification), taken directly from the bottle at various time intervals after product opening, (L-R) week 1 (newly opened), week 5, week 9, month 15.



Figure 5.4: Colour photomicrograph images of Max Factor Miracle Pure Foundation (x135 magnification), taken directly from the bottle at various time intervals after product opening, (L-R) week 1 (newly opened), week 5, week 9, month 15.

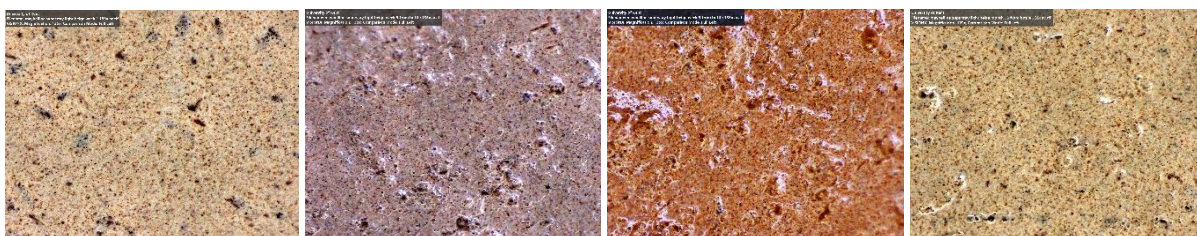


Figure 5.5: Colour photomicrograph images of Maybelline Superstay Foundation (x135 magnification), taken directly from the bottle at various time intervals after product opening, (L-R) week 1 (newly opened), week 5, week 9, month 15.

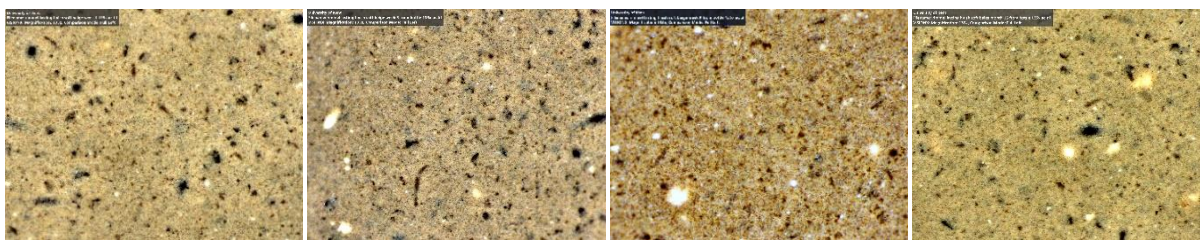


Figure 5.6: Colour photomicrograph images of Rimmel Lasting Finish Foundation (x135 magnification), taken directly from the bottle at various time intervals after product opening, (L-R) week 1 (newly opened), week 5, week 9, month 15.

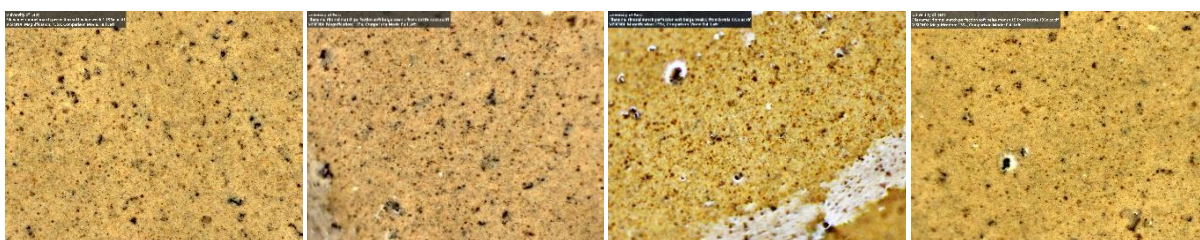


Figure 5.7: Colour photomicrograph images of Rimmel Match Perfection Foundation (x135 magnification), taken directly from the bottle at various time intervals after product opening, (L-R) week 1 (newly opened), week 5, week 9, month 15.

Nonetheless, given that only the L'Oreal and Maybelline foundations exhibited this drastic change in colour, while the other five foundations in the set displayed a more consistent appearance, potential causes were investigated. By inspecting the ingredients list for the L'Oreal and the Maybelline foundations it was found that, after water, dimethicone was the second most abundant and third most abundant ingredient for the two foundations respectively. The refractive index (RI) for water is around 1.3, whereas the RI for dimethicone is higher at around 1.5³. Conversely, components that featured strongly in the other foundation formulations included cyclopentasiloxane and trimethylsiloxysilicate, both of which have lower RI values of around 1.4³. It is hypothesised that this compositional difference between the foundations explains why the ambient light may have affected the two dimethicone-based products far more than the rest of the sample set.

It should be noted, however, that despite the stark difference in colour, the apparent texture of the formulations, the particle sizes and distributions, and the level of hydration, all appear consistent across these images. The level of hydration can be partly demonstrated by the shiny appearance of the products' surfaces, indicating that they were wet upon image acquisition.

5.3.2.2 Foundations “from slide”

Images were taken at weekly intervals from product opening until week 5 (one month), and then at monthly intervals to week 21 (five months). The last images taken of each sample were after 15 months of ageing on the microscope slide. As expected, the most noticeable change in the samples deposited onto glass slides, occurred within the first week. It is evident from each of the image series (Figures 5.8 through to 5.14) that the most obvious texture changes can be seen between the first and second images, corresponding to week 1 (newly opened) and one week (week 2) of ageing respectively. Less reliance is placed on colour changes between successive sample images, as it is acknowledged that differences in shade could be due to an artefact of lighting.

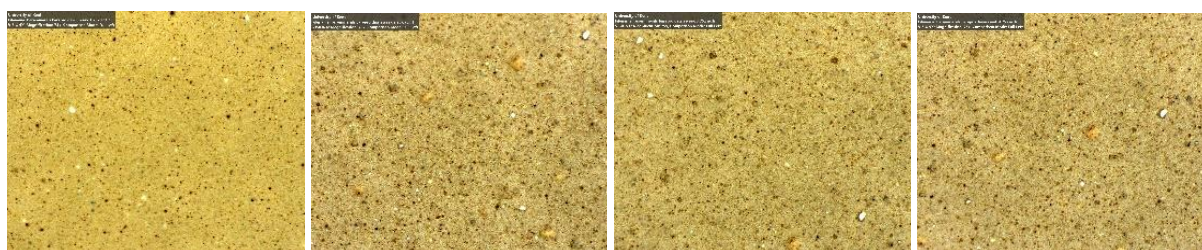


Figure 5.8: Colour photomicrograph images of BareMinerals BarePro Foundation (x75 magnification), taken at weekly intervals, showing a decrease in fluid content over time, (L-R) week 1 (newly opened), week 2, week 3, week 4.

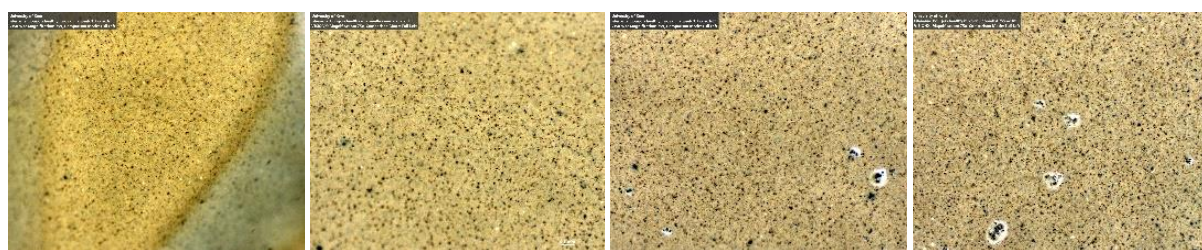


Figure 5.9: Colour photomicrograph images of Bourjois Healthy Mix Foundation (x75 magnification), taken at weekly intervals, showing a decrease in fluid content over time, (L-R) week 1 (newly opened), week 2, week 3, week 4.



Figure 5.10: Colour photomicrograph images of L'Oreal Infallible Foundation (x75 magnification), taken at weekly intervals, showing a marked decrease in fluid content over time, (L-R) week 1 (newly opened), week 2, week 3, week 4.

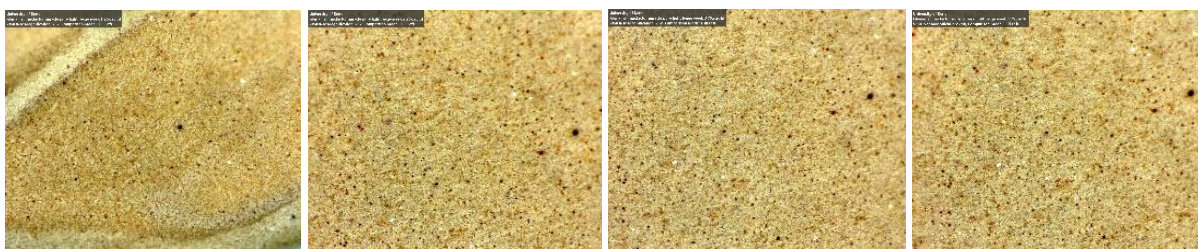


Figure 5.11: Colour photomicrograph images of Max Factor Miracle Pure Foundation (x75 magnification), taken at weekly intervals, showing a decrease in fluid content over time, (L-R) week 1 (newly opened), week 2, week 3, week 4.

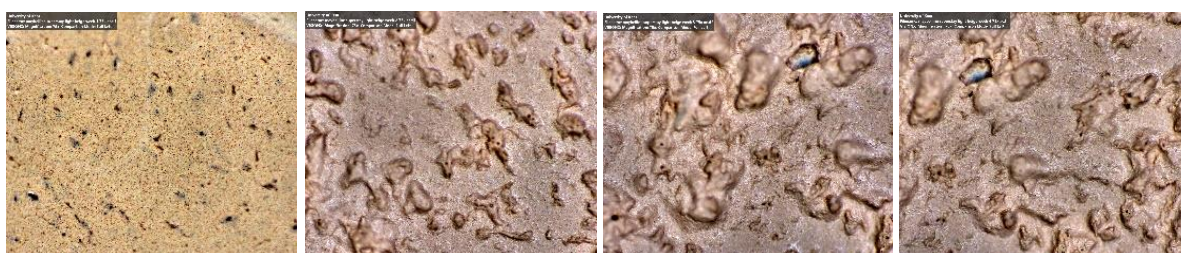


Figure 5.12: Colour photomicrograph images of Maybelline Superstay Foundation (x75 magnification), taken at weekly intervals, showing a marked decrease in fluid content over time, (L-R) week 1 (newly opened), week 2, week 3, week 4.



Figure 5.13: Colour photomicrograph images of Rimmel Lasting Finish Foundation (x75 magnification), taken at weekly intervals, showing a marked decrease in fluid content over time, (L-R) week 1 (newly opened), week 2, week 3, week 4.

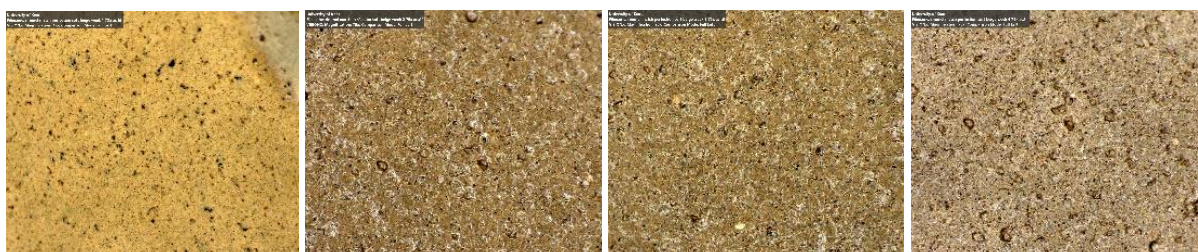


Figure 5.14: Colour photomicrograph images of Rimmel Match Perfection Foundation (x75 magnification), taken at weekly intervals, showing a decrease in fluid content over time, (L-R) week 1 (newly opened), week 2, week 3, week 4.

In Figures 5.9 and 5.11, the first images have noticeable areas that are out of focus at the edges of the micrographs. This is due to the viscous nature of these products; because the images were acquired immediately after transferring the product to the glass slide, they had not dried or settled. Hence the centres of the imaged areas are in focus and the edges are not.

The samples with the most marked decrease in fluid content and significant change to colour and texture were the L'Oreal, Maybelline and Rimmel Lasting Finish foundations, shown in Figures 5.10, 5.12, and 5.13 respectively. This higher loss of fluid content compared with the other samples correlates with the increased water content relative to oil in these formulations and indicates water evaporation. All three products are designed to be long-lasting and suitable for oily skin types, suggesting a stronger reliance on water-based components and inorganic pigments, and a lesser contribution from oily/organic components. Furthermore, all three are marketed by the manufacturers as “*sweat proof*”, “*transfer proof*”, “*heat proof*”, and “*humidity resistant*”; and all claim to be “*full coverage*” and “*oil-free*”. These statements corroborate the changes that are seen microscopically as the samples age.

In order to make direct comparisons with the “*from bottle*” images in Section 5.3.2.1, the x135 magnification images have been selected for the following discussion on the monthly ageing intervals. For clarity and to better highlight the relative differences over time, only images from weeks 1 and 5, and month 15 are displayed across Figures 5.15 to 5.21.

The BareMinerals foundation dried substantially within the first week – as is evident in Figure 5.8 – with little change thereafter. The images taken at week 5 and month 15 (Figure 5.15) also show no visible change and no additional structures compared to the week 2 images in Figure 5.8. This product showed the least amount of colour change over the 15-month period, although as previously stated, less emphasis was placed on subjective colour determinations due to fluctuations in the ambient lighting. Despite the lighting fluctuations between subsequent images for each sample, all the 15-month images for each sample were acquired on the same day (over the course of around two hours), meaning differences in colour at this timepoint can be compared between the different products.



Figure 5.15: Colour photomicrograph images of BareMinerals BarePro Foundation (x135 magnification), taken from the sample placed on a glass microscope slide and allowed to age in air and natural light, (L-R) week 1 (freshly opened), week 5, month 15.

The Bourjois foundation was one of the samples that appeared to discolour the most, turning from a sandy colour to mid-grey over the course of 15 months (Figure 5.16). This is a significant finding as it would have implications for casework that was based on a visual assessment of colour. The amount of sample analysed was comparable to the amount of product that a consumer might be expected to apply to their skin, however, this does not necessarily equate to the amount of product that may be transferred to a substrate. Cosmetic transfers are highly variable and dependent on the circumstances of the event, as discussed previously (Chapter 1, Section 1.3).

The sample showed a consistent appearance from product opening (week 1) up to the week 9 image, however, from week 17 pore-like structures started to appear, becoming more pronounced at week 21 and advancing further at month 15 into crevice-like deformities. After 15 months of ageing, the sample resembled the surface of the human brain, with the crevices appearing to contain liquid. It is hypothesised that the emulsifiers and/or stabilisers within this product's formulation had degraded, causing the separation of oil and water-based components of the emulsion.

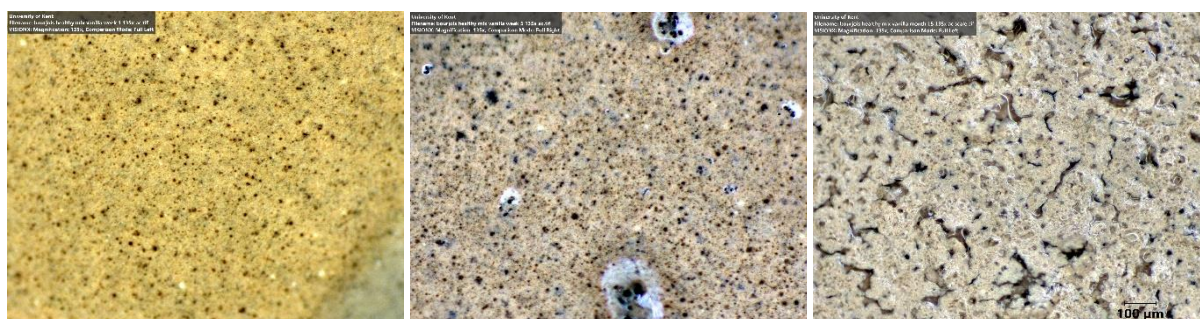


Figure 5.16: Colour photomicrograph images of Bourjois Healthy Mix Foundation (x135 magnification), taken from the sample placed on a glass microscope slide and allowed to age in air and natural light, (L-R) week 1 (freshly opened), week 5, month 15.

As was seen with the BareMinerals sample, the L'Oreal sample also showed a significant amount of drying in first week of ageing. The drying continued such that by month 15, desiccation cracks were visible across the surface of the L'Oreal sample (Figure 5.17). Due to the rapid and extensive drying, pigment particles started to separate from the matrix and became visible from week 2. These can be seen in both the week 5 and month 15 images of Figure 5.17 and have the appearance of multicoloured eyes peering out from dark caves. Despite both synthetic fluorphlogopite and calcium aluminium borosilicate pigments appearing on the ingredients list, given the spherical nature of these particles it is most likely they are borosilicate-based interference pigments. In keeping with the platy crystal nature of natural mica, synthetic fluorphlogopite also tends to occur as flakes⁴. As was the case with the Bourjois sample decolouration, this separation of pigment particles from the matrix would have implications in casework, as it may mean that the particles detach from the deposited cosmetic and are transferred elsewhere. Whilst these sorts of interference pigments are not exclusively used in decorative cosmetics (they are also found in certain automotive topcoats, luxury plastic packaging, and textile decorations), they are less likely to detach from their respective matrices if embedded into paints or plastics than a cosmetic preparation.



Figure 5.17: Colour photomicrograph images of L'Oreal Infallible Foundation (x135 magnification), taken from the sample placed on a glass microscope slide and allowed to age in air and natural light, (L-R) week 1 (freshly opened), week 5, month 15.

The Max Factor sample was quite interesting. Small bead-like structures started to become visible from week 9 and were very pronounced at month 15 (Figure 5.19). Inspection of the ingredients list revealed the presence of silica; amorphous silica microspheres are becoming increasingly common in cosmetics as a replacement for plastic microbeads since the EU ban on microplastics use⁵.

Surprisingly, the sample surface retained its greasy appearance despite the prolonged drying over 15 months and was the only sample from this set that appeared oily or wet all over. However, this product is marketed as a “*hybrid blend of skincare and foundation*”⁶ and contains ingredients such as sodium hyaluronate (a powerful humectant) and coconut oil (an emollient

and occlusive). Either or both of these components (plus many other of the components in this product) could be responsible for this sample's persistent greasy appearance.



Figure 5.18: Colour photomicrograph images of Max Factor Miracle Pure Foundation (x135 magnification), taken from the sample placed on a glass microscope slide and allowed to age in air and natural light, (L-R) week 1 (freshly opened), week 5, month 15.

Throughout this chapter thus far it has been identified that the Maybelline foundation is predominantly water-based. Therefore, it was not surprising to see a significant amount of drying (or dehydration) in the first week, leaving randomly sized and shaped lumps within the matrix, as can be seen in the week 5 image of Figure 5.19. No discernible change in appearance was seen thereafter, and no additional structures were visible, up to and including the month 15 images. This suggests that the vast majority of water loss occurred in the first week of sample deposition onto the glass slide and indicates that the formulation was relatively stable thereafter. It is not unreasonable to presume that the same product drying effect could be seen after application to the skin, particularly on dehydrated skin types, leaving a similar residue. Whether or not this residue then readily transfers to a substrate, a textile, or even the skin of another individual, remains to be tested.



Figure 5.19: Colour photomicrograph images of Maybelline Superstay Foundation (x135 magnification), taken from the sample placed on a glass microscope slide and allowed to age in air and natural light, (L-R) week 1 (freshly opened), week 5, month 15.

The Rimmel Lasting Finish Foundation had dried sufficiently by week 5 to reveal randomly distributed, thin greasy-looking striped structures of varying widths and lengths (the largest

measured ~ 0.5 mm), with rough edges (Figure 5.20). The general matrix and the mysterious striped structures appeared consistent until week 21. Then at some point between week 21 and month 15, the matrix dried further and the striped structures disappeared, leaving hollowed out areas with a greasy-looking floor. The origin and composition of these structures remains unknown.



Figure 5.20: Colour photomicrograph images of Rimmel Lasting Finish Foundation (x135 magnification), taken from the sample placed on a glass microscope slide and allowed to age in air and natural light, (L-R) week 1 (freshly opened), week 5, month 15.

The other Rimmel foundation (Match Perfection) behaved very differently, highlighting the varying formulations present between products of the same brand. This sample exhibited drying from deposition, becoming noticeable from week 3. The sample's appearance remained consistent up to week 21, but after 15 months the sample became desiccated and visible mineral structures could be seen protruding from the matrix. One of these structures is responsible for the blurred area in the month 15 image (Figure 5.21). A better focussed image of the same “star-like” structure was also taken using the x243 magnification and is shown in Figure 5.22 (centre). For spatial context and to show the variety, frequency, and distribution of other mineral structures, the x75 magnification was used to take an image with a larger field of view (Figure 5.22 left).

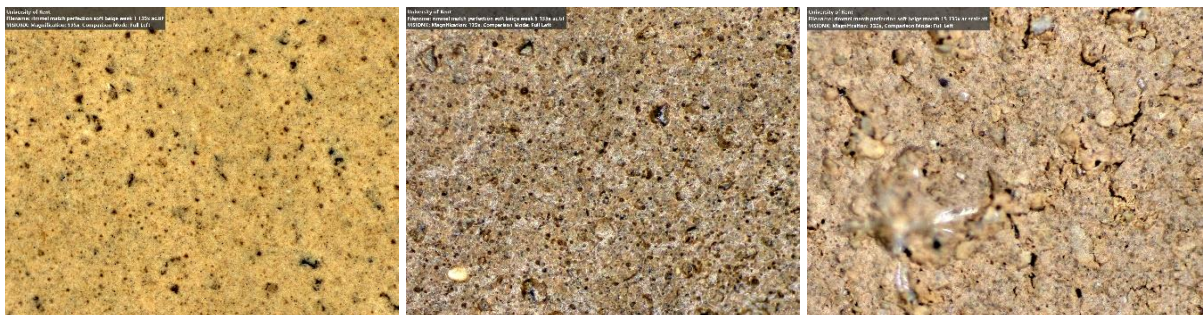


Figure 5.21: Colour photomicrograph images of Rimmel Match Perfection Foundation (x135 magnification), taken from the sample placed on a glass microscope slide and allowed to age in air and natural light, (L-R) week 1 (freshly opened), week 5, month 15.



Figure 5.22: Colour photomicrograph images of Rimmel Match Perfection Foundation taken from the sample placed on a glass microscope slide and allowed to age in air and natural light for 15 months, (left) image at x75 magnification showing several fibrous mineral structures, (centre) one of the mineral structures taken at x243 magnification, (right) two different mineral structures taken at x243 magnification.

Figure 5.22 (right) shows two differently sized and shaped structures at the microscope's highest magnification (x243). The structure at the top of this image resembles an amalgamation of cubic or prismatic crystals, whereas the “*star-like*” structure below it has an acicular or fibrous nature⁷. Inspection of the ingredients list for this product reveals sepiolite and montmorillonite; both of which are phyllosilicate minerals. Montmorillonite is primarily an aluminium silicate, defined as a smectite clay, that can form microcrystalline aggregates⁸. This is consistent with the amalgamation of the cubic crystals seen at the top of the image. Sepiolite is a hydrous magnesium silicate, chemically very similar to talc, that can form fibrous crystals⁹. It is proposed that the “*star-like*” crystal structure observed in the centre of this image is sepiolite. The presence of these crystal structures suggests that techniques such as XRD might be effective in characterising traces of dried foundation.

5.3.3 Characterisation using Raman spectroscopy

In keeping with the trend seen in Chapter 3, five of these seven foundations contained the anatase polymorph and only two contained the rutile polymorph (Bourjois and Maybelline). None of the foundations appeared to contain a mixture of the two polymorphs.

5.3.3.1 Foundations “*from bottle*”

Raman spectra were taken immediately upon product opening and designated as “*week 1*”. As previously stated, this protocol is different to the minimum two-week drying time used across Chapters 3, 4, and 6. Spectra were then taken of fresh aliquots from the original product packaging at the following intervals: week 5, week 17, month 15.

Initial spectra for each product are given across Figures 5.23 through to 5.29 and show a good level of spectral homogeneity, in keeping with the findings from Chapter 3. It should be noted

here that none of the spectra displayed have been processed, nor have they been stacked for clarity. This is deliberate as it demonstrates stability in the baseline and consistency in background fluorescence, as well as alignment of the peak positions and intensities. The spectra remained consistent throughout the 15-month period, with the exception of day-to-day instrumental variation (all spectra are given in the appendix, Figures A1-A7).

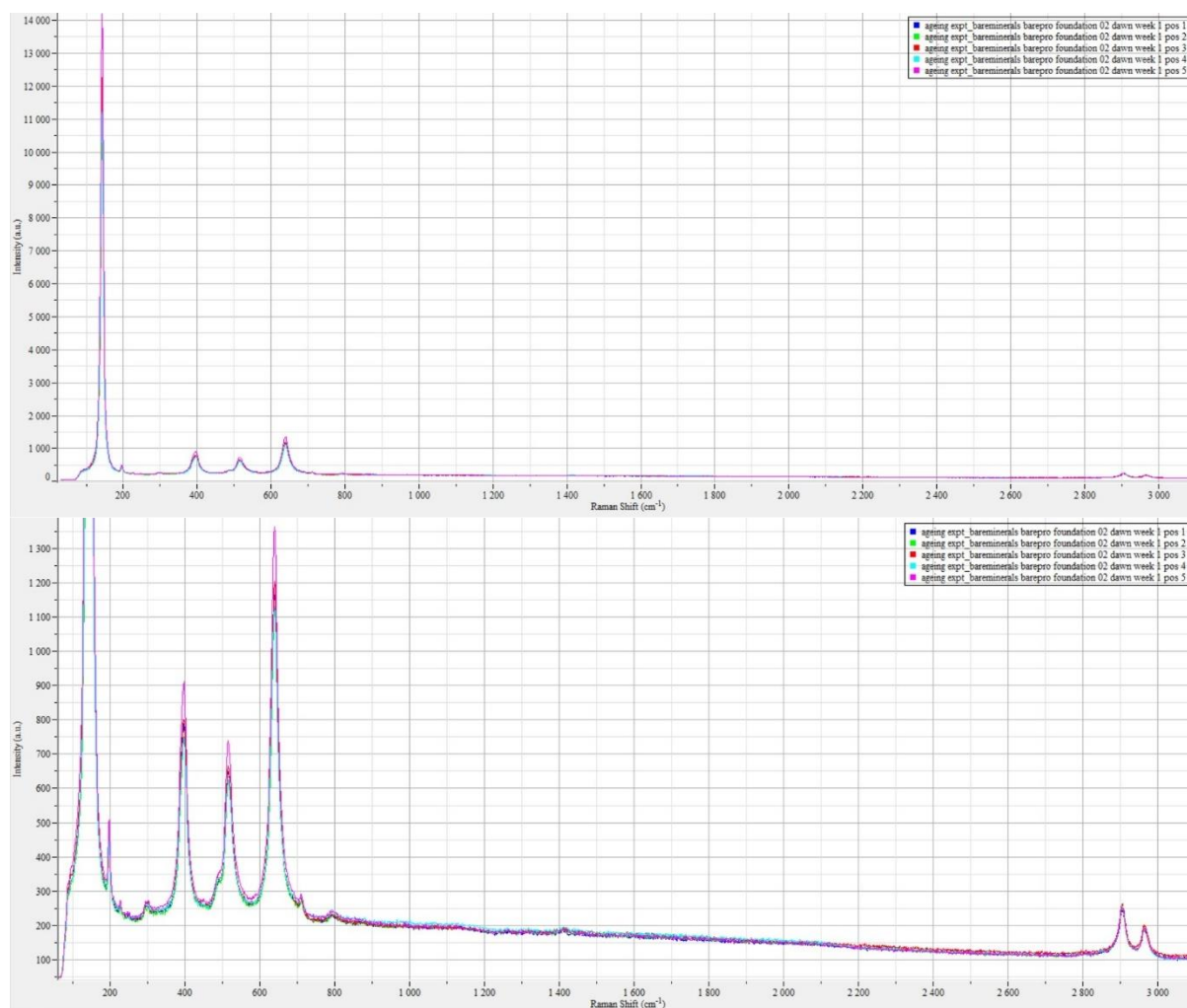


Figure 5.23: Raman spectra (unprocessed) of BareMinerals BarePro Foundation upon product opening, exhibiting spectral homogeneity across five replicates; (top) standard view, (bottom) magnified to show minor peaks.

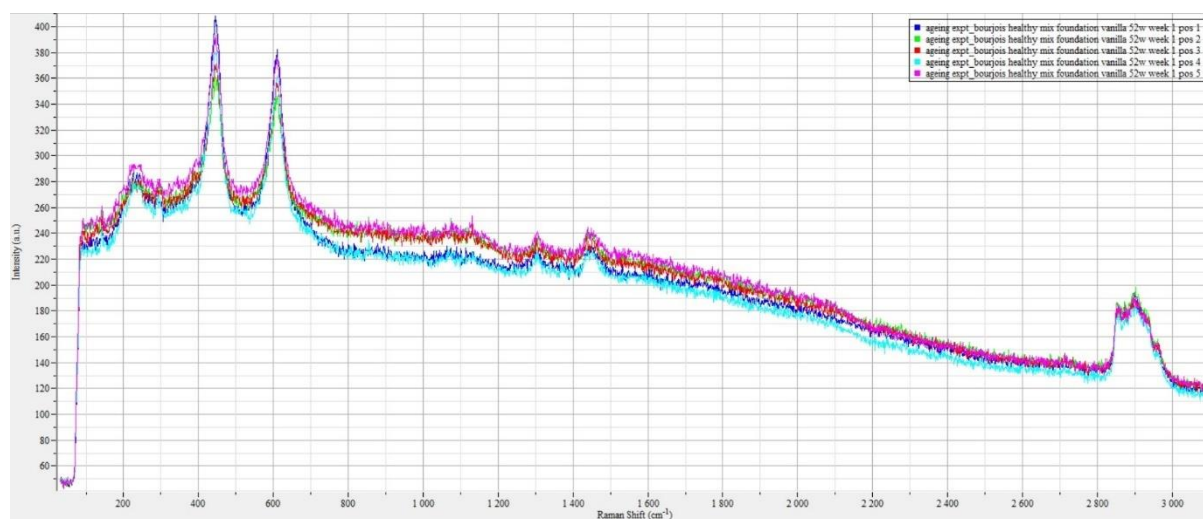


Figure 5.24: Raman spectra (unprocessed) of Bourjois Healthy Mix Foundation upon product opening, exhibiting spectral homogeneity across five replicates.

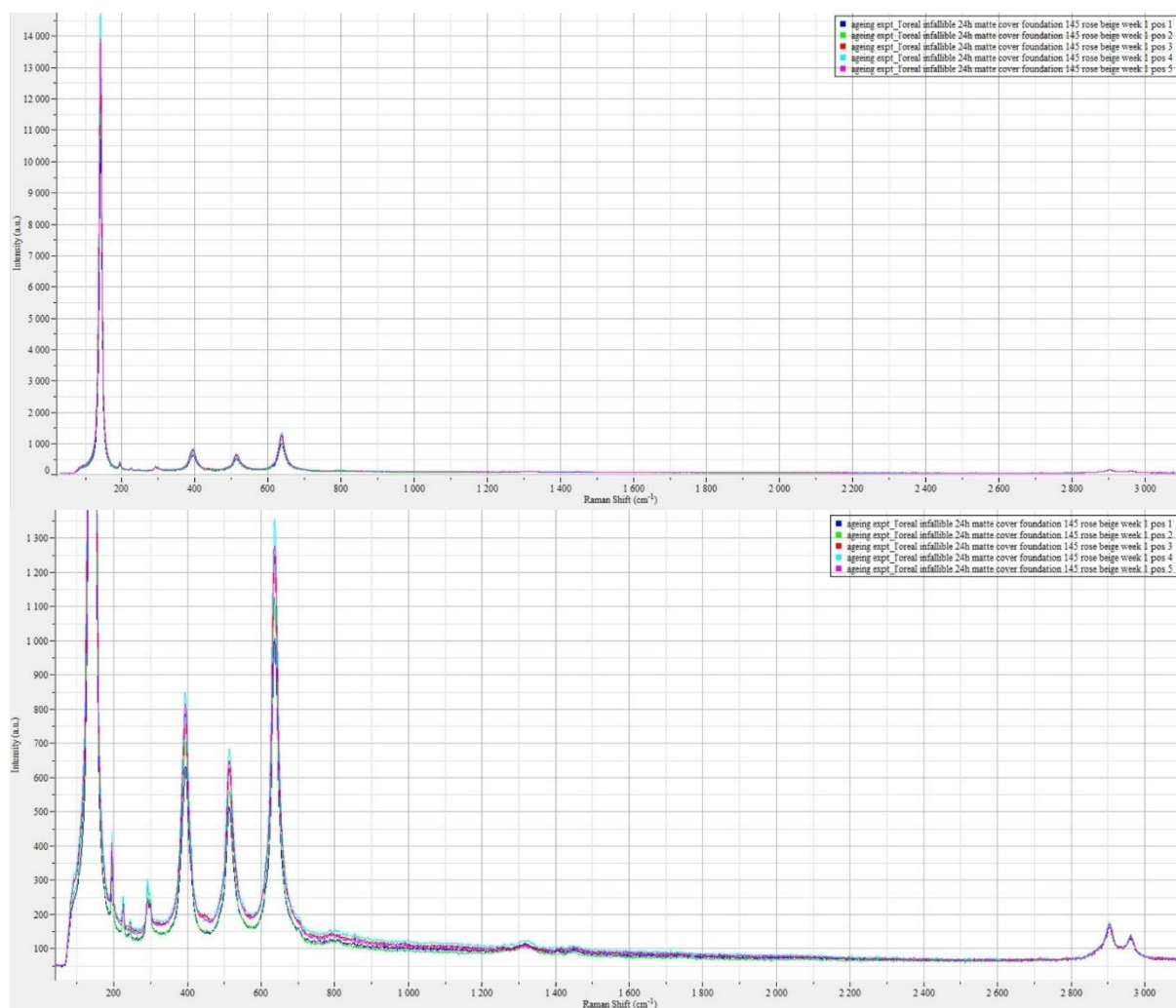


Figure 5.25: Raman spectra (unprocessed) of L'Oreal Infallible Foundation upon product opening, exhibiting spectral homogeneity across five replicates; (top) standard view, (bottom) magnified to show minor peaks.

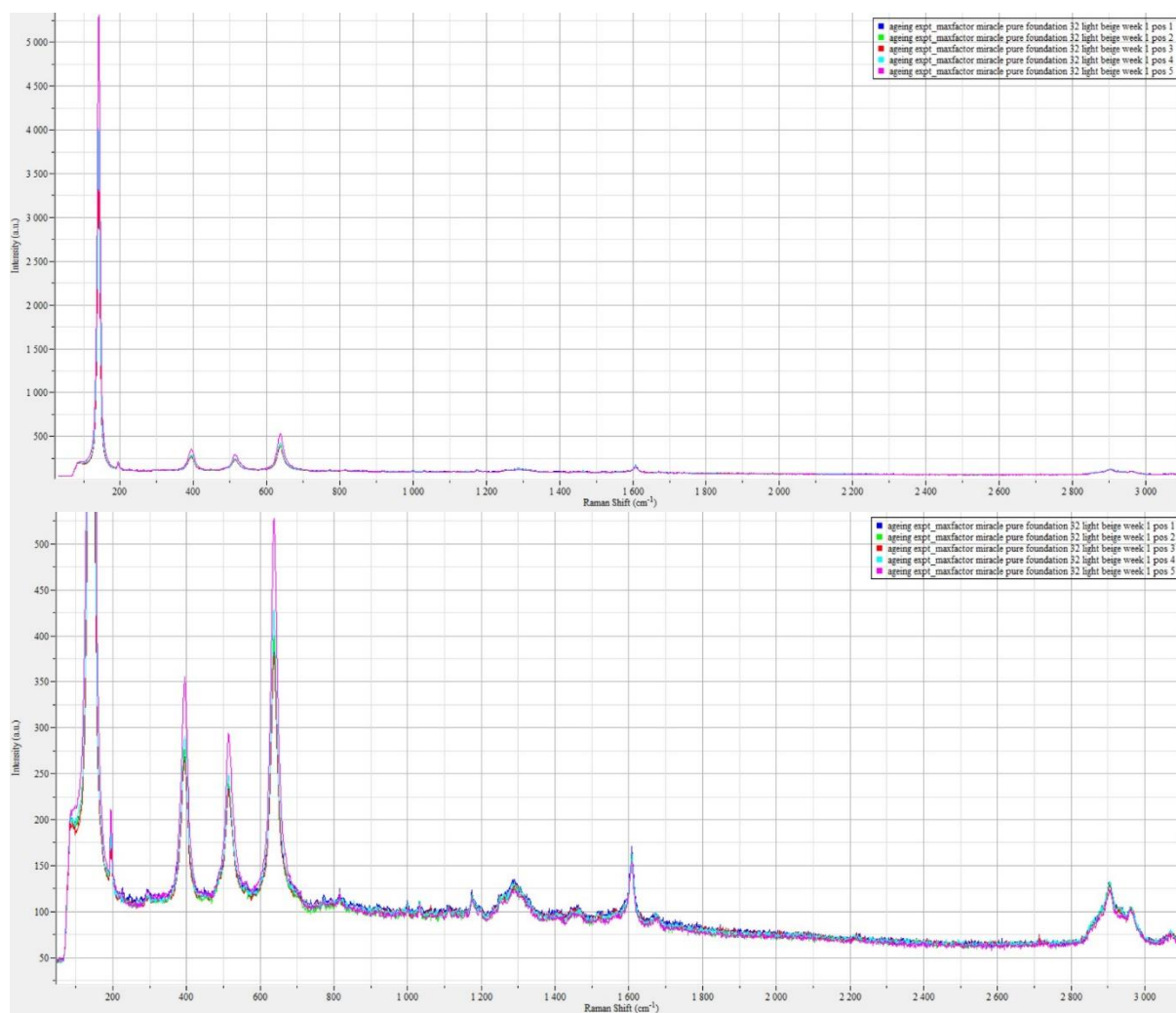


Figure 5.26: Raman spectra (unprocessed) of Max Factor Miracle Pure Foundation upon product opening, exhibiting spectral homogeneity across five replicates; (top) standard view, (bottom) magnified to show minor peaks.

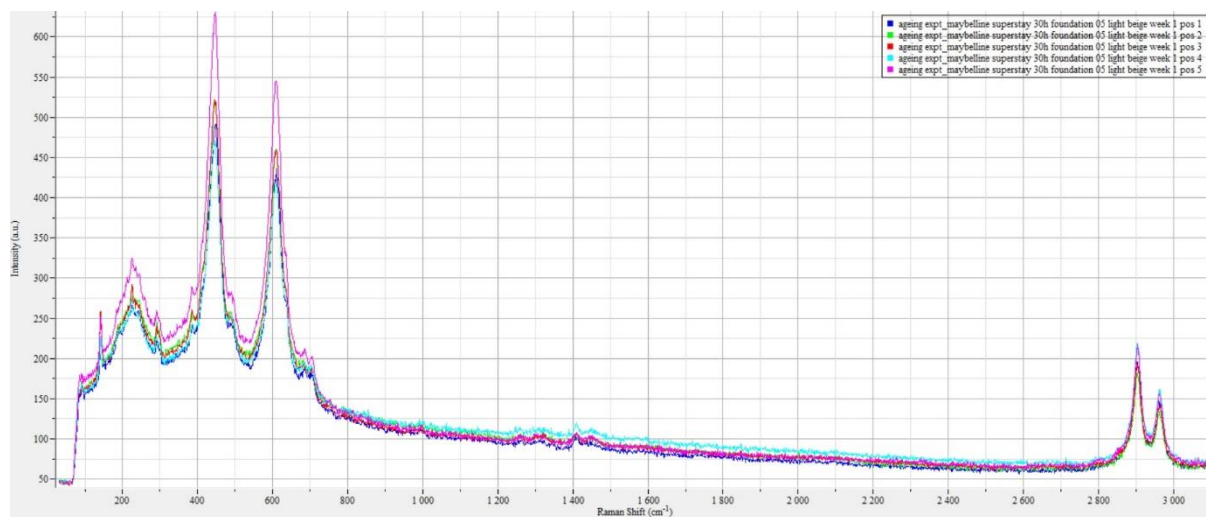


Figure 5.27: Raman spectra (unprocessed) of Maybelline Superstay Foundation upon product opening, exhibiting spectral homogeneity across five replicates.

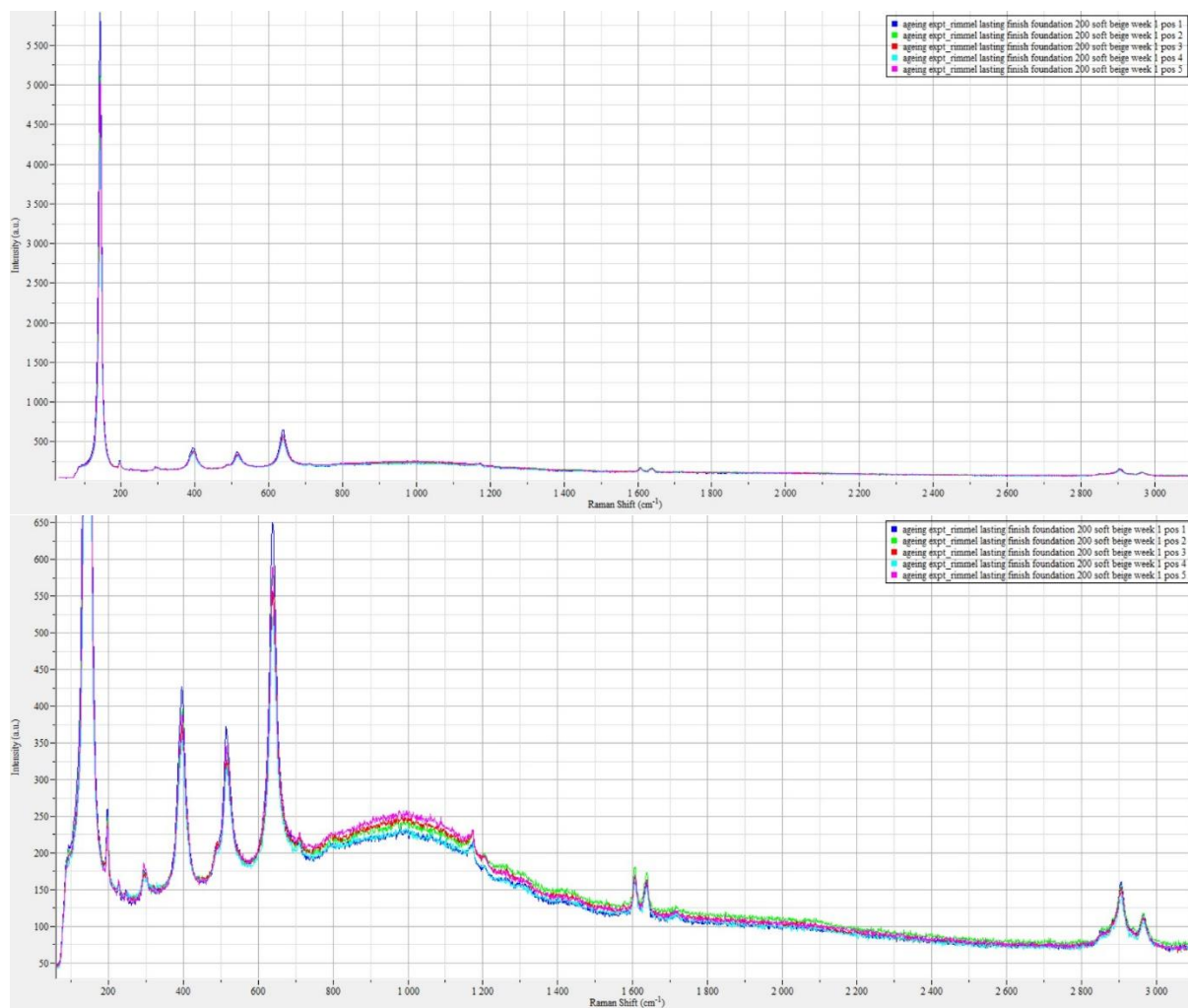


Figure 5.28: Raman spectra (unprocessed) of Rimmel Lasting Finish Foundation upon product opening, exhibiting spectral homogeneity across five replicates; (top) standard view, (bottom) magnified to show minor peaks.

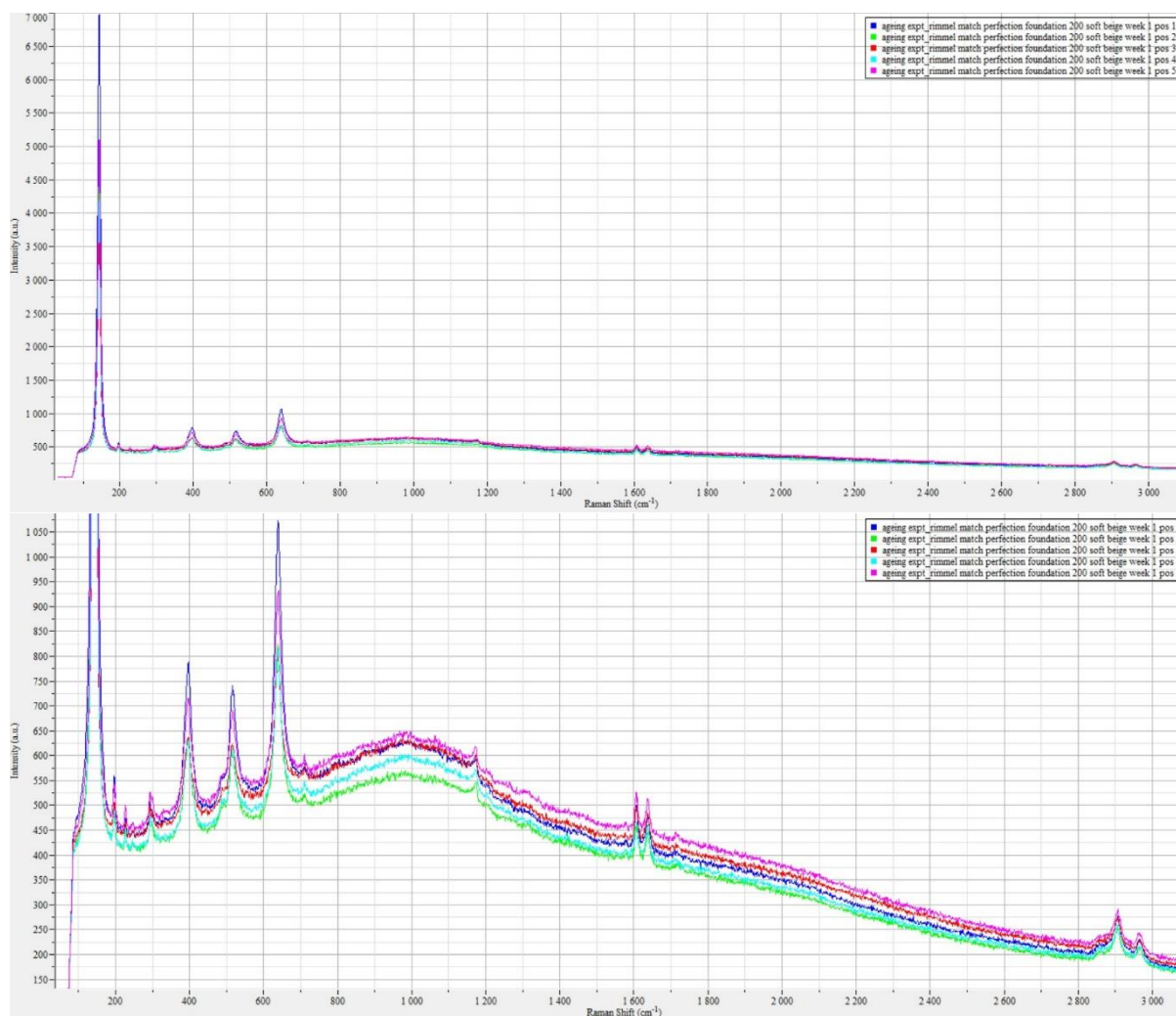


Figure 5.29: Raman spectra (unprocessed) of Rimmel Match Perfection Foundation upon product opening, exhibiting spectral homogeneity across five replicates; (top) standard view, (bottom) magnified to show minor peaks.

5.3.3.2 Foundations “from slide”

As anticipated, far more spectral variation was seen over time for the sample aliquots applied to glass slides that were allowed to air dry under natural light. However, not all products were as affected by the drying process, with spectral changes being practically undetectable for the L’Oreal foundation (Figure 5.30), and minimal for the Max Factor foundation (Figure 5.31). As discussed earlier in Section 5.3.2.2, microscopic imaging of the L’Oreal foundation revealed water evaporation from the sample over time. Given that water is a weak Raman scatterer (discussed in Chapter 2), it is conceivable that the loss of water from the matrix would not have a large impact on the resultant Raman spectrum.

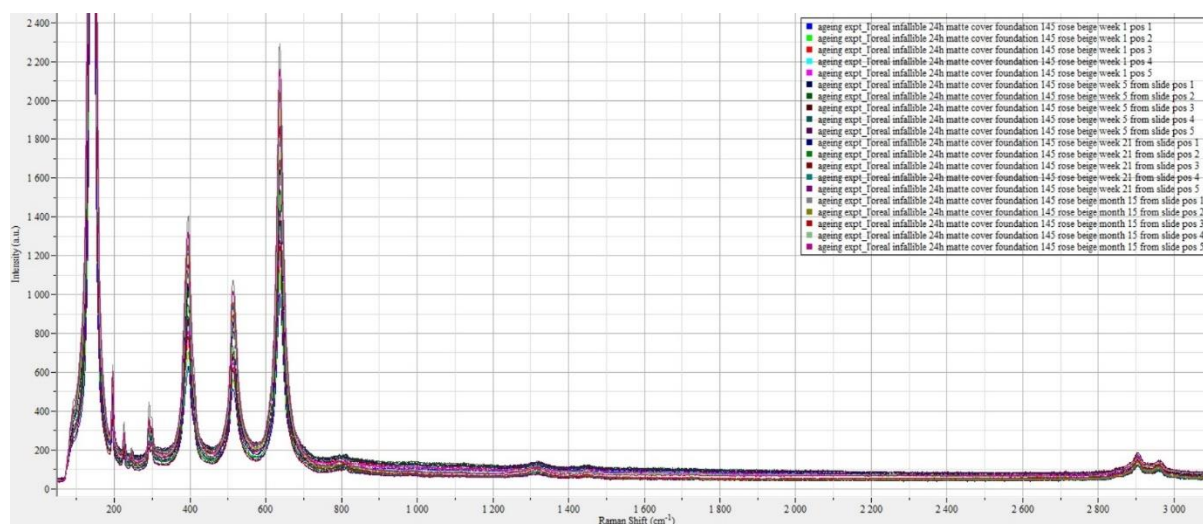


Figure 5.30: Raman spectra (unprocessed) of L’Oreal Infallible Foundation upon product opening, at week 5, at week 21 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.

Conversely, the Max Factor sample retained its greasy appearance through to the 15-month interval and appeared wet. However, it is unlikely this wet appearance relates to water content, as it was subjected to the same ageing conditions as the L’Oreal sample. As previously discussed, the Max Factor foundation is marketed as a “*hybrid blend of skincare and foundation*” and contains the emollient and occlusive ingredient: coconut oil. If the persistent greasy appearance of this product can be attributed to coconut oil, then it would stand to reason that the liquid fraction would not evaporate over a 15-month time period. Moreover, the inclusion of antioxidants and preservatives in this cosmetic product would largely safeguard against rancidity and spoiling of the coconut oil, therefore the chemical composition could be expected to remain stable and no change would be detected in its Raman spectrum.

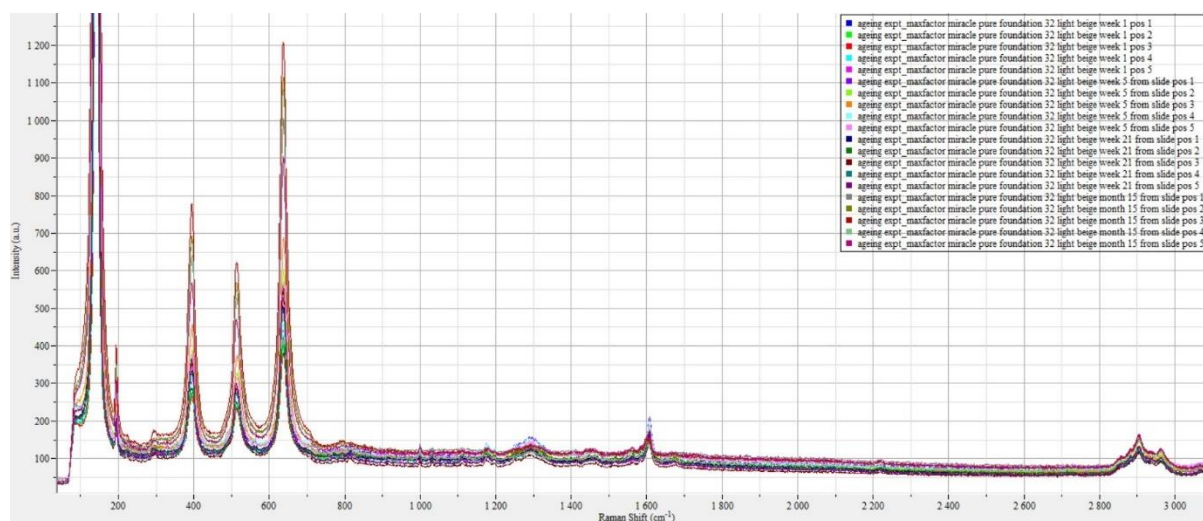


Figure 5.31: Raman spectra (unprocessed) of Max Factor Miracle Pure Foundation upon product opening, at week 5, at week 21 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.

Figures 5.32 and 5.33 show the two rutile-containing foundations, Bourjois and Maybelline, experienced an increase in the relative intensities of their mineral peaks in the 100-800 cm^{-1} region. Rimmel Lasting Finish lost the low intensity broad peak in the baseline between 700-1300 cm^{-1} from week 1, consistent with a loss of lecithin from the matrix, but remained consistent thereafter (Figure 5.34).

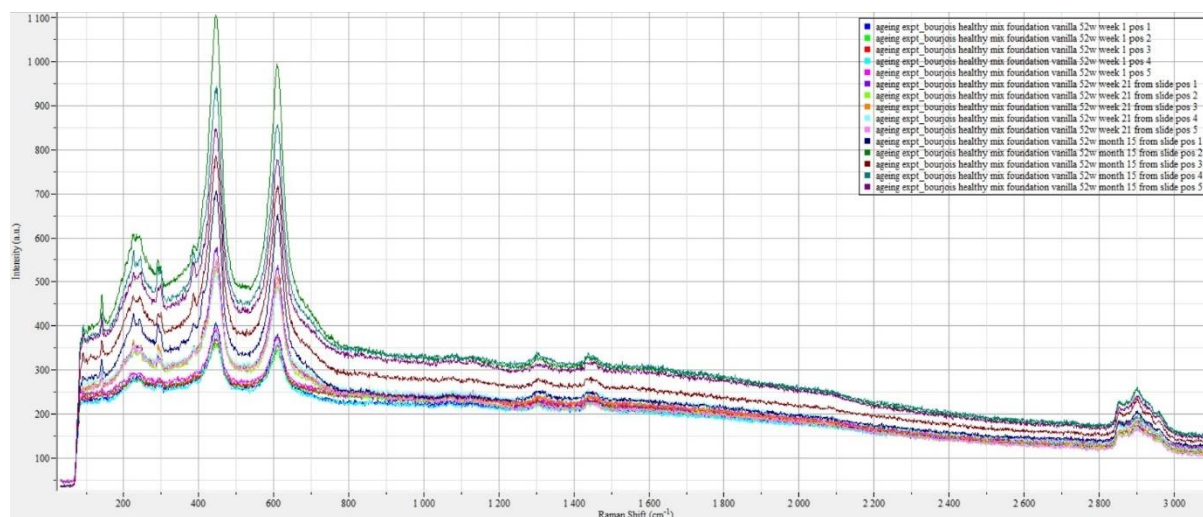


Figure 5.32: Raman spectra (unprocessed) of Bourjois Healthy Mix Foundation upon product opening, at week 21 and at month 15, exhibiting an increase in mineral peak intensities across the time interval.

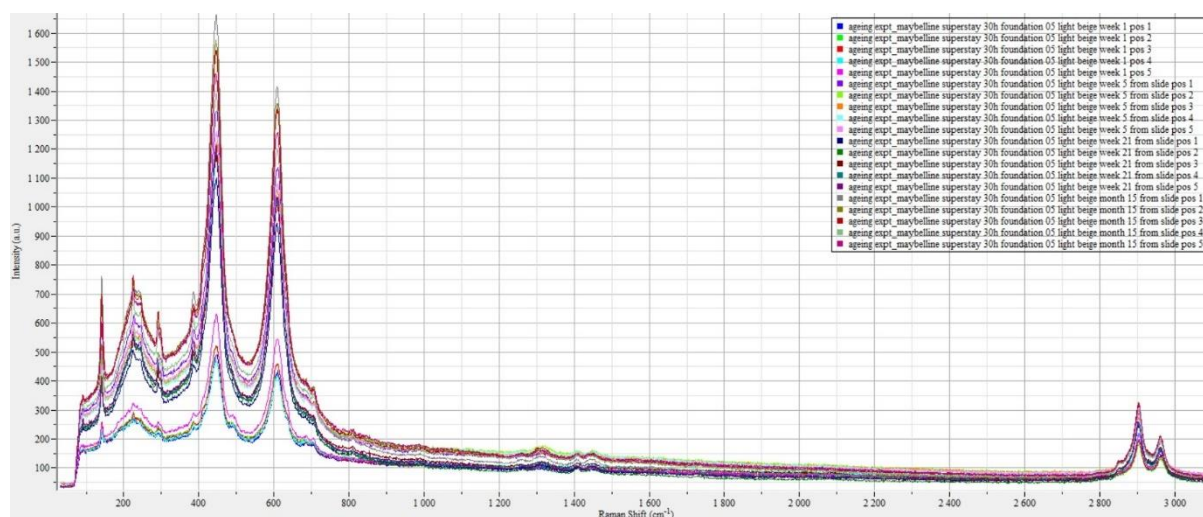


Figure 5.33: Raman spectra (unprocessed) of Maybelline Superstay Foundation upon product opening, at week 5, at week 21 and at month 15, exhibiting an increase in mineral peak intensities across the time interval.

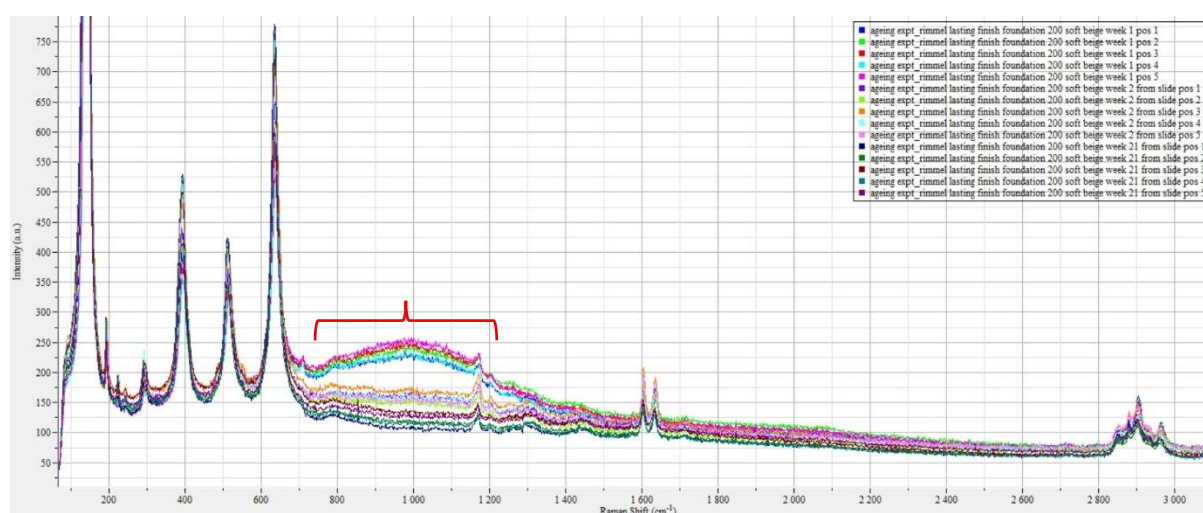


Figure 5.34: Raman spectra (unprocessed) of Rimmel Lasting Finish Foundation upon product opening, at week 2, and at week 21, exhibiting an increase in mineral peak intensities across the time interval. Red brace denotes low intensity broad peak.

The two products that exhibited the most spectral change and, therefore, seemed to be most affected by the ageing process were the BareMinerals and Rimmel Match Perfection foundations. With the BareMinerals product, each subsequent spectrum taken after the week 1 series showed a gradual increase of baseline fluorescence, consistent with the significant drying seen microscopically. This progressed to the point that for the month 15 analyses, a significant amount of photobleaching was required to suppress fluorescence and reveal meaningful peaks. As is evident from the spectra (Figure 5.35), despite this extensive

photobleaching, residual fluorescence remained as indicated by the “stepping” in the baseline at around 1200 cm^{-1} and again at around 2100 cm^{-1} .

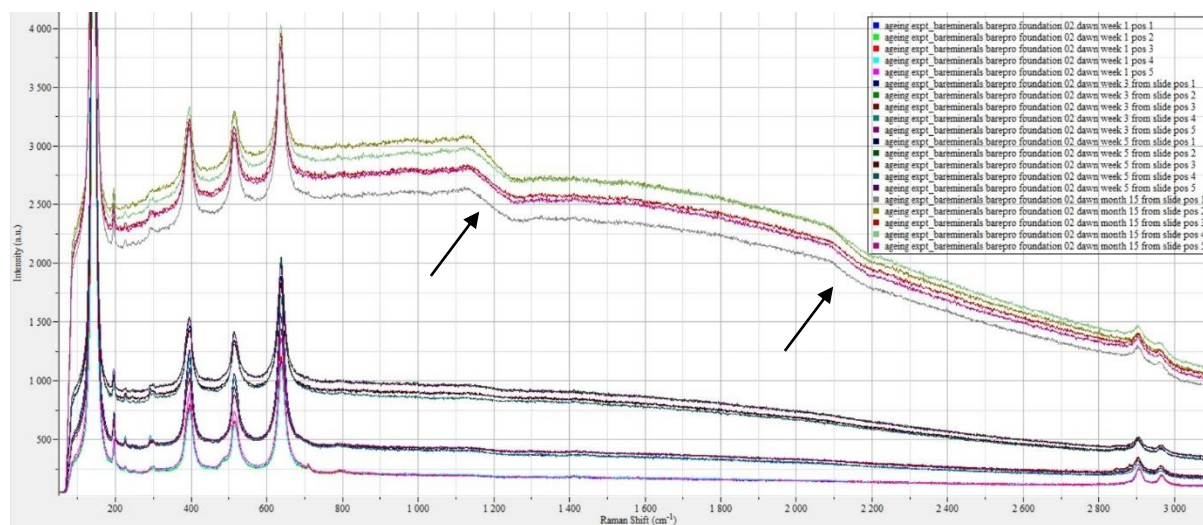


Figure 5.35: Raman spectra (unprocessed) of BareMinerals BarePro Foundation upon product opening, at week 3, at week 5 and at month 15, exhibiting a gradual increase of baseline fluorescence across the time interval. Black arrows denote “stepping” in the baseline.

The BareMinerals foundation contains four different preservatives (1,2-hexanediol, caprylyl glycol, sodium dehydroacetate, and phenoxyethanol) so should be relatively resistant to microbial and fungal growth. Nonetheless, it is the only foundation in this sample set to contain the ingredient, papain. Papain is an enzyme present in many cosmetics that derives from the papaya fruit. It serves as an exfoliant by breaking down skin cells and encouraging cell renewal, therefore, the papain could be responsible for the degradation of certain product components, which might give rise to the increased spectral fluorescence seen.

It is interesting to note that this pattern of increased baseline fluorescence was encountered with another BareMinerals sample in Chapter 3 but the origin of the fluorescence could not be ascertained at the time. Figure 3.22 shows an increase in the amount of baseline fluorescence in the spectrum for the Bareskin Foundation in shade Bare Natural and given that we also see this pattern for the aged BareMinerals BarePro Foundation above (Figure 5.35) it is conceivable that the pattern seen in Chapter 3 was due to the integrity of the sample’s packaging having been compromised.

The Rimmel Match Perfection sample lost the initial curved appearance in the baseline between 700-1300 cm^{-1} after week 1 (this product also contains a large proportion of lecithin as per ingredients list), whilst retaining the other spectral peaks up to and including the week 21 analyses. Yet at month 15, with the exception of the 1317 cm^{-1} peak attributable to red iron oxide, all the peaks had disappeared (1170 cm^{-1} , 1203 cm^{-1} , 1575 cm^{-1} , 1605 cm^{-1} , 1635 cm^{-1}

and 1710 cm^{-1}) as can be seen in Figure 5.36. This is associated with a loss of organic content and could be attributable to the loss of siloxanes, carbonyls, ethers, alcohols, amines or aromatics from the formulation, either via degradation or conversion into other functional groups. The peaks in the CH stretching region between $2800\text{--}3000\text{ cm}^{-1}$ do not appear to have changed, suggesting the preservation of alkane residues¹⁰.

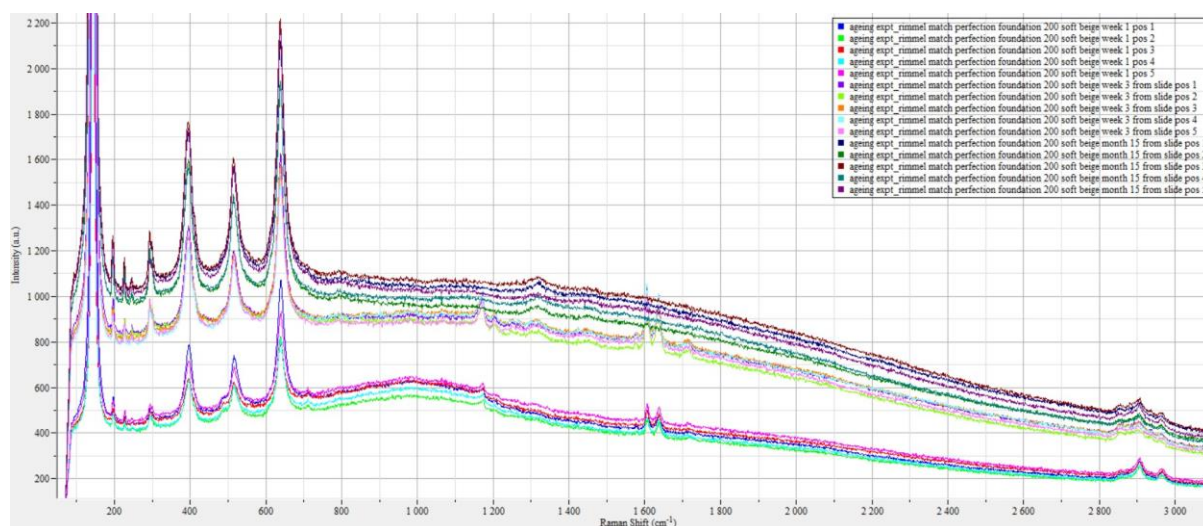


Figure 5.36: Raman spectra (unprocessed) of Rimmel Match Perfection Foundation upon product opening, at week 3, and at month 15, exhibiting a loss of spectral peaks across the time interval.

What is particularly interesting is that there does not seem to be a trend between the products' intended consumer market and the resultant Raman spectrum after ageing. It was anticipated that products marketed towards oily or combination skin, which contained high levels of humectants (such as the L'Oreal and Maybelline foundations) would behave opposingly to products marketed towards dry or sensitive skin (such as the Max Factor foundation) that contained high levels of emollients and occlusives. Unfortunately, no such patterns could be determined.

5.3.3.3 Inter-batch variation

One of the criteria used when selecting products for this ageing study was to purchase replicates of products that had already been analysed and included in Chapter 3, in order to check for inter-batch variations. Unfortunately, the Max Factor Miracle Match Foundation had since been discontinued and replaced with Miracle Pure, Miracle Second Skin, and Miracle Touch variants. A Max Factor Miracle Pure Foundation was purchased in the hope that perhaps only the product naming had changed, but inspection of the ingredients revealed a totally different chemical composition, with the main silicone component (second in ingredients list) being dimethicone, as opposed to cyclopentasiloxane for the old Miracle Match

foundation. The Miracle Pure foundation was also found to contain a variety of chemical sunscreens and many naturally derived ingredients – coconut, pea, and alfalfa – that were not found in the Miracle Match foundation, as well as an absence of talc.

The Maybelline Superstay 24H Foundation had been rebranded as a “30H” formulation, and again upon receipt of the product it was determined that the formulation had changed. The “24H” variant listed cyclopentasiloxane as its main component, followed by water and polypropylsilsesquioxane, whereas the “30H” variant listed water as its main component, followed by isododecane and dimethicone. It was also noted that the “24H” variant included ethylparaben and nylon-12, both of which are absent from the new formulation. It is assumed this is due to the increased concern of parabens acting as endocrine disruptors⁸ and the upcoming ban on plastics in cosmetic formulations⁵ respectively. The new “30H” formulation contains environmentally friendly alternative bulking agents to plastics such as cellulose, xanthan gum and various silica derivatives, alongside “*skin-friendly*” components such as sodium hyaluronate (a powerful humectant) and tocopherol (vitamin E). With regards to the mineral pigments, in addition to the standard CI 77891 (titanium dioxide) and CI 77491/77492/77499 (iron oxides), CI 77007 (ultramarine) also appears in the new “30H” variant, although it does not state whether this is the blue, pink, or violet shade.

The remaining five foundations from this dataset appeared to be consistent with previous purchased versions (used in Chapter 3) and thus were assumed to be comparable. An initial consideration for these comparisons was that the spectra from Chapter 3 were only acquired after the sample aliquot had been allowed to air dry for at least 14 days. Given that the results from this study indicate air drying affects different products inconsistently, it was decided that the “*week 3*” spectra for each sample (relating to 2 weeks worth of air drying on a slide) would be used as the comparators. Figures 5.37 and 5.38 demonstrate the importance of comparing spectra from samples that have been exposed to similar ageing conditions.

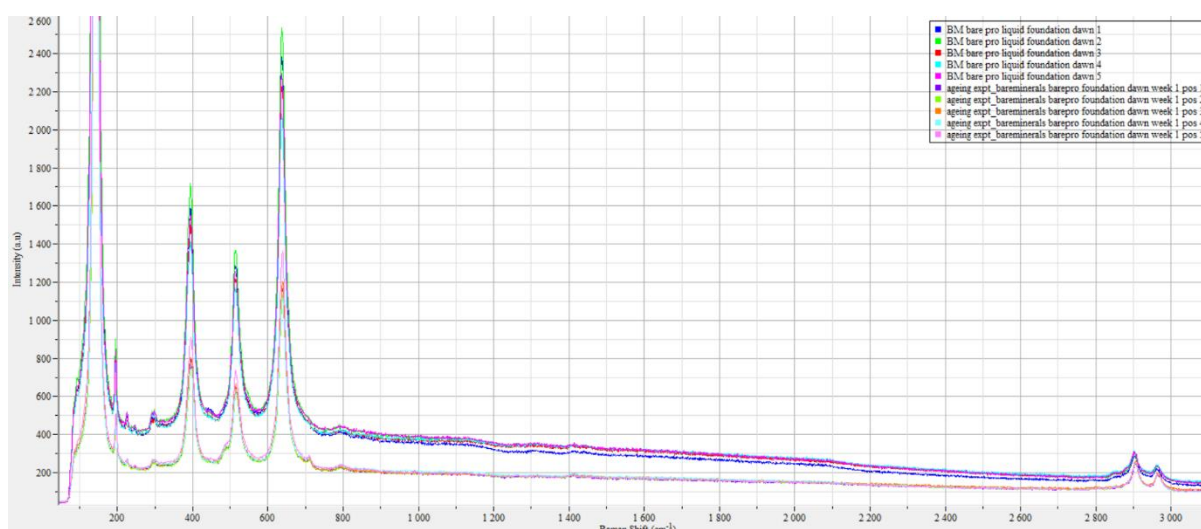


Figure 5.37: Raman spectra (unprocessed) of two BareMinerals BarePro Foundations in shade 02 Dawn (magnified to truncate “lattice” peak); top five spectra show the product purchased in 2020 and analysed after 14 days air drying, bottom five spectra show the product purchased in 2023 and analysed immediately upon opening packaging.

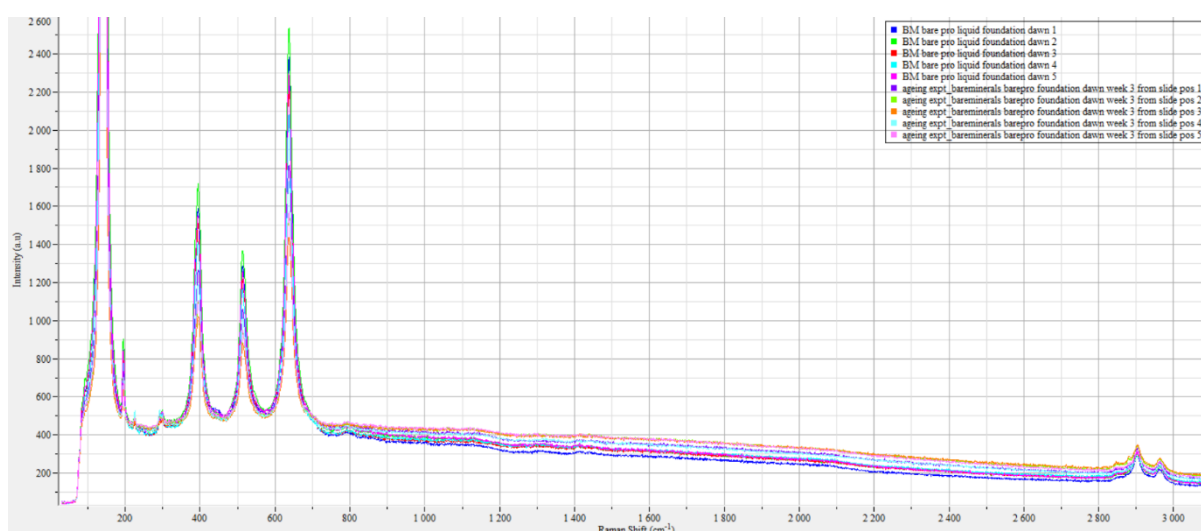


Figure 5.38: Raman spectra (unprocessed) of two BareMinerals BarePro Foundations in shade 02 Dawn (magnified to truncate “lattice” peak); one purchased in 2020 and the other in 2023, both analysed after 14 days of air drying on a glass slide.

Closer inspection of the ingredients lists for the two Bourjois foundations revealed that despite a lack of advertising on the product packaging, the formulation had clearly changed. This raises an important point to consider for forensic cosmetic analysis going forwards: some companies heavily market their “*new and improved*” formulations when altering very little, whilst others change their formulations significantly and choose not to advertise it. This would cause great difficulty tracking formulation changes for the purposes of keeping any cosmetic reference databases or spectral libraries up to date. The changes in this product’s formulation

are evident from the spectral differences seen in the mid- (between 1200-1600 cm^{-1}) and CH regions (between 2800-3000 cm^{-1}) of Figure 5.39.

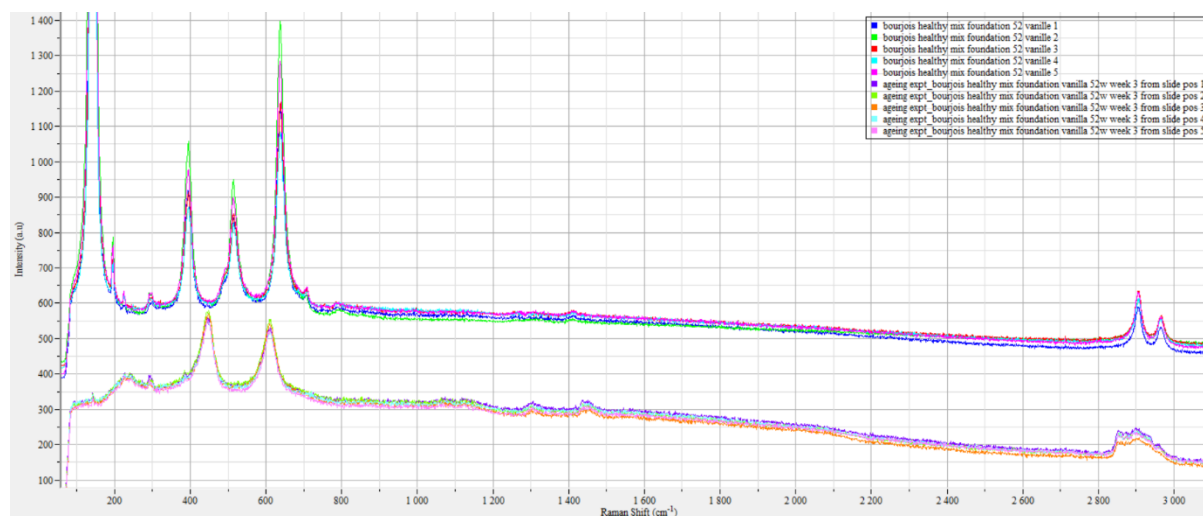


Figure 5.39: Raman spectra (unprocessed but stacked for clarity) of two Bourjois Healthy Mix Foundations in shade 52 Vanilla (magnified to truncate lattice peak); top five spectra show the product purchased in 2019, bottom five spectra show the product purchased in 2023, both analysed after 14 days of air drying, showing a clear discrepancy in the titanium dioxide polymorph used and minor spectral differences in the mid-region and CH region.

However, the most surprising finding from comparing the Bourjois inter-batch spectra was the discovery that the titanium dioxide polymorph had changed. As can be seen in Figure 5.39, the product purchased in 2019 clearly contains anatase, yet the product purchased in 2023 that forms part of this chapter's sample set is obviously rutile dominant. This is another important point to note: titanium dioxide is only ever listed in cosmetic ingredients as either “titanium dioxide” or “CI 77891”, but never gives the specific polymorph. Therefore, the manufacturers can change from using one of the main polymorphs to the other, or even use a combination of the two, and this would be totally undetectable from the product packaging, and perhaps even from the product itself. This change in polymorph may relate to the findings by Pur Cosmetics (discussed in Chapter 3) that anatase caused skin damage when irradiated with UV light while rutile did not¹¹.

Incidentally, the reformulation of the Maybelline foundation from “24H” to “30H” variant also came with a noticeable change of titanium dioxide polymorph used: anatase to rutile (Figure 5.40). Whether these changes are coincidental or related to the specific properties of the two polymorphs is unknown. Nonetheless, due to the specificity and sensitivity of Raman spectroscopy, and the very different spectral patterns gained with both polymorphs, this change would be easily ascertainable using this technique. This further demonstrates the

utility of Raman spectroscopy, either in isolation or within a multi-technique analytical workflow.

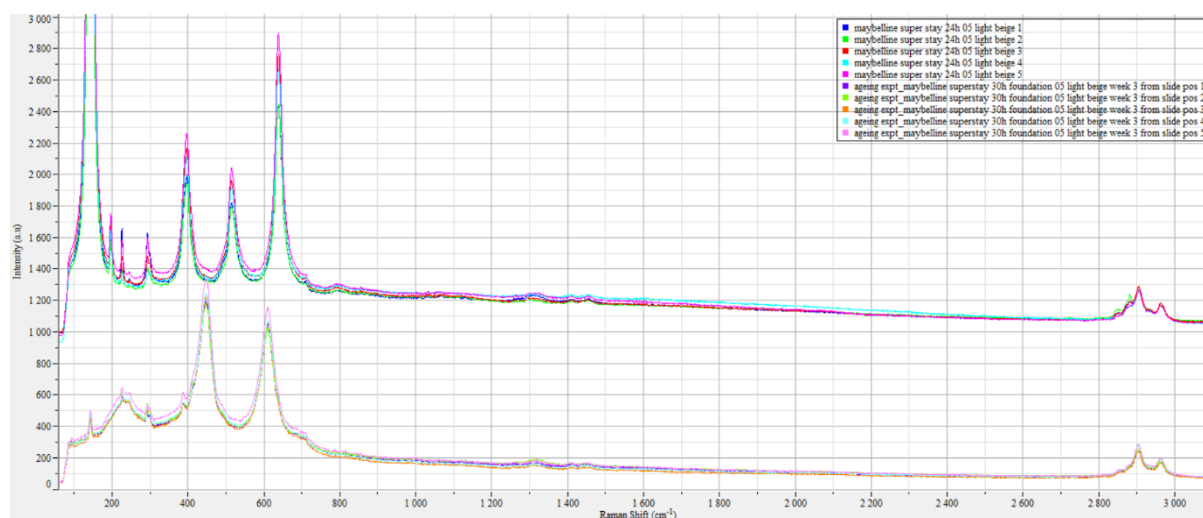


Figure 5.40: Raman spectra (unprocessed but stacked for clarity) of two Maybelline Superstay Foundations (magnified to truncate lattice peak); top five spectra show the “24H” variant purchased in 2020, bottom five spectra show the “30H” variant purchased in 2023, both analysed after 14 days of air drying, showing a clear discrepancy in the titanium dioxide polymorph used.

As expected, comparisons between the pairs of the remaining four products – BareMinerals, L’Oreal, and both Rimmel foundations – revealed very few, if any, spectral differences (Figures 5.41 and 5.42. The pair of products with the largest discernible difference between batches were the Rimmel Match Perfection foundations, as can be viewed in Figure 5.43, but these only related to variations in fluorescent baseline intensities.

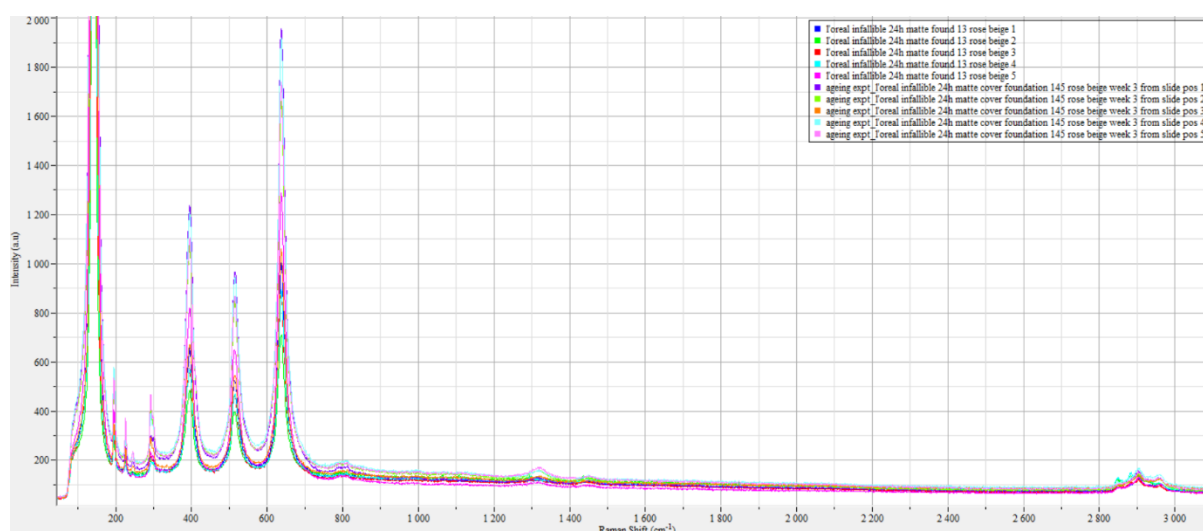


Figure 5.41: Raman spectra (unprocessed) of two L'Oreal Infallible Foundations in shade 145/13 Rose Beige (magnified to truncate lattice peak); one purchased in 2020 and the other in 2023, both analysed after 14 days of air drying on a glass slide.

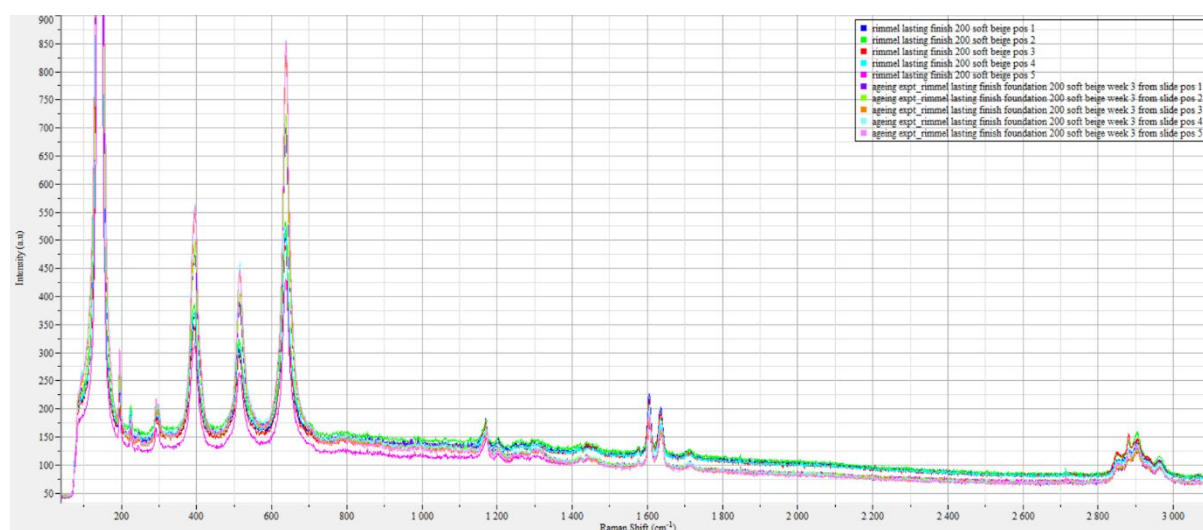


Figure 5.42: Raman spectra (unprocessed) of two Rimmel Lasting Finish Foundations in shade 200 Soft Beige (magnified to truncate lattice peak); one purchased in 2019 and the other in 2023, both analysed after 14 days of air drying on a glass slide.

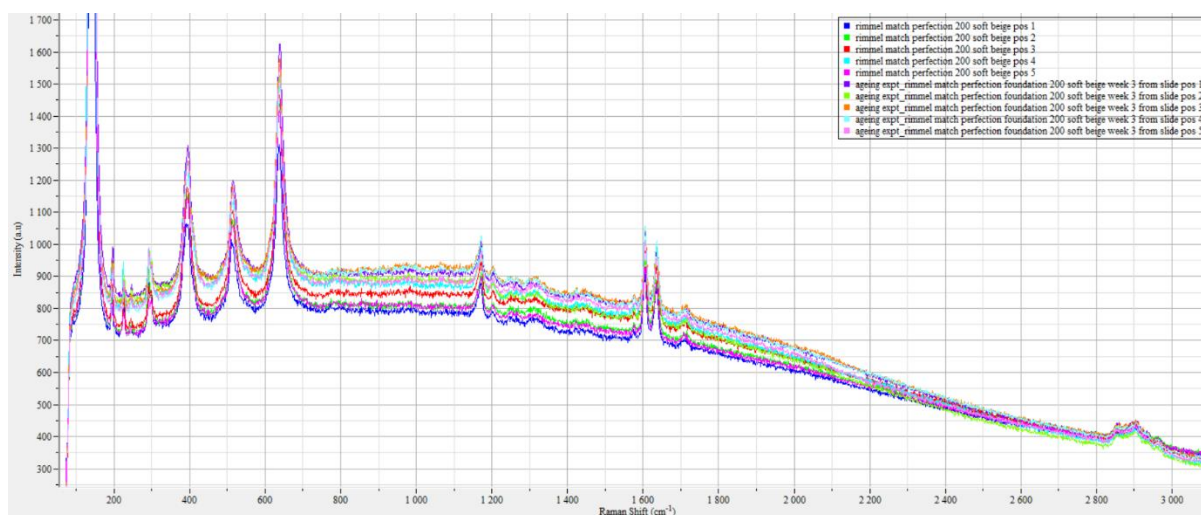


Figure 5.43: Raman spectra (unprocessed) of two Rimmel Match Perfection Foundations in shade 200 Soft Beige (magnified to truncate lattice peak); one purchased in 2019 and the other in 2023, both analysed after 14 days of air drying on a glass slide.

5.3.4 Exploratory data analysis

It was decided that owing to the dataset treatments in Chapter 3, there was little added benefit to creating an overall PCA model of all seven products analysed in this Chapter. Instead, the products should be subjected individually to PCA, in order to better model the relative differences in ageing between the seven products. Therefore, a separate PCA matrix was created for each foundation by importing into the Unscrambler software all five replicate Raman spectra for both the aliquots “*from bottle*” and the “*from slide*” samples, at every time interval. Category variables were then setup in order to perform grouping within the resultant models according to whether they represented the “*from bottle*” or “*from slide*” spectra, the time interval, or a combination of the two variables. The same pre-processing technique described in Chapter 2, a Standard Normal Variate (SNV), was used to transform the data, to maintain consistency across all sections of this project.

Initially PCA models were created using only the “*from bottle*” spectra for each sample and then projecting the “*from slide*” spectra onto those models. However, it was found that due to the low number of “*from bottle*” spectra, the resultant PCA models exhibited poor explained variances and validation errors. Furthermore, once the “*from slide*” spectra were projected onto those calibration data, the residual variances showed poor congruence between the two datasets, (indicating a poor fit to the calibration model), particularly where the products had been more affected by the ageing process. Thus, it was decided that all spectra for each foundation would be used to construct the respective PCA models for maximum data mining purposes.

Scree plots are not included but the values for the first three PCs are given. The loadings plots for each product were fairly featureless after PC1, with many of them only showing the “*lattice*” peak as the discriminating spectral peak, therefore these are omitted from this Chapter. Owing to the lack of discriminating features shown in the loadings plots from PC2 onwards, only PC1 vs. PC2 scores plots are included.

5.3.4.1 BareMinerals BarePro Foundation

The BareMinerals PCA scree plot revealed that 68% of the data was accounted for in the first PC, rising sharply to 97% for PC2 and to 99% for PC3. This indicated the model was reliable and carried meaningful information, and also that inclusion of further PCs was unnecessary. It should be noted at this point that owing to a fault with the Raman spectrometer during acquisition of the week 9 “*from slide*” spectra, only two of the five replicates could be obtained and therefore the model may be skewed by the lack of data at this time interval.

As can be seen in the scores plot in Figure 5.44, there is a distinct separation between spectra collected from different origins, with very little overlap. All spectra taken from bottle aliquots over time are associated with positive scores along PC1 and differ only by their PC2 score. The loadings for PC1 relate to the anatase response and the uniformity of the baseline, with negative scores indicating fluctuations in the baseline. This corroborates what was seen spectrally in Section 5.3.3.2, with the degree of baseline fluorescence increasing and becoming more unstable with advanced ageing on the slide.

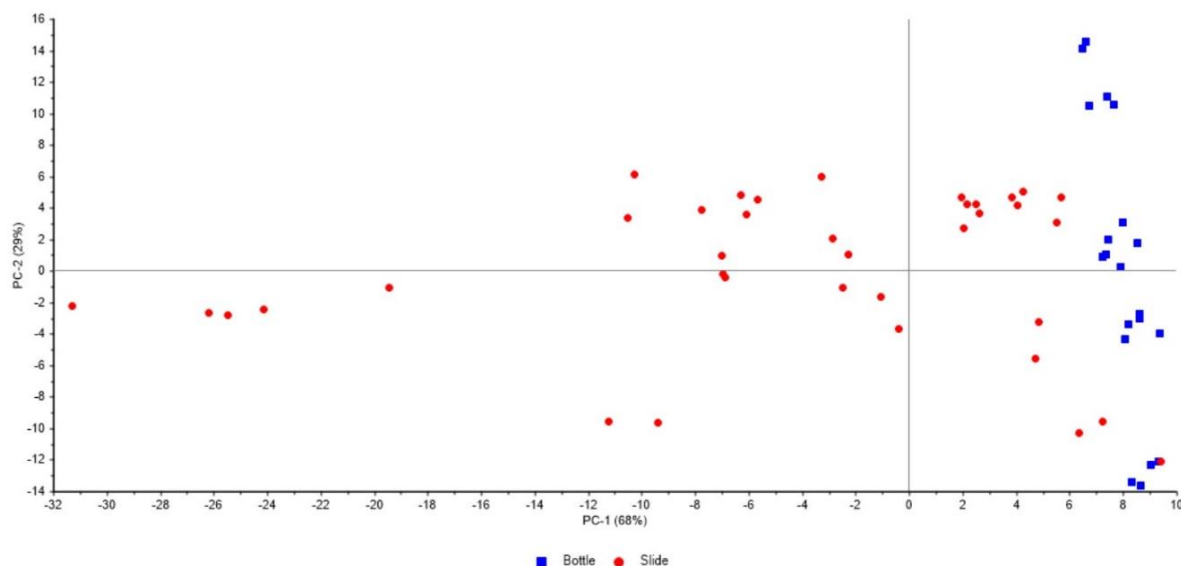


Figure 5.44: PCA scores plot (PC1 vs. PC2) of all spectra from the BareMinerals BarePro Foundation, grouped by origin.

Further sample grouping to account for the time interval reveals that it is indeed the oldest sample aliquot on the slide which has the most negative score along PC1, and thus appears at the extreme left of the plot (Figure 5.45). The magnitude of the negative score itself also tells us that that the spectra are significantly different to the rest; again, this corroborates what was seen spectrally. Replicate spectra cluster well indicating that regression modelling may be an appropriate next step¹².

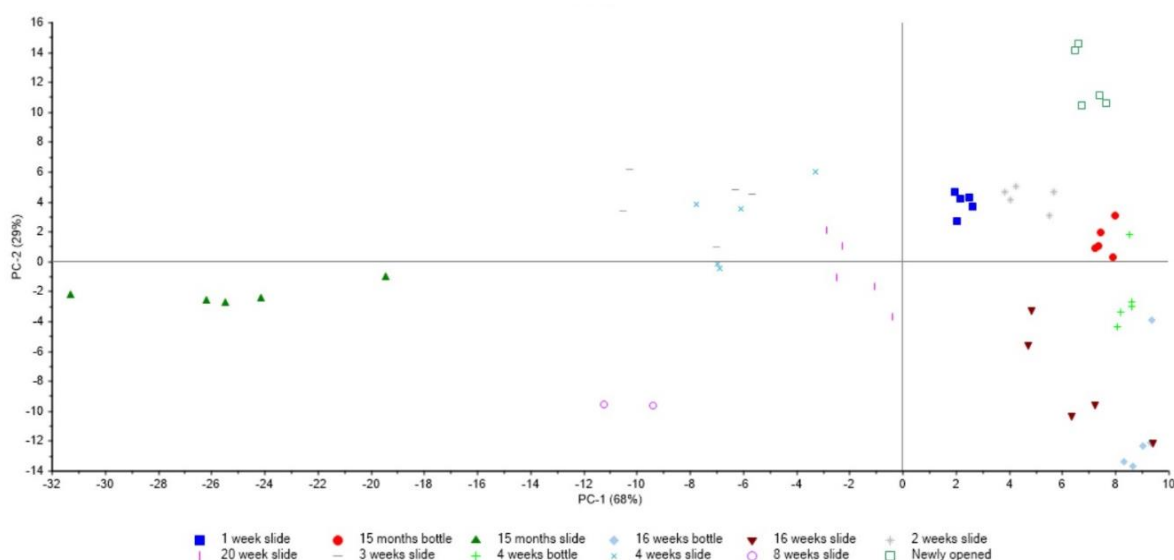


Figure 5.45: PCA scores plot (PC1 vs. PC2) of all spectra from the BareMinerals BarePro Foundation, grouped by time interval and origin.

Another trend that can be ascertained from this plot is that the product seems to be changing very slightly within the bottle after opening, with the spectra relating to the newly opened product having the highest scores along PC2 and subsequent aliquots taken from the bottle showing a decrease in PC2 scores. This is an important point to note for two reasons: firstly, because this suggests that the product is chemically changing within the sealed packaging after opening, which adds an additional variable and another layer of complexity to forensic cosmetic analysis, and secondly, because the spectral variation between these replicates was undetectable by eye and it was assumed that the product was not altering within the original packaging. This highlights the superior data analysis achieved when combining analytical techniques with chemometric methods.

The spectra taken from both the bottle and the slide after 16 weeks of ageing, and the two spectra taken after eight weeks of ageing, may be anomalous owing to their positions on the scores plot. As previously stated, there was a spectrometer fault during acquisition of the week 9 “on slide” sample, which meant that no data could be collected as planned for the 12-week

ageing interval. Thus, the next set of data collected were at the 16-week interval (this applied to all samples, not just the BareMinerals product) but the fault may have also adversely affected the week 17 spectra. Inspection of the influence plot reveals that one of the two spectra acquired for the week 9 “on slide” sample, bears a high residual value in both PC3 and PC4, meaning that it is not poorly modelled. Therefore, the spectra taken just before the spectrometer fault may also be dubious.

5.3.4.2 Bourjois Healthy Mix Foundation

The explained variance plot for PCA of the Bourjois foundation gave cumulative values of 81%, 86%, and 89% for the first three PCs. Whilst PC4 accounted for a further 2% variance, inspection of the loadings indicated the onset of random noise and therefore, error. Figure 5.46 shows a good degree of discrimination between spectra taken from the “from bottle” and “from slide”, with all the “from bottle” spectra having negative scores for PC1 and the majority of the “from slide” spectra having positive scores along PC1. The loadings for PC1 are dominated by the rutile spectral pattern, therefore it can be inferred that the “from slide” spectra feature a heavier presence of rutile relative to the other spectral features. This is exactly what we see in the spectral series and which was discussed in Section 5.3.3.2.

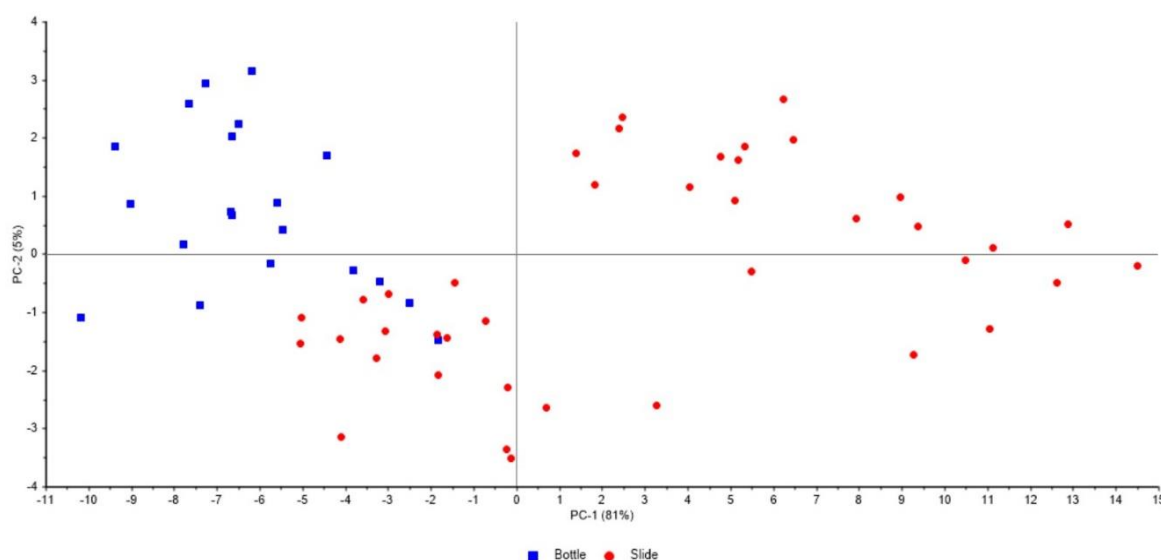


Figure 5.46: PCA scores plot (PC1 vs. PC2) of all spectra from the Bourjois Healthy Mix Foundation, grouped by origin.

Sample grouping by both origin and time interval confirms that the spectrum with the highest PC1 score belongs to the sample aged for the longest time: 15 months (Figure 5.47). However, this plot also shows that for most of the replicates the spectral repeatability has decreased, when compared with the BareMinerals foundation discussed in the previous Section. This

perceived decrease in repeatability may be due to a slightly more heterogeneous matrix or due to the titanium dioxide polymorph used in the formulation. As has been previously discussed in Chapter 3, the Raman spectrum for anatase features an incredibly intense “*lattice*” peak that dominates the spectrum but is absent in the Raman spectrum for rutile. This means that small spectral differences elsewhere in an anatase-containing spectrum may be effectively down-weighted but will not in the absence of the “*lattice*” peak, i.e. in a rutile-containing spectrum. Here, small spectral differences may be amplified, giving rise to the lower repeatability observed. This decrease in repeatability is most marked for the sample that was aged for eight weeks (week 9 “*from slide*”), which correlates with the time of the spectrometer fault, and could be a result of decreased instrumental performance just prior to the fault being detected.

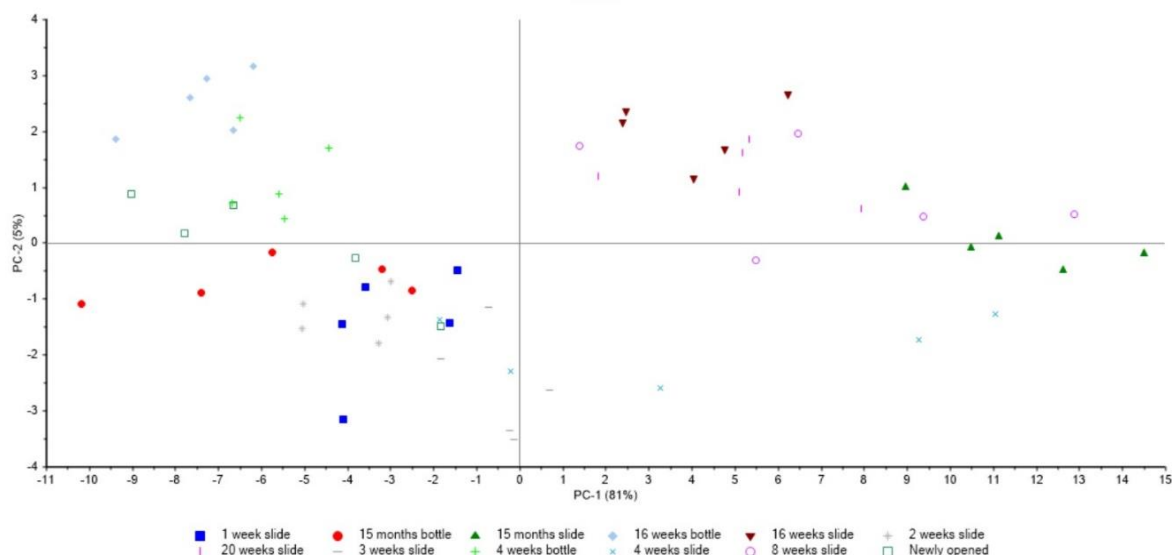


Figure 5.47: PCA scores plot (PC1 vs. PC2) of all spectra from the Bourjois Healthy Mix Foundation, grouped by time interval and origin.

Despite the perceived decrease in spectral repeatability (or sample heterogeneity issues), there appears to be less chemical change taking place within the packaging itself after product opening; the sample aliquots taken at varying time intervals appear consistent, indicating that ageing within the product packaging is less of an issue. Whether this relates to the specific chemistry of the product, the preservatives used, or the actual packaging itself, cannot be concluded from these limited data, but it warrants further investigation.

5.3.4.3 L'Oreal Infallible Foundation

In keeping with the visual analysis of the spectral data, there was very little variability amongst the various spectra taken for the L'Oreal foundation. The explained variance plot corroborates this with cumulative values of 95%, 99%, and 100% for the first three PCs. Contrary to the previous two products discussed, there is no definite separation here between spectra emanating “*from bottle*” or “*from slide*” (Figure 5.48). What is particularly curious though, is the outlying eight spectra on the far right of the scores plot.

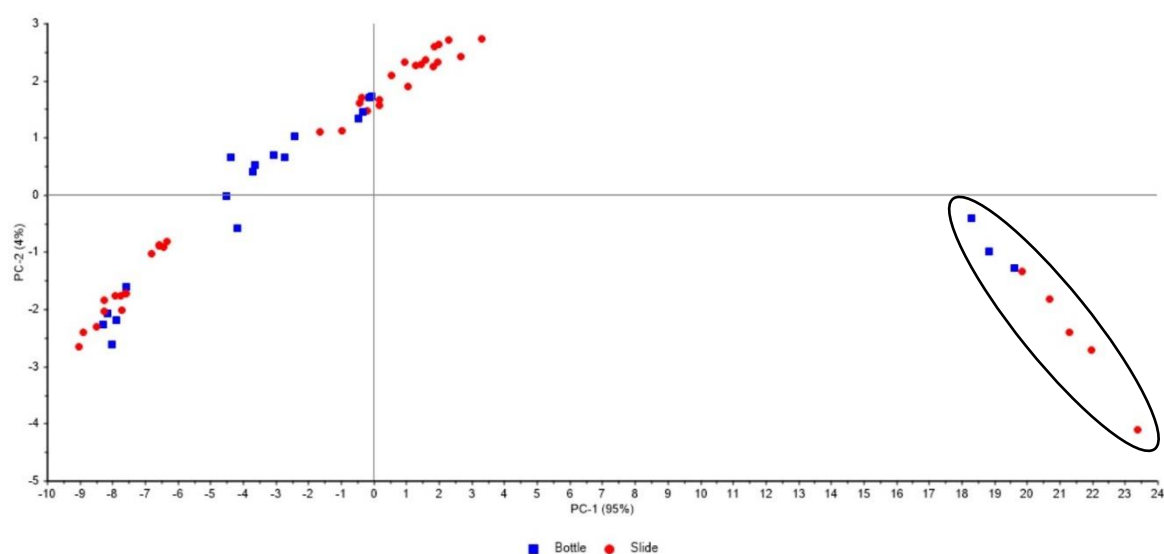


Figure 5.48: PCA scores plot (PC1 vs. PC2) of all spectra from the L'Oreal Infallible Matte Cover Foundation, grouped by origin. Outlying spectra marked with the black ellipse.

Sample grouping by time interval and origin (Figure 5.49) revealed that they all relate to spectra taken at the 17-week interval (representing 16 weeks of ageing) and therefore might also have been adversely affected by the spectrometer fault detected at the 9-week interval. However, two of the five replicates taken of the “*from bottle*” sample after 16 weeks of ageing, group with the majority of the spectra on the left-hand side of the scores plot, perhaps indicating that this separation is due to sample heterogeneity, despite this not being apparent from the spectra themselves. With the exception of the replicate spectra taken from the “*from bottle*” aliquots at the 17-week interval, the scores plot demonstrates a good level of experimental repeatability for this product at the various time intervals.

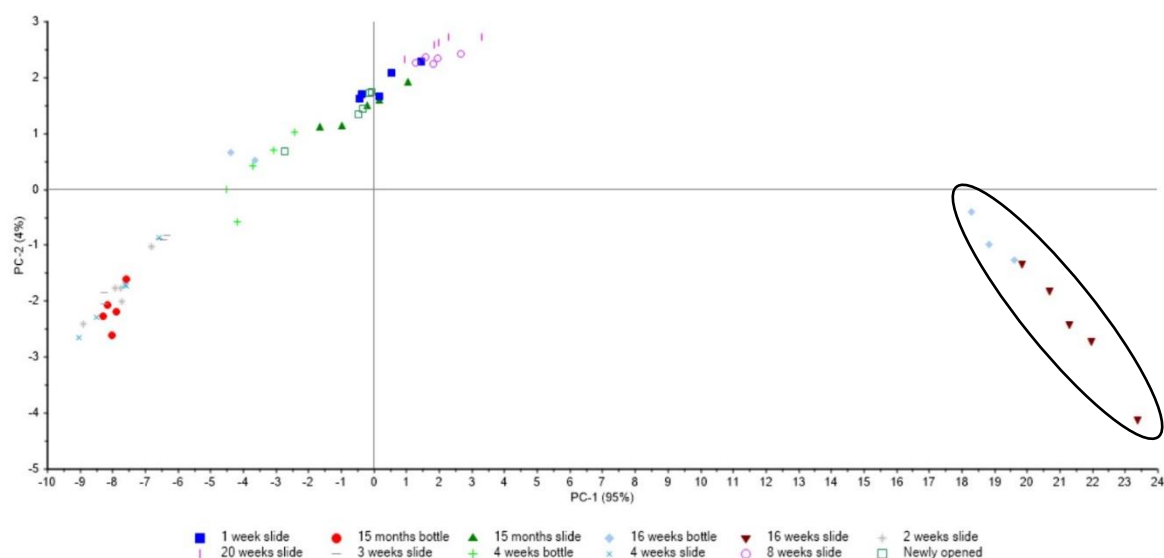


Figure 5.49: PCA scores plot (PC1 vs. PC2) of all spectra from the L'Oreal Infallible Matte Cover Foundation, grouped by time interval and origin. Outlying spectra marked with the black ellipse.

In an attempt to remove potential data points that might perturb the model, the eight outlying spectra were marked and the PCA was recalculated without these marked samples. The resultant score plot looked much better, with samples evenly spread about the origin as can be seen in Figure 5.50.

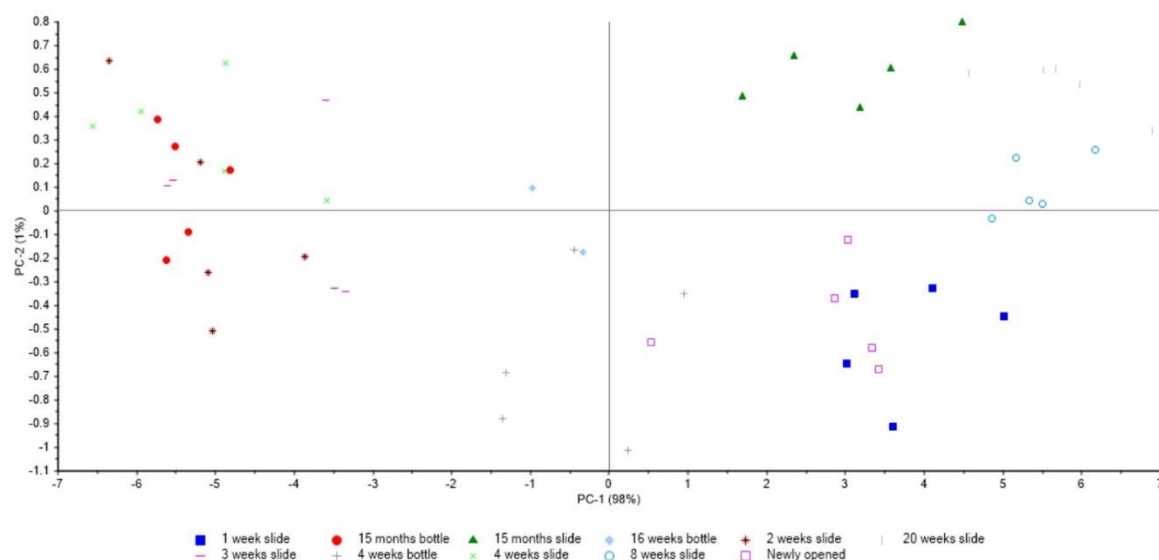


Figure 5.50: Recalculated PCA scores plot (PC1 vs. PC2) with outlying spectra removed for the L'Oreal Infallible Matte Cover Foundation, grouped by time interval and origin.

Nonetheless, the revised cumulative explained variance values (PC1 98%, PC2 99%, PC3 99%) show that the model contains no additional structure and is starting to introduce noise. The recalculation has also removed a lot of the model variability, indicating that whether those eight samples were outliers or not, their removal has not benefitted the resultant model, albeit has improved the accuracy.

5.3.4.4 Max Factor Miracle Pure Foundation

The Max Factor foundation showed the same peculiar cluster of outliers in the same region of the scores plot as the L'Oreal foundation in the previous Section (Figure 5.51). Surprisingly, these outliers were also found to be the “*from slide*” spectra, taken at the same time interval (16 weeks), casting further doubt on the validity of this spectral subset (Figure 5.52). Initial cumulative explained variance values were: PC1 86%, PC2 93%, PC3 99%, indicating a robust model. The same PCA recalculation without marked samples operation was carried out and this altered the cumulative variance to PC1 80%, PC2 96%, and PC3 97%. Whilst this shows the first three PCs of the recalculated model no longer account for as much of the structure in the data, it was found that the validation values tracked those of the calibration much better, indicating fewer errors found during the cross-validation procedure.

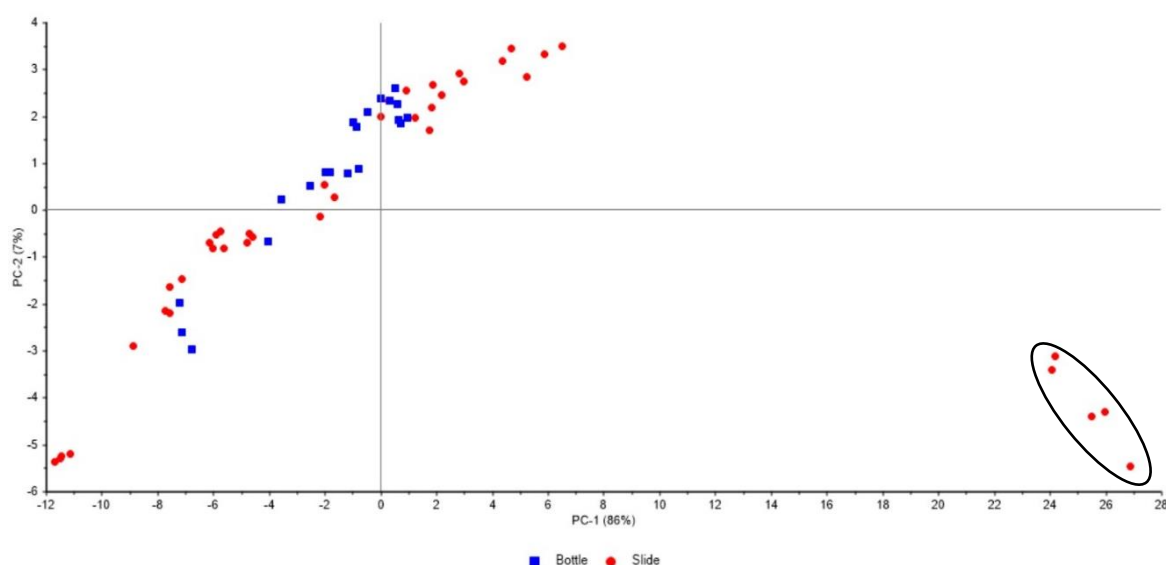


Figure 5.51: PCA scores plot (PC1 vs. PC2) of all spectra from the Max Factor Miracle Pure Foundation, grouped by origin. Outlying spectra marked with the black ellipse.

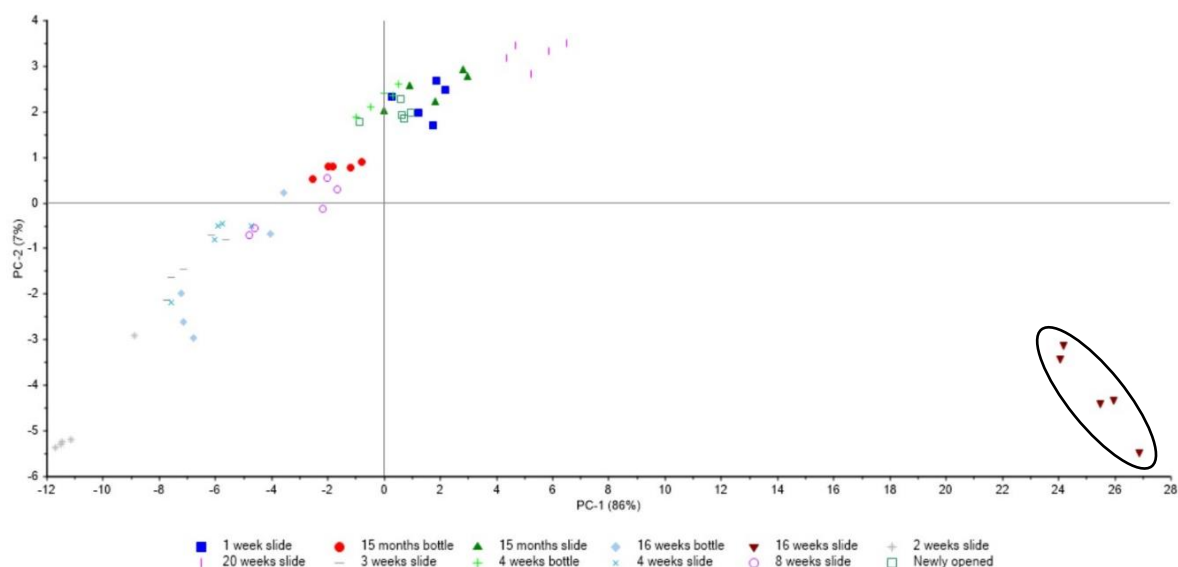


Figure 5.52: PCA scores plot (PC1 vs. PC2) of all spectra from the Max Factor Miracle Pure Foundation, grouped by time interval and origin. Outlying spectra marked with black ellipse.

The revised PCA scores plot shows a more symmetrical spread of data and facilitates a better trend assessment (Figure 5.53). Whilst there is still no clear divide between the “from bottle” and “from slide” spectra, it is now apparent that the “from bottle” spectra cluster in the centre of the plot, leaving the “from slide” spectra dotted around the edges. This lack of obvious ageing effect is echoed with the persistent greasy visual appearance (Figure 5.18) and the minimal spectral changes discussed in Section 5.3.3.2.

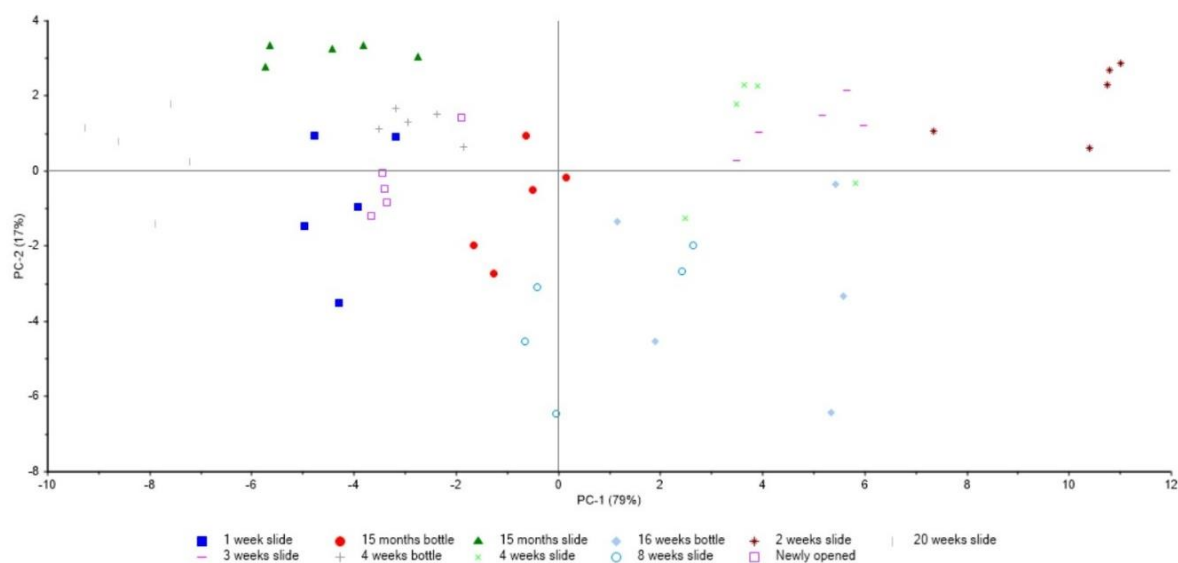


Figure 5.53: Recalculated PCA scores plot (PC1 vs. PC2) with outlying spectra removed for the Max Factor Miracle Pure Foundation, grouped by time interval and origin.

5.3.4.5 Maybelline Superstay 30H Foundation

The explained variance values for the PCA of the Maybelline foundation were surprisingly low with only 52% of the data's structure modelled in PC1, rising to 75% when adding PC2, and to 88% with the addition of PC3. Despite this apparent lack of structure retained within the first three PCs the resultant scores plot displays a clear distinction between spectra obtained “*from bottle*” and “*from slide*” along PC2, as is evident in Figure 5.54.

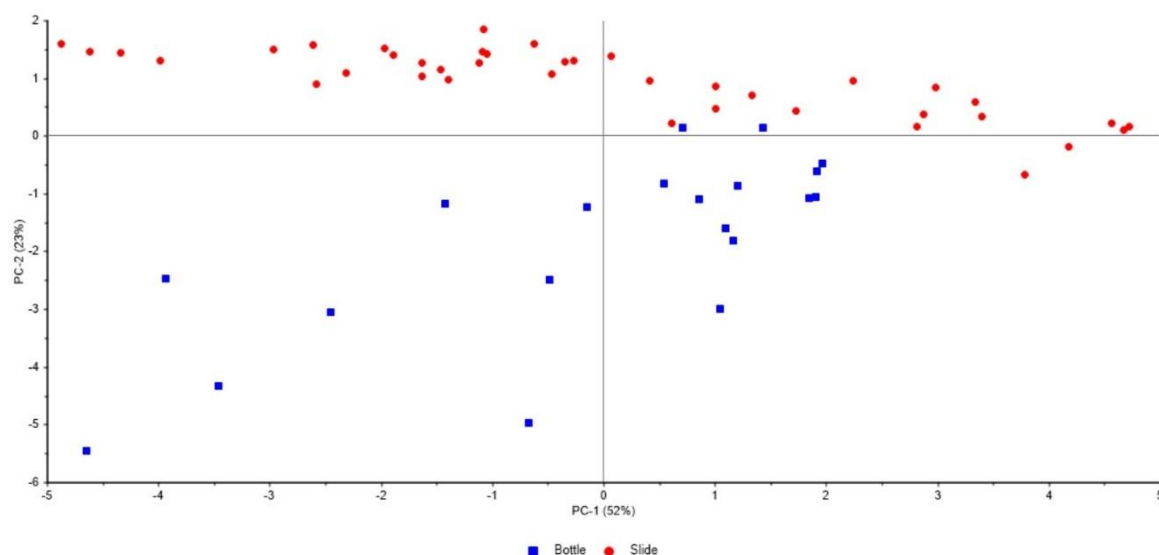


Figure 5.54: PCA scores plot (PC1 vs. PC2) of all spectra from the Maybelline Superstay 30H Foundation, grouped by origin.

The loadings for PC2 show a positive correlation with the rutile peaks and a negative correlation with the doublet peak in the CH region, ergo, spectra that appear along the negative scores for PC2 will have relatively smaller rutile peaks and larger “CH” region peaks. This is exactly what we see when comparing the spectra (Section 5.3.3.2): ageing seems to result in a loss of silicones and/or organics from the product matrix, increasing the relative intensity of the mineral peaks.

Application of the combined sample grouping reveals a trend across PC1 for both the “*from bottle*” and “*from slide*” spectra: the newly opened and freshly deposited samples are associated with negative PC1 scores, but as the samples age (either in the bottle or on the slide) the spectra move to the right and their PC1 scores become positive (Figure 5.55). The loadings for PC1 show positive correlations for both increased mineral content (rutile and iron oxide pigments) and increased baseline fluorescence, corroborating the spectral changes seen with increased product ageing. It should be noted here that the amount of mineral content is not increasing as the product ages, more that the spectral contribution from non-mineral

sources (that may be via evaporation or degradation) is decreasing and therefore the relative intensities of these components are changing.

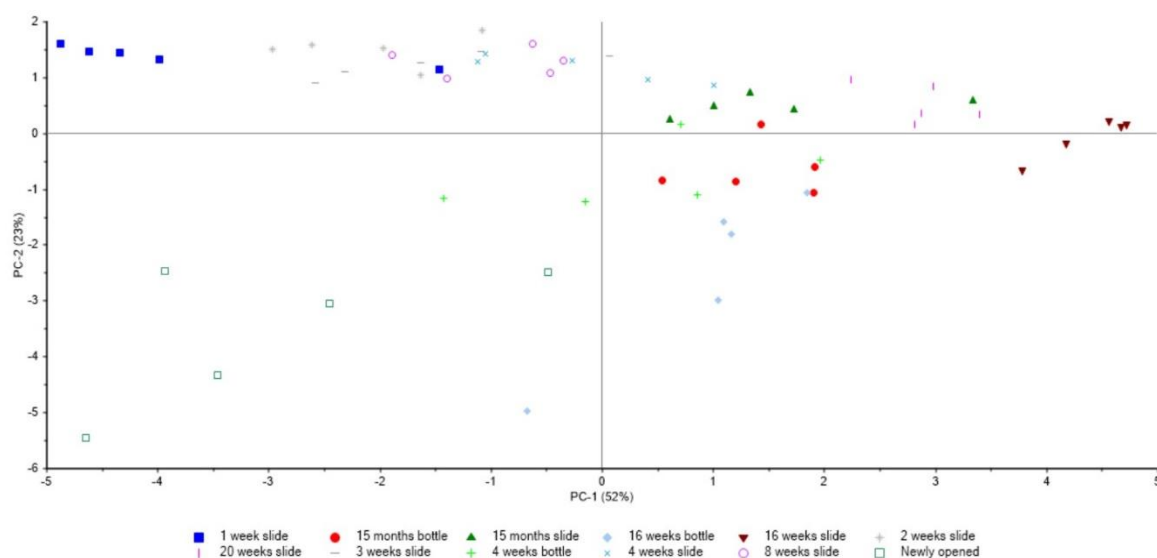


Figure 5.55: PCA scores plot (PC1 vs. PC2) of all spectra from the Maybelline Superstay 30H Foundation, grouped by time interval and origin.

An additional finding was that the repeatability for the “*from bottle*” spectra was inferior to the “*from slide*” spectra, with the “*newly opened*” product being the worst culprit. This is likely due to evaporation of volatile components in the minutes following an aliquot being taken from the packaging, whereas this would not represent as much of an issue if the sample had been air drying on a glass slide for a period of time.

5.3.4.6 Rimmel Lasting Finish Foundation

The same scores plot problem that was seen with the L’Oreal and Max Factor foundations was also observed with the Rimmel Lasting Finish Foundation. A cluster of highly loaded spectra in the top right corner of the plot compressed and skewed the rest of the data to the left-hand side of the plot (Figure 5.56). With this in mind and contrary to expectations, cumulative explained variance values for this PCA were 82%, 93%, and 99% for the first three PCs, indicating a model that contained plenty of data structure. Furthermore, cross-validation values showed good correlation with the calibration data, confirming a robust model. Nonetheless, these extreme samples will have strongly influenced the direction and magnitude of PC1 so their removal is warranted.

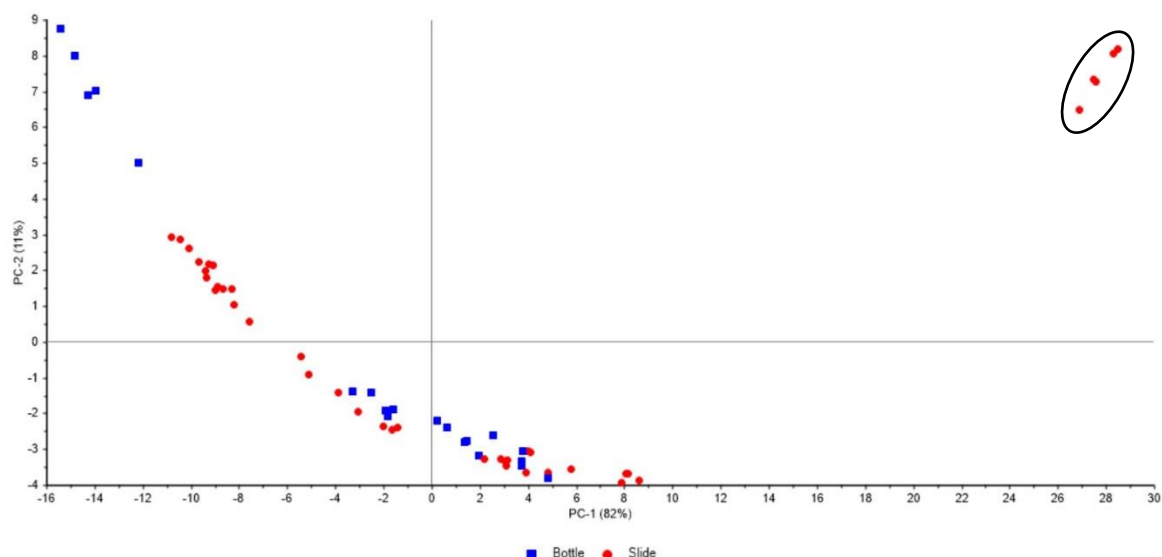


Figure 5.56: PCA scores plot (PC1 vs. PC2) of all spectra from the Rimmel Lasting Finish Foundation, grouped by origin. Outlying spectra marked with the black ellipse.

Figure 5.57 confirms that the highly loaded spectra belong to the same category as the previous outlying spectra: the aged for 16 weeks “from slide” samples. Hence, these five replicate spectra were removed from the model and the PCA recalculated. Revised explained variance values of 82%, 97%, and 99% for the first three PCs were reported. In addition, the loadings for PC2 looked far more meaningful, verified by the increase in explained variance for PC2.

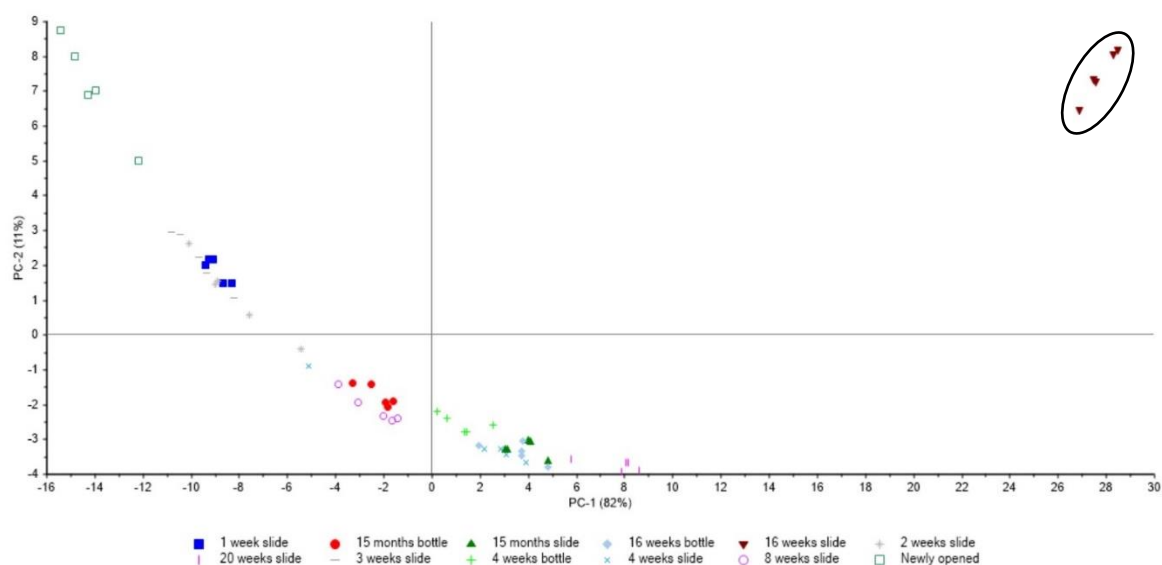


Figure 5.57: PCA scores plot (PC1 vs. PC2) of all spectra from the Rimmel Lasting Finish Foundation, grouped by time interval and origin. Outlying spectra marked with black ellipse.

In the original PCA scores plot (Figure 5.56) there did not appear to be a trend when sample grouped by origin, however, in the recalculated version (Figure 5.58) all the product spectra taken “*from slide*” have positive PC2 scores, while the majority of “*from bottle*” have negative scores. PC2 loadings show a heavily weighted negative correlation to the spectral region thought to relate to lecithin content, and a moderate positive correlation to peaks in the “CH” region (2800-3000 cm^{-1}). This confirms the spectral assessment; the lecithin peak is only visible when the product is taken directly “*from bottle*” but disappears within the first week of ageing. Therefore, all the “*from bottle*” spectra should possess this broad peak and conversely, all the “*from slide*” spectra should be devoid of this peak.

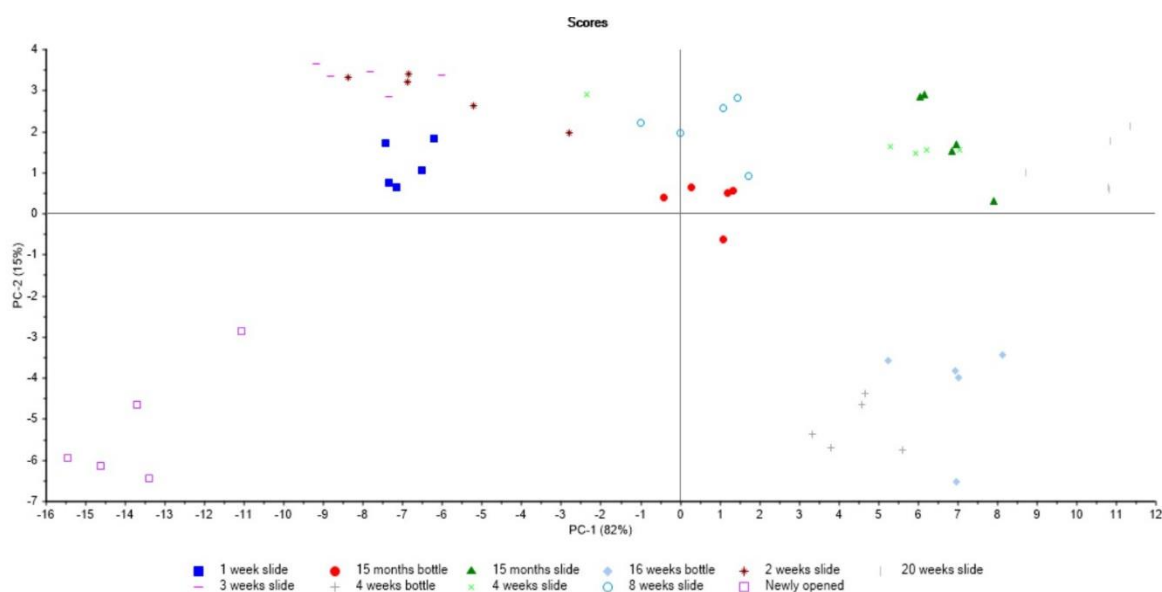


Figure 5.58: Recalculated PCA scores plot (PC1 vs. PC2) with outlying spectra removed for the Rimmel Lasting Finish Foundation, grouped by time interval and origin.

There is also separation seen across the various time intervals, with a gradual movement across PC1 (negative to positive scores) as ageing progresses, for both sample origins. The loadings for PC1 are relatively featureless and only show a positive correlation to the “*lattice*” peak. Given that titanium dioxide is the third most populous ingredient in this product (here it is used as an active ingredient, as a mineral sunscreen, rather than a white pigment), small fluctuations in relative titanium dioxide content caused by the ageing process will be readily apparent.

5.3.4.7 Rimmel Match Perfection Foundation

PCA of the Rimmel Match Perfection Foundation gave a scores plot that initially appeared to be unaffected by outliers (Figure 5.59). Cumulative explained variances were 67%, 90%, and

97% for the first three PCs, with good validation to calibration agreement. Sample grouping by origin demonstrated a pleasing separation between the “*from bottle*” and “*from slide*” spectra, with all but one of the “*from bottle*” spectra falling within the negative values along PC2 and the majority of “*from slide*” spectra appearing in the positive region of PC2 (Figure 5.59).

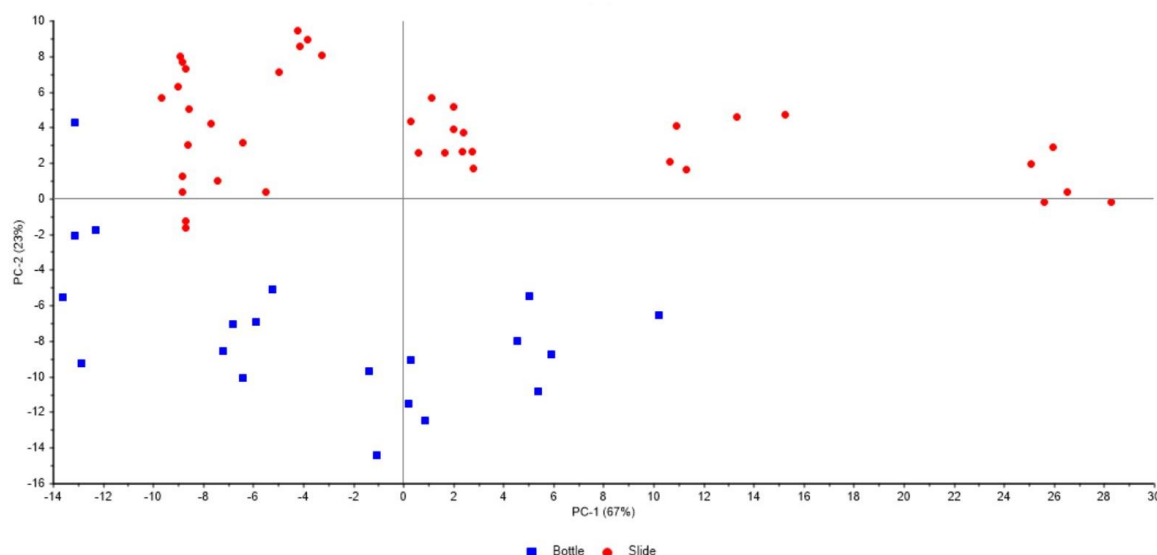


Figure 5.59: PCA scores plot (PC1 vs. PC2) of all spectra from the Rimmel Match Perfection Foundation, grouped by origin.

Inspection of the PC2 loadings revealed similar variables and weightings to the Rimmel Lasting Finish Foundation discussed in the previous Section: a strong negative lecithin response and an even stronger positive “*lattice*” peak. One can infer from this that the aliquots taken “*from bottle*” all displayed the broad lecithin peak but a less intense “*lattice*” peak and the opposite would be true for the aged samples on slides. This can be verified by visual inspection of the relevant spectra and is discussed further in Sections 5.3.3.1 and 5.3.3.2.

Application of sample grouping by both origin and time interval revealed some interesting observations. Firstly, each set of five replicate spectra clustered well together, indicating a good level of repeatability and secondly, the potentially erroneous spectra collected after 16 weeks of ageing did not appear to be extreme in this case, nor did they appear to perturb the model (Figure 5.60). Nonetheless, their removal from the model was trialed and the resultant recalculated PCA was investigated. The explained variance for PC1 decreased to 55% and the validation accuracy also suffered. Furthermore, the revised scores plot appeared almost identical to the original version, suggesting that the 16-week aged spectra did not warrant removal in this case.

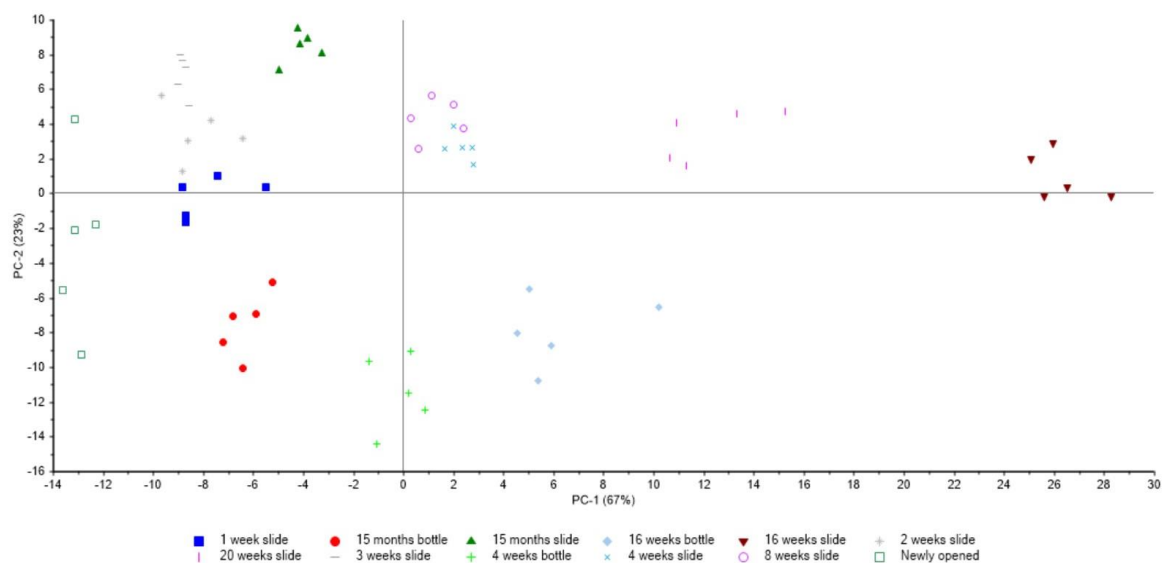


Figure 5.60: PCA scores plot (PC1 vs. PC2) of all spectra from the Rimmel Match Perfection Foundation, grouped by time interval and origin.

There does not seem to be a definite trend across PC1 in respect of sample ageing; the 15-month aged “from slide” spectra appear closer to the 1-week, 2-week, and 3-week aged “from slide” samples than the 4-week, 8-week, 16-week, and 20-week aged “from slide” samples. The same lack of trend across the aliquots taken “from bottle” is also demonstrated. Loadings for PC1 show only a positive correlation for the “lattice” peak, suggesting that the intensity of the “lattice” peak is variable and not necessarily related to the ageing process.

5.4 Conclusions

This Chapter investigated the effects of passive ageing (air and natural/office light) on seven new cosmetic foundations purchased from the UK market. The samples were deliberately chosen to represent the same product type, formulation, and shade and differed only by product line. The samples were analysed directly upon product opening to assess the extent of product drying and/or degradation over time and were not permitted to dry as samples had been throughout Chapters 3 and 4.

An improved optical microscope was purchased during this project, which enabled colour photomicrographs of a far better resolution and higher magnification to be obtained for the visual discrimination of the samples. Visual analysis of each product was carried out for both the aged samples deposited onto glass slides, and from fresh aliquots taken from the product packaging at various time intervals.

Across all samples the most obvious signs of ageing were detected after one week of deposition onto the glass slides. After 15 months of ageing, the most marked difference in visual appearance was seen among the foundations marketed towards oily skin types or those claiming to be “*long-lasting*” formulations. Given that these products were much lower in oil content, this indicated a loss of water from the matrix.

At 15 months ageing, the Bourjois sample had significantly discoloured; this would have significant implications for colour assessment in forensic casework. At the same time interval, the L’Oreal sample had dried so substantially that the spherical borosilicate glass interference pigments were detaching from the overall matrix, and the Max Factor sample had dried enough such that silica microspheres were evident. Again, this has implications for casework as these spherical microparticles may become separated from their deposited trace and transferred elsewhere. The Rimmel Match Perfection sample revealed the presence of multiple mineral crystals after 15 months of ageing. These appeared in various cubic and fibrous structures and denoted the inclusion of montmorillonite and sepiolite respectively.

Raman spectra taken of each sample “*from bottle*” showed excellent repeatability and product stability over the 15-month period. However, spectra taken of the deposited samples “*from slide*” revealed striking differences between the seven products. Spectral changes were almost undetectable for the L’Oreal and Max Factor samples, presumably due to a loss of weakly Raman scattering water molecules, while the Bourjois and Maybelline samples exhibited increases in the relative mineral peak intensities. Both Rimmel samples experienced a loss of their lecithin content denoted by the absence of the broad distinguishing peak, but the Rimmel Match Perfection sample also suffered a loss or conversion of other organic components. The most significant spectral change over 15 months was demonstrated by the BareMinerals sample, which saw a huge increase in baseline fluorescence that was not readily overcome via photobleaching.

Inter-batch variation studies were undertaken for six of the seven samples by comparison with the 2-week aged spectra. Surprisingly, the Bourjois and Maybelline products displayed an obvious switch from the anatase to the rutile polymorph, despite this not being apparent from the packaging. No discernible differences were observed between batches of the BareMinerals, L’Oreal, or either of the Rimmel products, suggesting consistent formulations.

Individual PCA models were created for each sample, with category variables relating to origin (“*from bottle*” or “*from slide*”) and ageing interval set up. Interestingly, slight variations between the “*from bottle*” spectra were apparent on the scores plots, despite these changes being imperceptible by eye and reinforces the value of chemometric methods when analysing spectral data.

Clear separations between the “*from bottle*” and “*from slide*” spectra were evident for four of the samples (BareMinerals, Bourjois, Maybelline, and Rimmel Match Perfection), but not for the other three (L’Oreal, Max Factor, and Rimmel Lasting Finish).

A fault with the Raman spectrometer at the 9-week interval seemed to cause some spectral discrepancies amongst the 9-week and 17-week spectra (and also prohibited acquisition of the 13-week interval spectra) denoted by outlying samples in the PCA scores plots. Removal of these outliers and subsequent PCA recalculations resulted in vastly improved scores that exhibited better sample clustering.

The excellent sample clustering according to origin and age observed among replicates for the BareMinerals and both Rimmel products indicate that they may be suitable for regression modelling techniques, such as partial least squares regression (PLSR).

5.5 References

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Chapter Six: Raman microspectroscopic analysis and chemometric interpretation of donated facial cosmetic samples – insights into “*real world*” samples

6.1 Introduction

After assessing the extent of chemical variability across a variety of facial cosmetic products (Chapter 3), the impact of sample heterogeneity on Raman spectral repeatability (Chapter 4), and the controlled ageing of a selection of foundations (Chapter 5), the next aim was to ascertain how cosmetic samples from a “*real-world*” population might be expected to behave and how their Raman spectra would compare to the models created for the new samples.

This study examined old, donated samples, discarded shop testers, and finished products that were destined to be thrown away. The exact ages of the samples were unknown; the shop testers were being discarded at the three-month interval but all other donated samples (from friends and family) could not be dated accurately. It is known, however, that some of these donations were over ten years old. In addition, this study included 120 different samples spanning various brands, representing all seven product types, all five formulation types, and a range of different shades.

6.2 Experimental

The same method used throughout the project was applied to this batch of samples. All samples were prepared, analysed and characterised as described in the experimental section of Chapter 2.

6.2.1 Samples

For this Chapter, facial cosmetic samples were acquired via donations: from friends and family, as expired testers from a local Boots store, or as finished products with minimal residue remaining. The sample population consisted of 120 products that comprised 84 foundations, 16 concealers, seven tinted moisturisers (including BB and CC creams), two finishing powders, two bronzers, three highlighters and six primers (a full list is given in the appendix).

A large proportion of these donated samples (56) were the expired testers; as such, the range of brands, product types and shades could not be chosen. Nonetheless, it did mean that there were batches of samples from the same manufacturer and product line that differed only by shade. This enabled the comparison of spectra for differences in pigment concentration. The sample set spanned across 22 different brands and within those brands, 78 different product

lines were represented. As was the case with the sample sets used in Chapters 3 and 4, these samples also ranged from the budget market (average product cost £1.00), through to the mainstream market (average product cost £12.00) and up to the premium market (average product cost £39.00).

The full range of product formulations were represented across the sample set, with 94 liquids (including gel consistencies), 14 mousses (aerated lightweight semi-solid), four creams (either stick based or compact style), three loose powders, and five pressed powders.

6.3 Results and discussion

6.3.1 Preliminary considerations

Armed with the knowledge gained in Chapter 5 that foundations degrade over time after opening and if left exposed to the air, it was reasonable to assume that many, if not all of the donated samples' chemistry would have altered from their original formulations. The degree of chemical alteration or sample decomposition would not only be affected by the sample's age (or the amount of time the product had been open) but also the product type. For example, powdered cosmetics can be expected to have better stability over time than their liquid counterparts; similarly, anhydrous formulations resist degradation far better than aqueous formulations, albeit can be prone to oil rancidity.

In addition to the age of the product (which could not be accurately determined for any of the donated samples), the amount of use would also play a part in its level of degradation or chemical alteration, as it can be expected that the product would have been open for longer if used more frequently. Amongst the expired testers that were donated as a series of varying shades, it was common to note increased usage of the medium shades and lower usage of the extreme shades, i.e. the very pale or very dark shades. One can infer from this that the medium shades would have been opened more frequently, and therefore exposed to the air or contamination sources for longer, potentially increasing sample degradation. Unfortunately, due to the unknown time intervals and usage of each product, it would be impossible to state whether spectral differences arose as a result of ageing/use or were intrinsic to that sample.

Undoubtedly, all samples would have been exposed to varying degrees of contamination either via the introduction of foreign substances or by sample mixing. As was the case with sample age and usage, both the level of contamination and the type thereof, was unknown. This would have implications for spectral interpretation and subsequent chemometric modelling, as it is possible that this extraneous information would interfere with the sample spectrum. Furthermore, given that the source of any contamination would be unknown, reference samples could not be used to perform spectral subtractions. It is also important to

mention that shop testers are often not treated with the same amount of care as consumers' own products; for example, lids not closed properly, products mixed within containers or sampled with dirty hands, and therefore may represent a worst-case scenario.

There may be storage variations to consider as well. The shop testers are subjected to fluorescent lighting but not often to natural light or UV radiation, as privately owned products might. Testers are also stored in much smaller containers than their full-sized counterparts, and often do not have an airtight lid. Fluctuations in ambient temperature and relative humidity may impact on cosmetic samples. These are not variables that have been investigated during this thesis but it is reasonable to assume they would have an effect on products nonetheless. It is also worth noting that certain cosmetic packaging is more effective at maintaining formulation stability and mitigating sample degradation, such as opaque containers that reduce UV exposure, or pump style dispensers that minimise air exposure and product contamination.

Whilst these sample variances cause difficulty in modelling spectral differences and proposing reasons for chemical discrepancies seen, the donated sample set does represent trace evidence that might be encountered at a scene more accurately. It is impossible to encounter cosmetics as a form of associative trace, as a pristine sample, given that it must have originated from the skin of one individual prior to its transfer onto another substrate.

Lastly, due to the extended nature of this project, many of the products analysed are no longer manufactured or available in the UK. Some products have been superseded with improved formulations while others have been discontinued entirely. This will have implications for future analyses of these products, to further understanding of transfer, persistence, and activity level propositions.

6.3.2 Characterisation using optical microscopy

6.3.2.1 Foundations

The foundations represented the majority of the samples assessed in this Chapter and encompassed all five previously described product formulations. The visual macroscopic and microscopic appearances of all samples was consistent with what was seen among the new samples described in the previous Chapters. Figure 6.1 shows three examples of unremarkable, visually homogeneous samples, that differ slightly by shade.

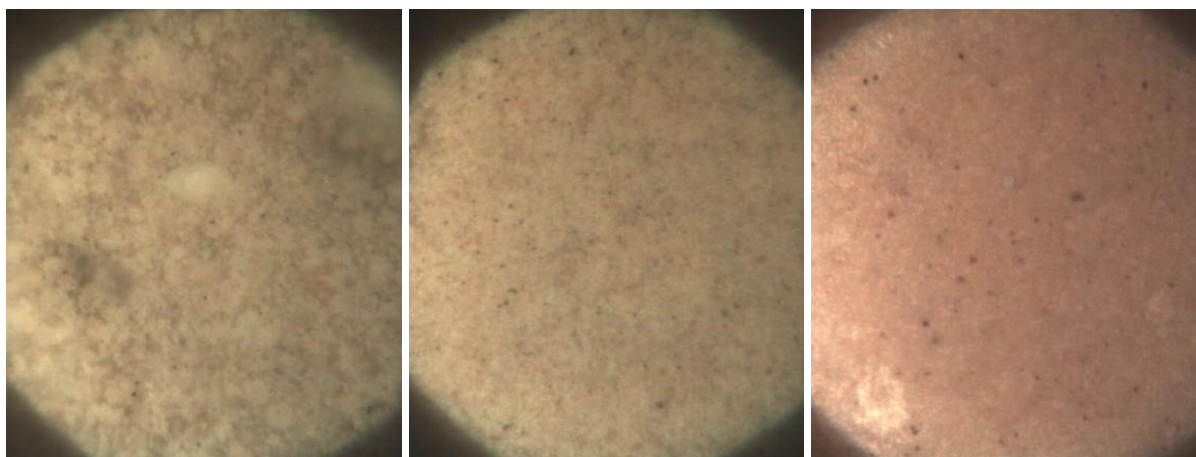


Figure 6.1: Colour photomicrograph images of donated foundation samples showing varying shades but visually homogeneous appearances, (L-R) Lacura Liquid Foundation in shade 10 Transparent, Estee Lauder Double Wear Foundation in shade Pure Beige, Revlon Even Out Makeup in shade 02 Nude Beige.

Despite all samples having been used, opened for varying amounts of time, and potentially contaminated, there was no obvious evidence of this when compared to the range of new samples. For example, no extraneous hairs, fibres or other exogenous material were apparent. As was seen across the samples examined in Chapters 3 and 4, microscopic differences between the various formulations were also evident, with the mousse textured samples giving rise to focusing difficulties due to the undulating surface topologies (Figure 6.2).

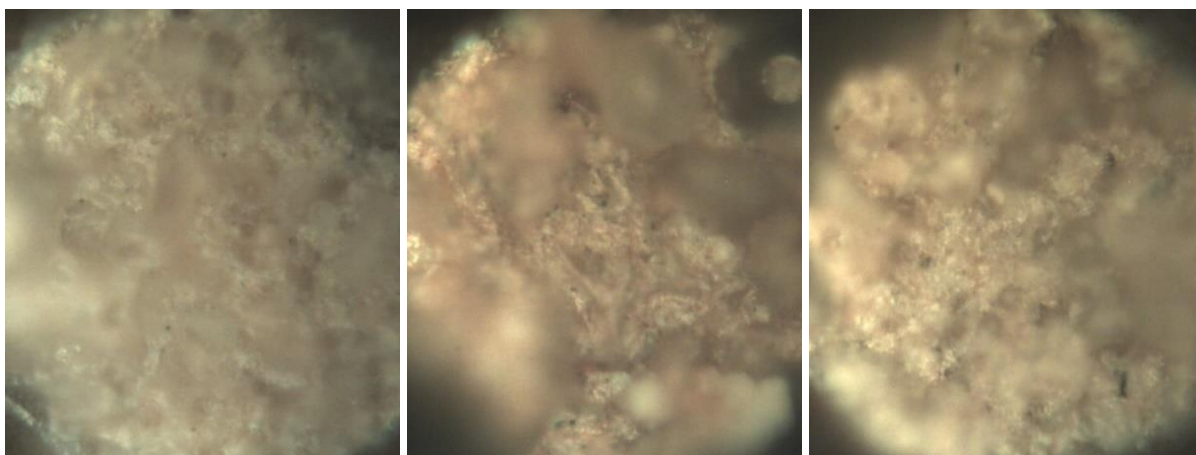


Figure 6.2: Colour photomicrograph images of donated mousse-based foundation samples illustrating differences in surface topologies that gave rise to focusing difficulties, (L-R) Bourjois Mineral Matte Mousse Foundation in shade 81 Tendre Ivoire, L'Oreal Matte Morphose Soufflé Foundation in shade 140 Creamy Natural, Boots No.7 Beautifully Matte Mousse Foundation in shade Cool Ivory.

Due to the number of donated testers containing a series of different shades from the same product line, the effect of colour could be investigated. The macroscopic differences between subsequent shades were very subtle and nearly imperceptible, but the microscopic differences were more readily ascertained by the increase of pigment particles, such as black iron oxide (denoted by the black flecks in Figure 6.3), with darkening shade.

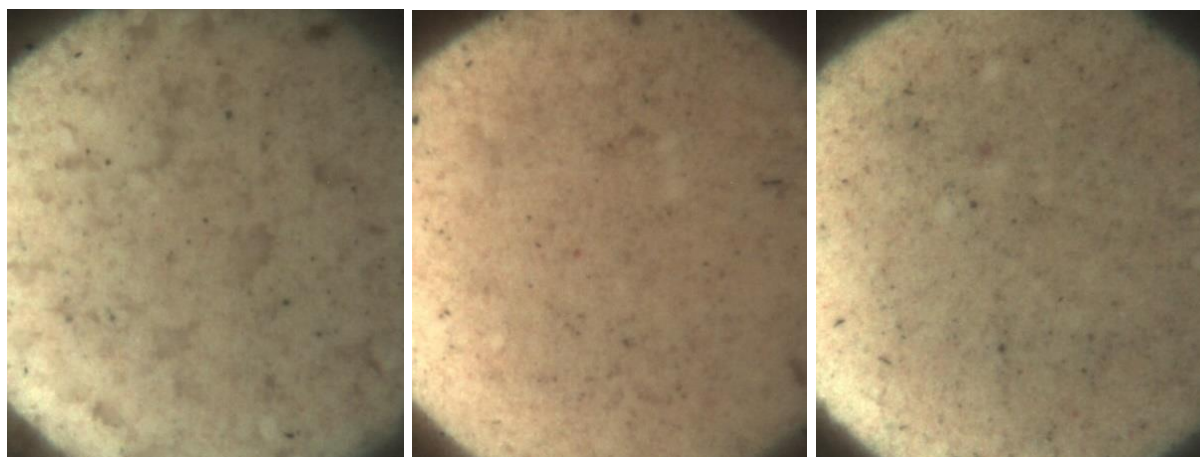


Figure 6.3: Colour photomicrograph images of a series of donated Bourjois foundation samples illustrating an increase of pigment particles with darkening shade, (L-R) Bourjois Comme Après 10 Heures De Sommeil Foundation in shade 71 Abricote, Bourjois Comme Après 10 Heures De Sommeil Foundation in shade 72 Rose, Bourjois Comme Après 10 Heures De Sommeil Foundation in shade 73 Beige.

6.3.2.2 Concealers

The 16 donated concealer samples comprised liquid and cream formulations exclusively, which enabled easier surface focusing. Consistent with the findings from Chapters 3 and 4, the concealers exhibited more visual similarities between samples than the foundations, presumably due to their limited purpose of hiding unwanted skin imperfections^{1,2}. The only microscopic discrepancies between the donated concealers were the occasional detection of spherical particles and/or mica flakes within formulations.

As can be seen in Figure 6.4, The Collection 2000 concealer contains spherical particles; unfortunately, owing to the lack of original packaging the ingredients list could not be found. Therefore, the nature of these particles could not be determined, but from assessing the ingredients list of more recent editions of this product, it is likely these particles are silica. The MAC concealer contains reflective flakes suggestive of either titanium dioxide coated mica or bismuth oxychloride. Inspection of the ingredients list confirms the presence of both components, but the Raman spectra indicate that mica is the more likely source. The Garnier

concealer contains neither spherical particles nor reflective particles but does contain the usual iron oxide pigment particles.

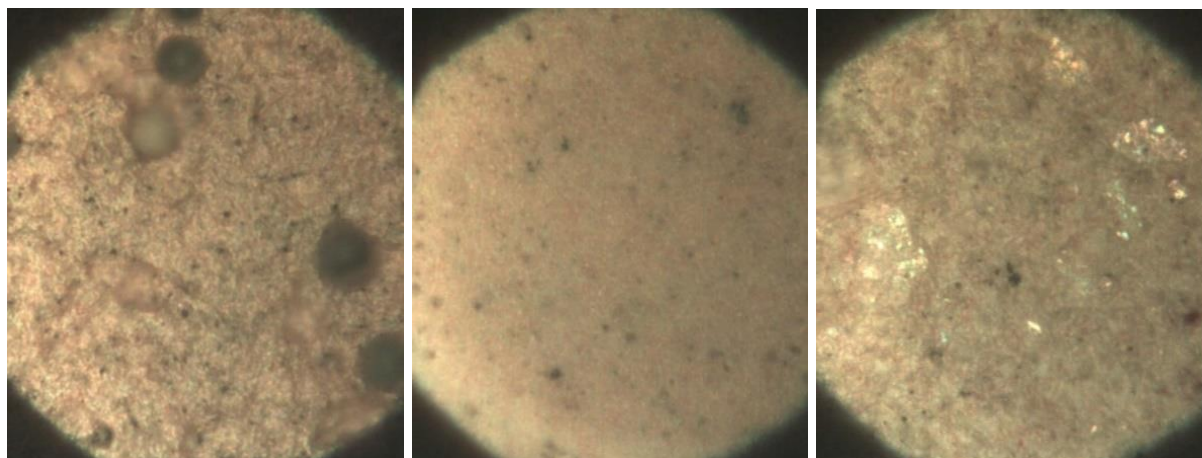


Figure 6.4: Colour photomicrograph images of donated concealer samples illustrating the presence or absence of certain particles, (L-R) Collection 2000 Lasting Perfection Concealer in shade Deep 4, Garnier Anti-dark Circles Roll on Concealer (no shade specified), MAC Select Cover Up Concealer in shade NW20.

6.3.2.3 Tinted moisturisers

Surprisingly, despite all seven tinted moisturiser samples being liquid formulations, there seemed to be more visual variation than was previously seen across the new tinted moisturiser samples discussed in Chapter 3. Whether this is an incidental finding that relates to the products investigated (as none of these products were present in Chapter 3) or is a result of potential ageing/contamination of the samples, could not be ascertained. It may also be the case that due to the smaller number of donated samples in this set compared to the new samples (n=17), any discrepancies in appearance would be more noticeable.

Of particular note are the black thread-like inclusions seen in the Clinique CC Cream (Figure 6.5), which may represent an agglomeration of iron oxide pigment particles or may simply be contamination of the sample, perhaps with tiny hairs or fibres. Also of note is the variety of different coloured particles within the L'Oreal CC Cream. Inspection of the ingredients list reveals that in addition to the standard inorganic pigments: titanium dioxide, the trio of iron oxides and bismuth oxychloride, this product also contains the organic pigments: yellow 6 lake (CI 15985), red 28 lake (CI 45410), yellow 5 lake (CI 19140), and blue 1 lake (CI 42090). It is unusual to see such a range of pigments in a complexion product, and even more unusual for organic lake pigments to be used preferentially to inorganic or mineral pigments^{3,4}.

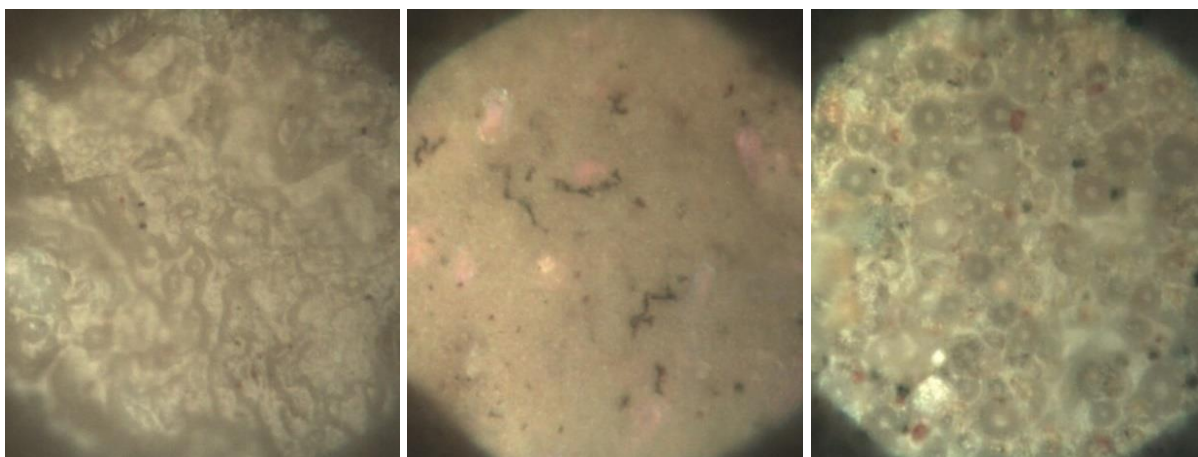


Figure 6.5: Colour photomicrograph images of donated tinted moisturiser samples illustrating differences in pigment particles, (L-R) Avon Face Perfecting Duo Tinted Moisturiser in shade Light, Clinique Moisture Surge CC Cream in shade Medium, L'Oreal Nude Magique CC Cream in shade Anti-dullness.

6.3.2.4 Finishing powders

There were only two finishing powders donated, one loose powder and one pressed powder. According to the ingredients list, the Golden Rose loose powder is predominantly corn starch (*Zea Mays*) based, with lesser proportions of silica, synthetic fluorophlogopite and mica. The photomicrograph image (Figure 6.6) is consistent with a mixture of these components, as is evidenced by the corn starch-based and mica-based finishing powders examined in Chapter 3 (Figure 3.16).

The appearance of the Rimmel pressed powder (Figure 6.6) is similar to that of the Fashionista Catwalk 360 Pressed Powder encountered in Chapter 4 (Figure 4.4). Comparison of both products' ingredients lists confirms the major components (i.e. the first three appearing in the list) as talc, mica, and magnesium stearate.

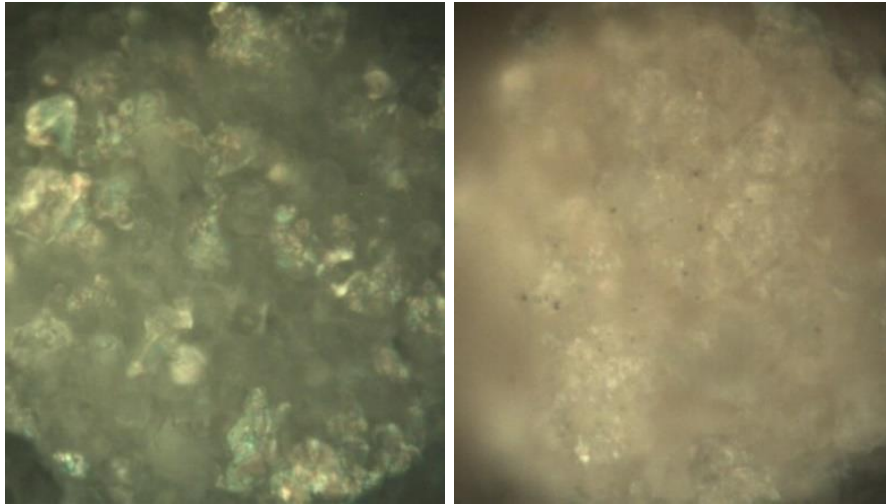


Figure 6.6: Colour photomicrograph images of the donated finishing powder samples, (L-R) Golden Rose Finishing Powder in shade Translucent (loose powder), Rimmel Stay Matte Pressed Powder in shade 001 Transparent.

6.3.2.5 Bronzers

As was the case with the donated finishing powders, only two donated samples were bronzers; coincidentally these also consisted of one loose powder and one pressed powder. Their appearances were very similar as can be seen in Figure 6.7, with the exception of slightly larger titanium dioxide coated mica flakes in the BareMinerals bronzer. However, this is congruous with the appearance of the BareMinerals Ready Bronzer shown in Figure 4.7, so may be a feature of bronzers manufactured by this brand.

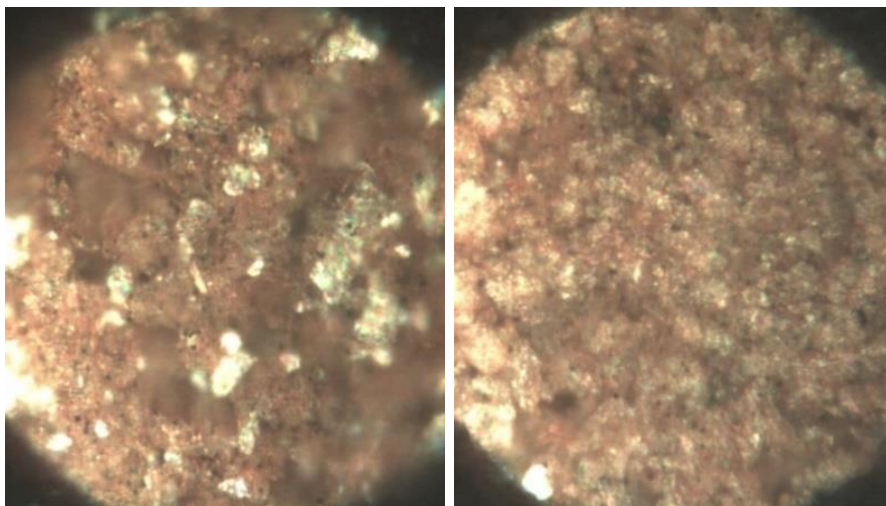


Figure 6.7: Colour photomicrograph images of donated bronzer samples, (L-R) BareMinerals All Over Face Colour in shade Warmth, Diorskin Terra Bella Sun Powder in shade 003 Bronze Tan.

6.3.2.6 Highlighters

The highlighters comprised a limited set of three liquid samples, images of which are shown in Figure 6.8. With the exception of the red hue in the Boots No.7 Highlights Illuminating Lotion, the visual appearances are similar to each other, and also to the new set of highlighters assessed in Chapter 4 (Figure 4.8). Despite the larger sizes of the reflective flakes in the Highlights Lotion, their morphologies are not suggestive of borosilicate glass-based interference pigment, as these tend to display sharper well-defined edges⁵. Regrettably, the ingredients list was not available for this product and it has been discontinued since analysis, so this assertion could not be verified.

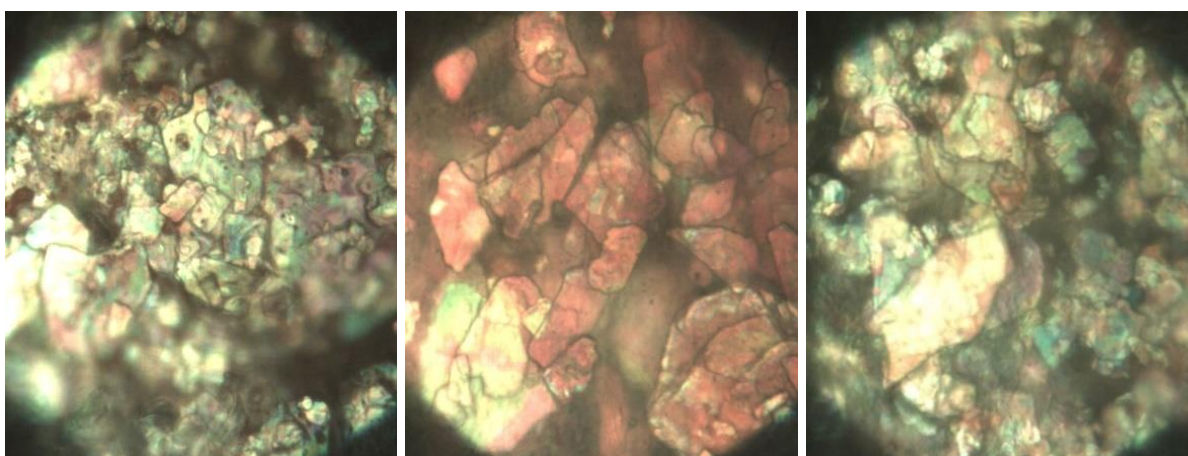


Figure 6.8: Colour photomicrograph images of donated highlighter samples illustrating a large proportion of interference pigment particles, (L-R) Boots No.17 Skin Glow Face Illuminator in shade Pink Flash, Boots No.7 Highlights Illuminating Lotion (no shade specified), Boots No.7 Skin Illuminator in shade Peach.

6.3.2.7 Primers

The six liquid primer samples exhibited the most visual variability of all the product types despite five of them belonging to the same brand, BareMinerals (Figure 6.9). This may stem from the fact that primers serve different purposes according to consumer need. Some primers are colourless but provide a base for foundation to adhere to, prolonging makeup wear, whereas others are pigmented (e.g. the BareMinerals BB Primer Cream), offering enhanced coverage. Some may contain interference pigment particles for a brightening effect, such as those seen in the Avon Invisible Light Illuminating Makeup Base, or not if the wearer wishes to achieve a mattified look. Others may double up as a moisturiser (BareMinerals Prep Step Moisture Set Cream), a serum, an SPF, an oil absorber, a colour corrector, a pore refining agent, and many more. This further highlights the intricacies of these types of cosmetics, and

when considering these products are designed to be applied in a layered sequence with other decorative cosmetics, the resultant chemical complexity is vast.

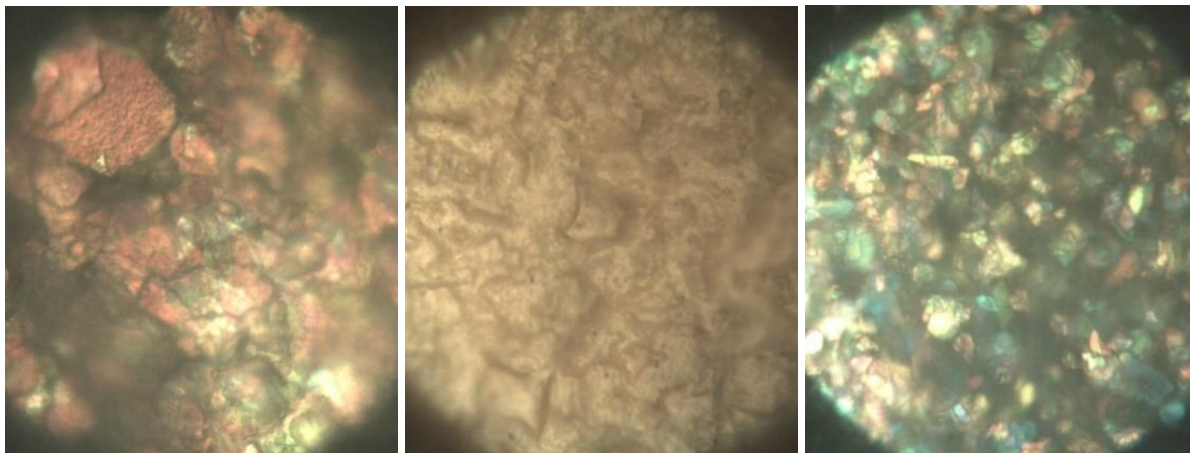


Figure 6.9: Colour photomicrograph images of donated primer samples illustrating differences in matrix colour and pigment particles, (L-R) Avon Invisible Light Illuminating Makeup Base (no shade specified), BareMinerals BB Primer Cream in shade Light, BareMinerals Prep Step Moisture Set Cream (no shade specified).

6.3.3 Characterisation using Raman spectroscopy

Visual assessment of the Raman spectra revealed that some samples exhibited spectral homogeneity whilst others were spectrally heterogeneous. However, as discussed in Section 6.3.1, due to the unknown usage and level of contamination of the donated samples it was felt that splitting them into two groups, (as the new samples were in Chapters 3 and 4) would not aid sample discrimination as the cause of the spectral heterogeneity was uncertain. In other words, any spectral deviations in replicate measurements could be due to the effects of ageing or contamination, rather than being an intrinsic property of the sample itself.

6.3.3.1 Foundations

The 84 donated foundations were represented by 45 different product lines and a variety of shades from “ivory” through to “deep”. Across the foundation samples, 68 were perceived as spectrally homogeneous and 16 spectrally heterogeneous. This is a much larger proportion of heterogeneous samples compared to the new foundations, where out of a total of 82 samples only five were found to be heterogeneous. This either suggests that the ageing and/or contamination of these donated samples has negatively affected their compositions or that this set of samples happened to represent products that were inherently heterogeneous.

As previously mentioned, due to receiving shade series for various product lines, the effect of increasing pigment concentration on the Raman spectra could be assessed. The Bourjois

Healthy Mix Foundation samples spanned six different shades and their Raman spectra are given in Figure 6.10. The increasing yellow iron oxide concentration, denoted by the peaks at 92 cm^{-1} , 243 cm^{-1} , and 298 cm^{-1} , is evident as each shade becomes darker, as is the broad peak centred at 1305 cm^{-1} , indicative of increasing red or brown iron oxide content.

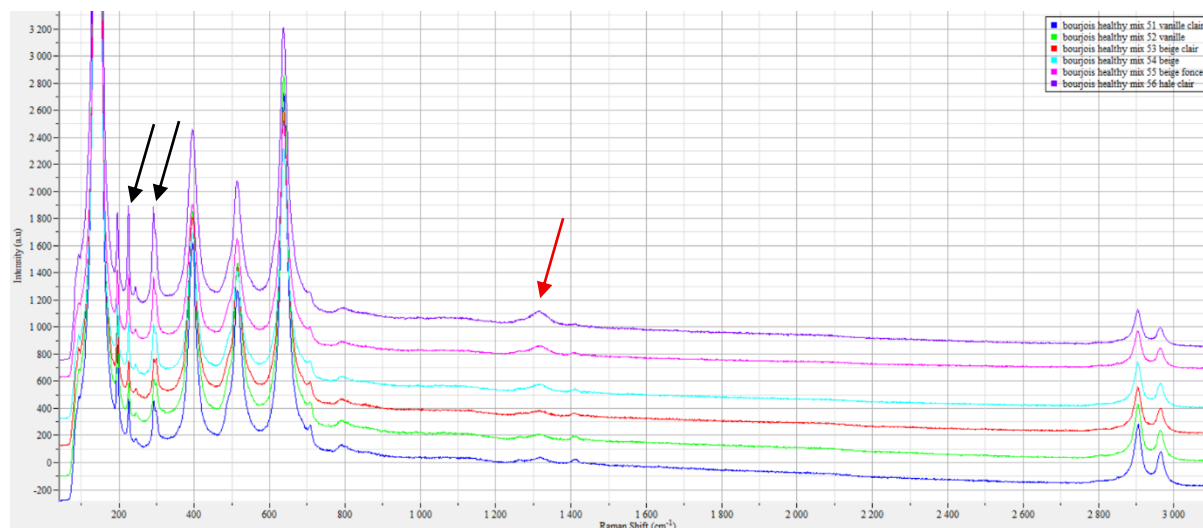


Figure 6.10: Raman spectra (unprocessed but stacked for clarity) of donated Bourjois Healthy Mix Foundations in shades: 51 Vanille Clair, 52 Vanille, 53 Beige Clair, 54 Beige, 55 Beige Foncé, and 56 Hale Clair, showing a gradual increase in the yellow (marked with the black arrows) and red/brown (marked with the red arrow) iron oxide peak intensities with darkening shade.

Fortunately, a proportion of the donated cosmetic samples were from the same brand and product line, and in the same shade as the new samples already purchased. Figure 6.11 compares the Raman spectra of three L'Oreal Infallible 24H Matte Foundations in the shade Rose Beige. One sample was donated as the tube was nearly empty and the other two samples were both purchased new. Interestingly, the new product purchased in 2020 displayed different packaging to the product purchased in 2023, and the numbering of the shade had changed (13 to 145), despite the name of the shade remaining constant.

Notwithstanding these changes to the packaging design and product numbering, the spectra of both new foundations are consistent with each other. The donated used sample, however, tells a different story. As can be seen across these five replicate spectra, there are observable differences in both the presence (or absence) of peaks in the $1000\text{ cm}^{-1} - 1700\text{ cm}^{-1}$ range and in the CH region, and also the amount of fluorescence in the baseline. This increase in baseline fluorescence with advanced ageing was demonstrated by the BareMinerals BarePro Foundation discussed in Chapter 5, so these spectral changes could be attributed to the donated sample's age.

However, what is most unexpected is that the L’Oreal Infallible sample purchased in 2023 was used as another of the controlled ageing samples in Chapter 5 and the spectral changes seen over 15 months were negligible. This contradicts what is shown here and suggests factors other than time are contributing to these spectral deviations. It may be that the owner of this product added water into the tube to rehydrate the aged sample or encourage the last remnants out of the packaging. It could be due to storage conditions or contamination with other products. Alternatively, it may simply have been a different chemical composition to start with. Given that this product changed between 2020 and 2023, and a recent internet search reveals the product has since changed again to “L’Oreal Infallible 32H Matte Cover”, this assertion is entirely possible.

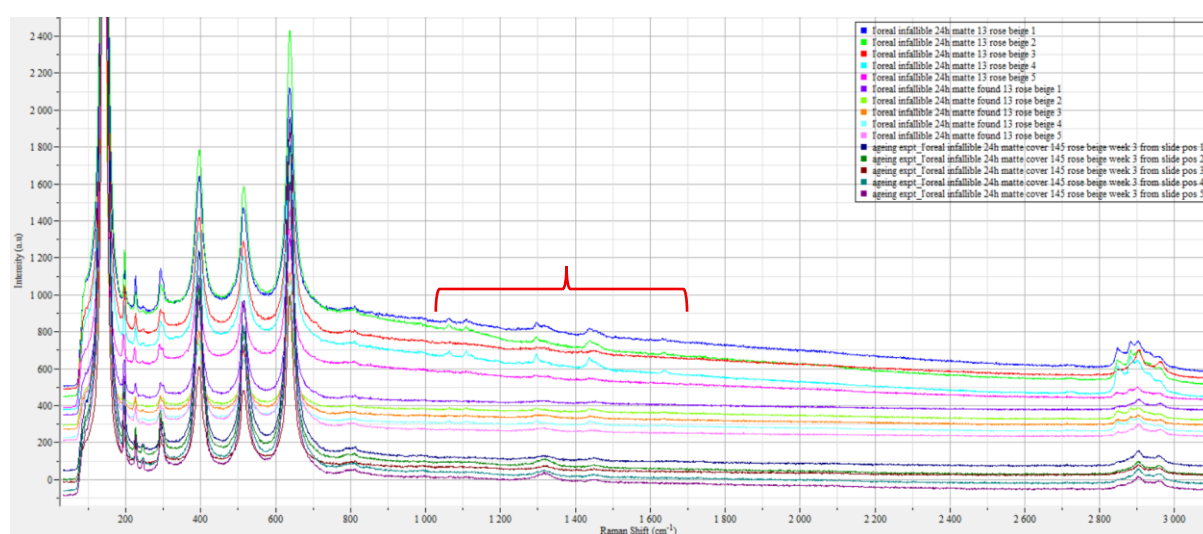


Figure 6.11: Raman spectra (unprocessed but stacked for clarity) of three L’Oreal Infallible 24H Matte Foundations in shade 13/145 Rose Beige: donated sample (top five spectra), new sample purchased in 2020 (middle five spectra), new sample purchased in 2023 (bottom five spectra). Red brace denotes spectral range with observable differences between products.

The same comparison could be made between three Rimmel Lasting Finish Foundations in shade 200 Soft Beige, as one was an expired tester and the other two were purchased in 2020 and 2023 respectively. Figure 6.12 confirms that the two new versions of this product exhibit excellent spectral homogeneity and repeatability, while small differences are seen between replicate spectra for the used tester. Nevertheless, these spectral differences are less obvious than those seen for the donated L’Oreal sample in Figure 6.11, and there are minimal fluctuations in the baseline slope.

The Rimmel Foundation purchased in 2023 was also used for the ageing study described in Chapter 5. The spectra presented here (Figure 6.12) are consistent with those findings; namely, that the spectra for this product changed minimally over a 15-month period. The

expired testers had been open for a maximum of three months, as confirmed by the store manager. Boots' regulations stipulate that all testers must be discarded and renewed every three months to minimise microbial growth and prevent cross-contamination of customers. Given that this tester could have therefore only been open for a maximum of three months, advanced ageing is not a potential variable, yet contamination of the sample could still have occurred. Since the spectra are largely consistent with the new counterparts, this suggests that the expired tester was not as contaminated or degraded as initially presumed.



Figure 6.12: Raman spectra (unprocessed but stacked for clarity) of three Rimmel Lasting Finish Foundations in shade 200 Soft Beige: donated sample (top five spectra), new sample purchased in 2020 (middle five spectra), new sample purchased in 2023 (bottom five spectra).

6.3.3.2 Concealers

The concealer samples (n=16) were represented by 15 different product lines. Only three samples were assessed as being spectrally heterogeneous. This is consistent with the new concealer set, where only four out of a total of 31 samples were found to be spectrally heterogeneous, suggesting that ageing and contamination did not feature as strongly in the sample variability.

Figure 6.13 gives the five replicate Raman spectra for two Maybelline Fit Me Concealers in the same shade: 15 Fair. The uppermost spectra were taken from the donated sample and the bottom spectra were taken from a newly purchased sample in 2020. It is evident from the fluorescent baseline that the donated sample has changed chemically. Whether that is due to ageing and contamination or represents a difference in ingredient composition cannot be verified, due to the lack of original box packaging for the donated sample. However, the increased baseline fluorescence is consistent with the results obtained for the BareMinerals foundation in the ageing experiments described in Chapter 5.

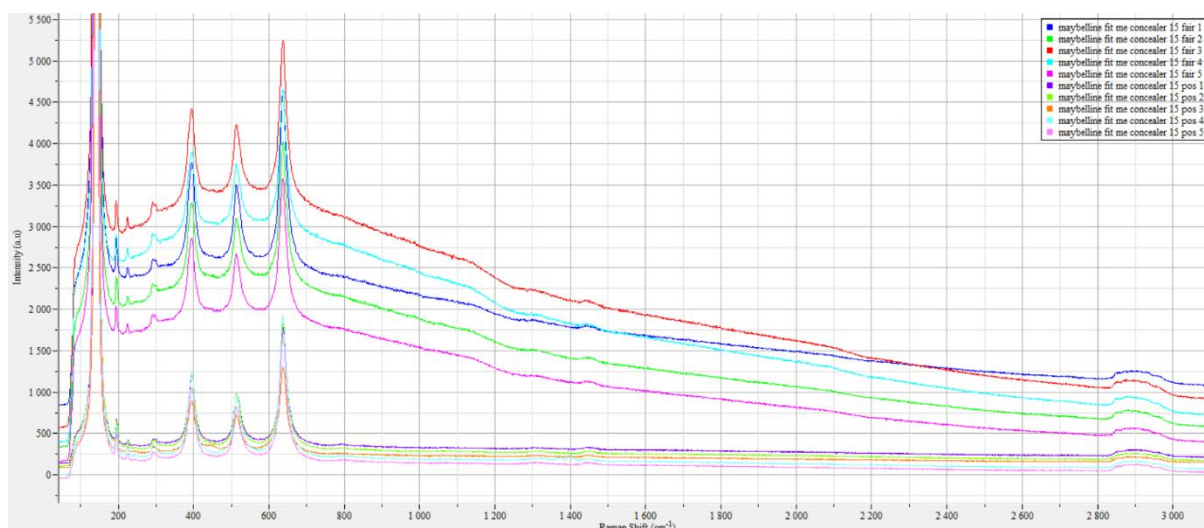


Figure 6.13: Raman spectra (unprocessed but stacked for clarity) of two Maybelline Fit Me Concealers in shade 15 Fair: donated sample (top five spectra), new sample purchased in 2020 (bottom five spectra).

Figure 6.14 again shows the increase in baseline fluorescence when comparing a used tester Rimmel Match Perfection Concealer with a new version of the same product, in the same shade. The new sample was discussed in Chapter 4, as it was identified as being spectrally heterogeneous. Hence, the spectral heterogeneity seen across replicates for the donated sample can be explained as an intrinsic property of this product, rather than attributed to the used nature of the sample. However, the fluorescence is likely linked to either the ageing process (in keeping with the results from Chapter 5) or the presence of exogenous material, and indicates this sample has been subjected to contamination such as skin cells.

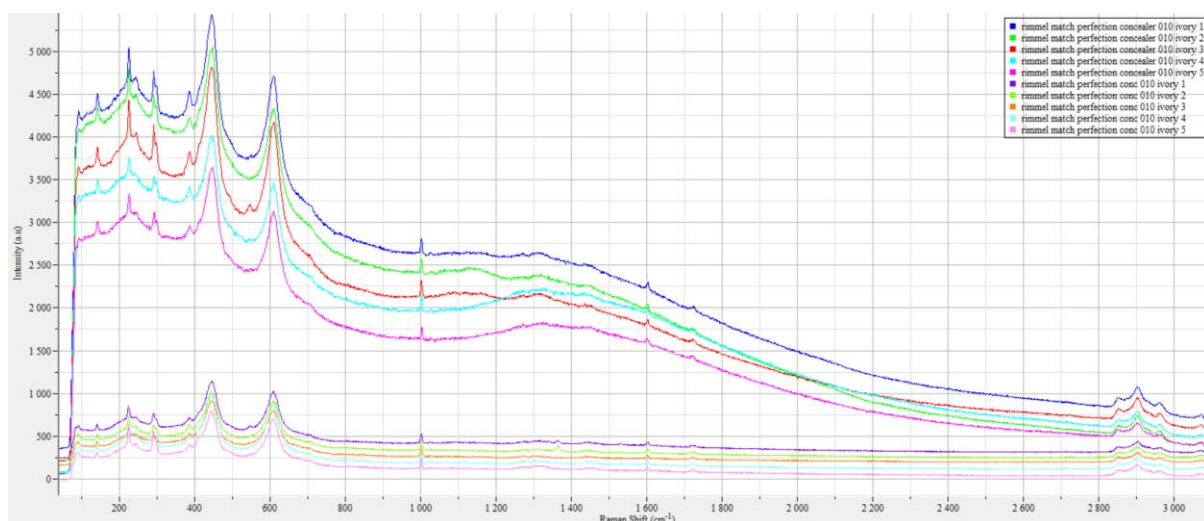


Figure 6.14: Raman spectra (unprocessed but stacked for clarity) of two Rimmel Match Perfection Concealers in shade 010 Ivory: donated sample (top five spectra), new sample purchased in 2020 (bottom five spectra).

6.3.3.3 Tinted moisturisers

The donated tinted moisturiser sample set was limited but a comparison could still be made between one of the products and a similar new product from Chapter 3. Figure 6.15 shows two variants of the product line Boots No.7 Beautiful Skin BB Cream. The donated sample was for dry/very dry skin and the newly purchased sample was for normal/oily skin. In spite of this difference the spectra appear very similar, again suggesting that the donated sample was minimally affected by use. The differences between peak intensities in the fingerprint region for the donated sample can be attributed to focusing upon different mineral pigments within the matrix, for example, iron oxide or titanium dioxide. Nonetheless, there is a noticeable increase in the baseline fluorescence of the donated sample and thus, similarly to the distinction between the two samples in Figure 6.14, this is likely to represent either product ageing or contamination of a biological nature.

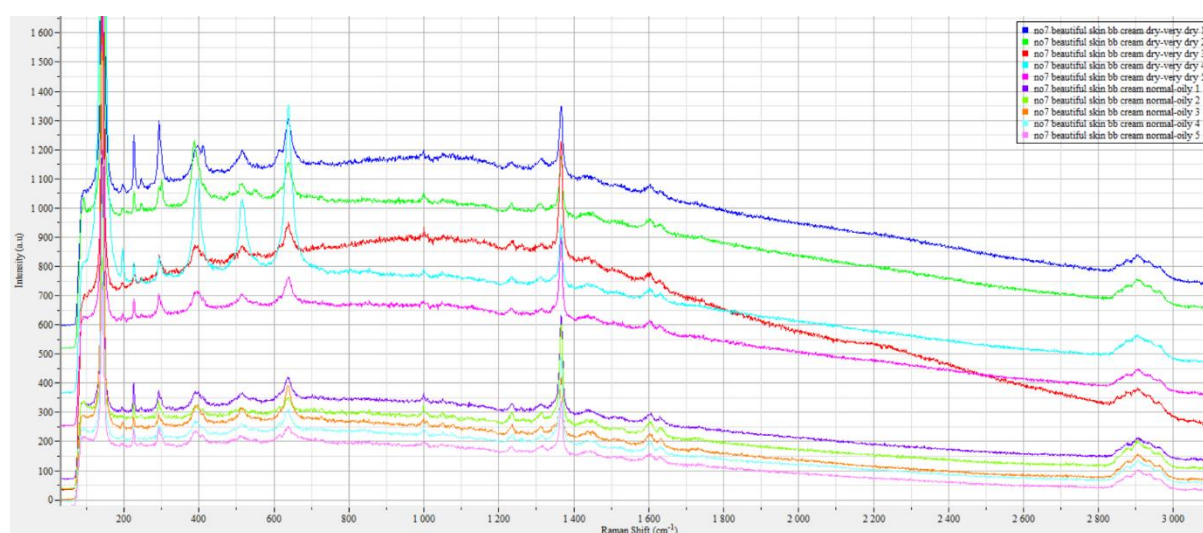


Figure 6.15: Raman spectra (unprocessed but stacked for clarity) of two Boots No.7 Beautiful Skin BB Creams: donated sample in variant dry/very dry (top five spectra), new sample purchased in 2020 in variant normal/oily (bottom five spectra).

6.3.3.4 Finishing powders

No spectral comparisons could be made between either of the two donated finishing powders with newer counterparts as neither could be purchased. The Golden Rose Finishing Powder was purchased in Poland and the Rimmel Stay Matte Pressed Powder was very old and had since been discontinued from the Rimmel range.

Figure 6.16 displays the spectra obtained for the five replicate measurements of the Golden Rose sample. The cause of the fluorescent baseline and lack of discriminating features cannot be ascertained; these may represent advanced degradation or may be representative of the

sample's constitution. A possible explanation for the spectral profiles seen is the presence of kaolinite, a naturally occurring mineral (otherwise known as China clay), often added to finishing powders for its physical properties⁶. Kaolinite functions as an opacifier, an oil absorber, and a preservative. It also imparts a smooth appearance to the skin and can prolong makeup wear, as it withstands the effects of perspiration and humidity⁷. The reference standard spectrum for kaolinite is given in Figure 6.17 and displays a fluorescent baseline with only one visible “*lattice*” peak. A similar naturally mineral that is common to many cosmetic face powders is magnesium aluminium silicate. This ingredient absorbs oil and imparts a smooth appearance as kaolinite does, but also acts as a thickening agent, viscosity controller, and anti-caking agent⁸. The reference standard spectrum for magnesium aluminium silicate is also given in Figure 6.17.

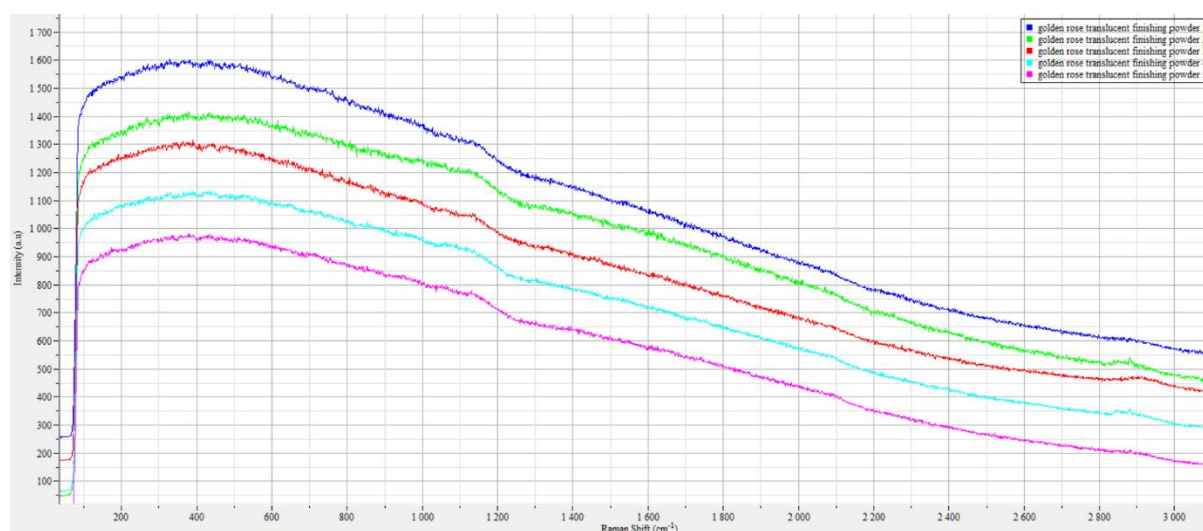


Figure 6.16: Raman spectra (unprocessed but stacked for clarity) of donated Golden Rose Finishing Powder in shade Translucent, displaying a fluorescent baseline and a lack of discriminating features.

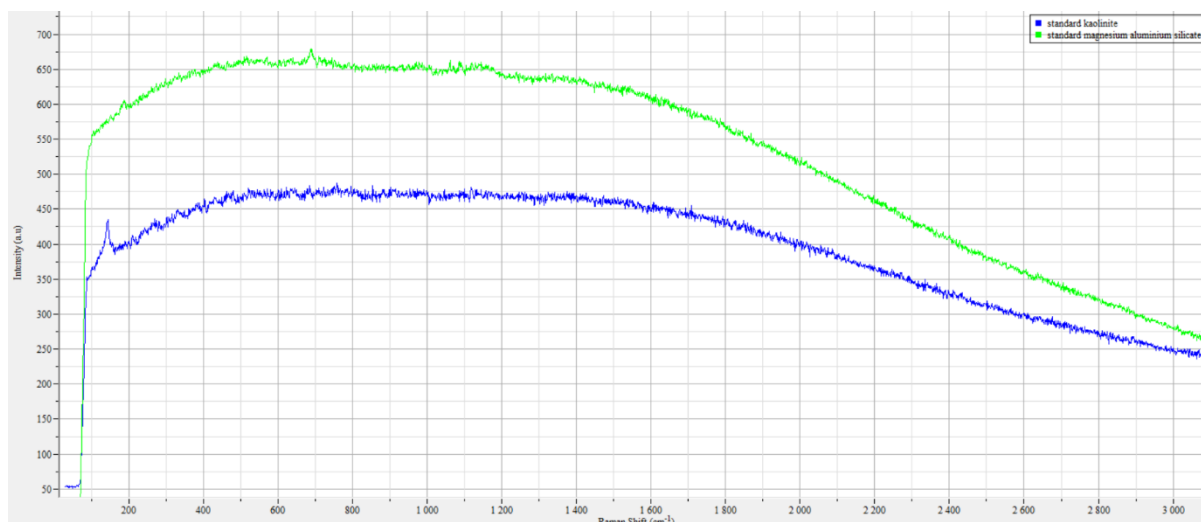


Figure 6.17: Raman spectra (unprocessed) of the reference standards for kaolinite (bottom) and magnesium aluminium silicate (top), displaying fluorescent baselines and a lack of discriminating features.

The replicate spectra for the other donated finishing powder sample are shown in Figure 6.18. This sample is not devoid of spectral features but does exhibit some heterogeneity. Once more, the cause of this heterogeneity cannot be determined due to the lack of comparator and ingredients list, but in light of the fact this is a pressed powder, it is likely that the formulation is giving rise to these inconsistencies. The peaks that differ in intensity relate to the pigment goethite (the reference spectrum for which is given in Figure 4.23) and further corroborates that these differences are due to focusing on specific pigment particles, rather than any effects of ageing or use.

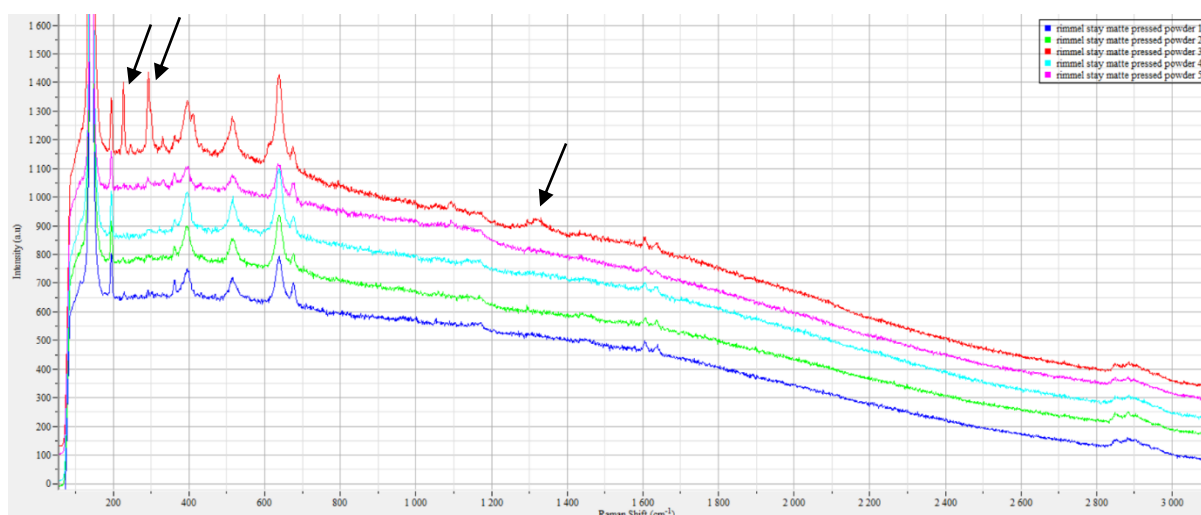


Figure 6.18: Raman spectra (unprocessed but stacked for clarity) of Rimmel Stay Matte Pressed Powder in shade 001 Transparent, exhibiting spectral heterogeneity across the five replicates. Black arrows denote peaks associated with goethite content.

6.3.3.5 Bronzers

When viewing the photomicrograph images for the two donated bronzer samples it appeared that their compositions were similar. However, when plotting their spectra it was evident that their ingredients were not (Figure 6.19). Both samples have a high proportion of red/brown iron oxide – denoted by the broad peak centred at 1305 cm^{-1} – which is unsurprising given their shades (“warmth” and “bronze tan”), but there are clear differences in organic content. The ingredients list for the BareMinerals sample was fortuitously printed on the underside of the sample pot and is given as: mica, bismuth oxychloride CI 77163, iron oxides CI 77499, CI 77491, CI 77492, ergo, an entirely inorganic composition. Conversely, the presence of peaks in the 2900 cm^{-1} region of the Dior spectra are suggestive of an organic component, but this could not be verified due to a lack of original packaging and the fact this product has since been discontinued. Nonetheless, as a pressed powder formulation it is likely to derive from organic binders such as isopropyl myristate, cetyl palmitate, or acrylates copolymer⁹.

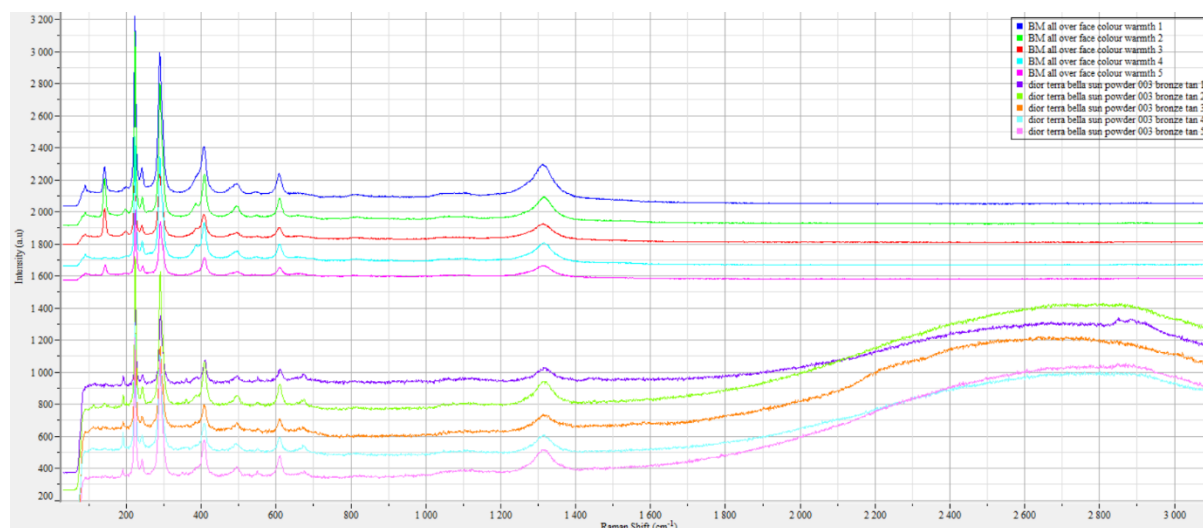


Figure 6.19: Raman spectra (unprocessed but stacked for clarity) of two donated bronzer samples: BareMinerals All Over Face Colour in shade Warmth (top five spectra) and Diorskin Terra Bella Sun Powder in shade 003 Bronze Tan (bottom five spectra), showing a strong red/brown iron oxide response in the 1305 cm^{-1} region.

The spectral homogeneity exhibited by the BareMinerals sample could be due to the simple ingredients list, or the fact this product is a loose powder (the Dior sample is a pressed powder), or due to a lack of contamination. Owing to the exclusive mineral composition it is unlikely this sample would succumb to ageing effects and therefore the length of time since opening is less relevant.

The Dior sample almost certainly has a more complex chemical composition owing to pressed powders requiring some sort of binder (usually a zinc/magnesium stearate or plant-based oil)

to bring the solid particles together into what is known as a “*cake*”, to be then placed into the traditional powder compact tin container⁹. The sample may also have degraded over time; this sample was very old so this is particularly applicable. It is also pertinent to note the differing modes of sample delivery: the Dior sample’s compact powder style means that the bulk of the sample is always open to contamination, whereas all BareMinerals loose powders (this sample included) are packaged into a pot with a sifter sealed into the top. This means only the amount of cosmetic required is shaken out of the container and into the lid to be used. This ensures the bulk of the product remains concealed within the pot, thereby minimising contamination. Any or all of these differences could be responsible for the spectral heterogeneity seen among the Dior replicates in Figure 6.19.

6.3.3.6 Highlighters

Remarkably, every highlighter analysed during the course of this thesis, whether new or old/donated, was found to be spectrally heterogeneous. This suggests that this is an intrinsic property of the product type and is unsurprising given the compositional nature and visual appearance of these products. Figure 6.20 compares two of the three highlighters received as donations: a Boots No.7 Skin Illuminator in shade Peach, and a Boots No.17 Skin Glow Face Illuminator in shade Pink Flash. The spectra for both samples are very similar, despite the slight differences in relative peak intensities, and suggest complicated organic compositions.

This raises further questions about the suitability of microscopic techniques for the analysis of highly heterogeneous samples such as these. One solution would be to utilise the spectral reduce transformation discussed in Section 2.3.3 of Chapter 2. Another would be to use a microscope objective with a lower magnification to increase the field of view. Alternatively, automatic Raman mapping may be employed for comprehensive analysis of these types of samples, but that is dependent upon the availability of instruments capable of mapping. Furthermore, acquisition of a Raman map takes considerably longer than five manual replicate spectra, and is also more complex to interpret, which would have implications in casework.

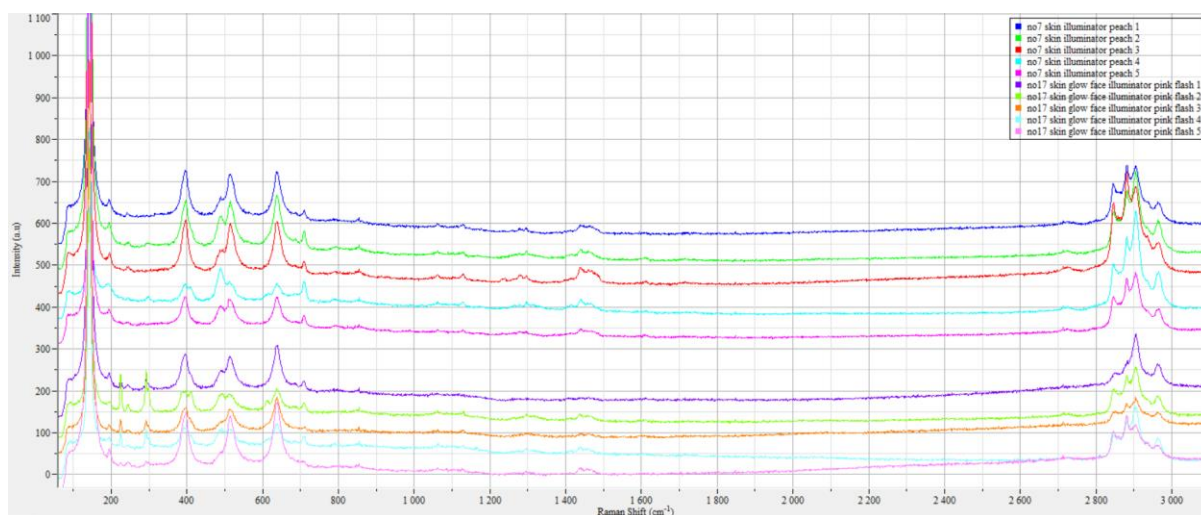


Figure 6.20: Raman spectra (unprocessed but stacked for clarity) of Boots No.7 Skin Illuminator in shade Peach (top five spectra) and Boots No.17 Skin Glow Face Illuminator in shade Pink Flash (bottom five spectra).

6.3.3.7 Primers

Regrettably, none of the six primers donated to the study matched with the single new primer sample contained in Chapter 3. Nevertheless, comparisons could still be drawn between these donated samples and the reference spectra obtained for the raw cosmetic standards. To this end, the BareMinerals Primetime Original Primer was contrasted with the reference spectrum for dimethicone 500 (polydimethylsiloxane). As can be seen in Figure 6.21, all of the peaks for the dimethicone standard were present in the primer sample's spectra, notably: 157 cm^{-1} , 191 cm^{-1} , 490 cm^{-1} , 686 cm^{-1} , 708 cm^{-1} , 789 cm^{-1} , 862 cm^{-1} , 1263 cm^{-1} , 1410 cm^{-1} , 2800 cm^{-1} , 2905 cm^{-1} , and 2965 cm^{-1} . Peak assignments for these are given in Table 6.1.

Inspection of this product's ingredient list reveals that dimethicone is the third component by weight, and therefore makes up a large proportion of the composition¹⁰. However, the first and second most abundant components of the primer are cyclopentasiloxane and dimethicone crosspolymer respectively, and therefore the majority of this product comprises various silicones¹⁰. Socrates notes that it is difficult to distinguish between cyclic and linear siloxanes as their peak regions overlap¹¹ and therefore the peaks we see in the primer sample's spectra could derive from any of these three main ingredients. Given that the remainder of the ingredients encompass various vitamins and botanical extracts, e.g. aloe, camomile, and liquorice, it is reasonable to presume their small contribution to the Raman spectrum will be overwhelmed by the silicon-containing compounds.

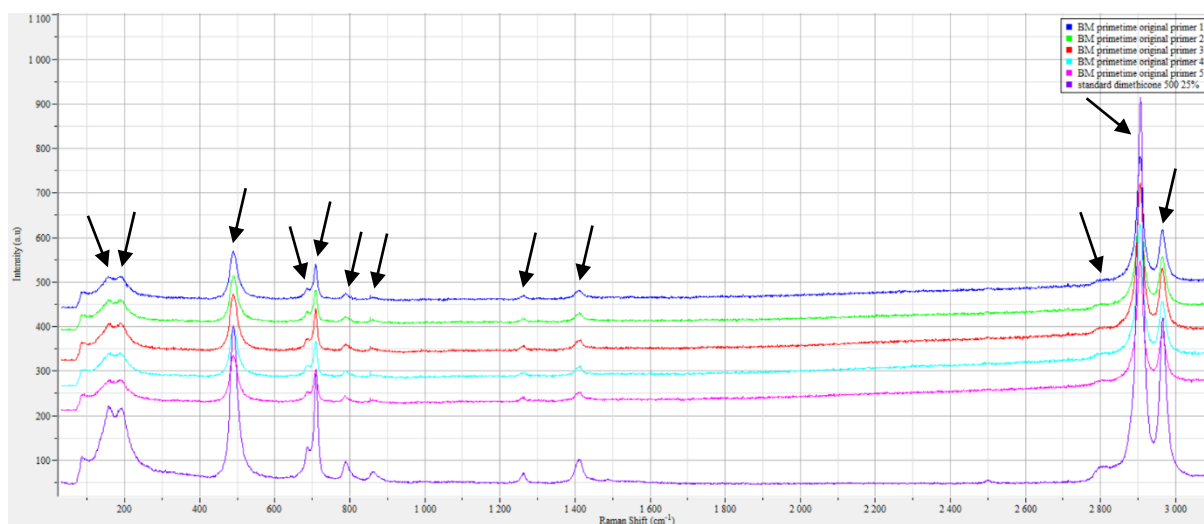


Figure 6.21: Raman spectra (unprocessed but stacked for clarity) of BareMinerals Primetime Original Primer with the reference standard for dimethicone 500. Black arrows denote congruent peaks.

Table 6.1: Raman spectral peak assignments for polydimethylsiloxane (PDMS)¹².

Raman shift (cm ⁻¹)	Vibration	Functional group
157 cm ⁻¹	C-Si-C twist deformation	Si-CH ₃
191 cm ⁻¹	C-Si-C scissor deformation	Si-CH ₃
490 cm ⁻¹	Si-O symmetric stretch*	Si-O-Si
686 cm ⁻¹	CH ₃ rocking	Si-CH ₃
708 cm ⁻¹	C-Si-C symmetric stretch	Si-CH ₃
789 cm ⁻¹	C-Si-C asymmetric stretch	Si-CH ₃
862 cm ⁻¹	CH ₃ rocking	Si-CH ₃
1263 cm ⁻¹	CH ₃ symmetric bend	Si-CH ₃
1410 cm ⁻¹	CH ₃ asymmetric bend	Si-CH ₃
2800 cm ⁻¹	CH ₃ asymmetric bend overtone	Si-CH ₃
2905 cm ⁻¹	CH ₃ symmetric stretch	Si-CH ₃
2965 cm ⁻¹	CH ₃ asymmetric stretch	Si-CH ₃

*The Si-O asymmetric stretch usually appears at 1100 cm⁻¹ but tends to disappear from the PDMS Raman spectrum with increasing molecular weight¹² such as the dimethicone 500 reference standard analysed here.

6.3.4 Exploratory data analysis

The five replicate Raman spectra for each sample were imported into the Unscrambler software and a PCA model created as detailed in Chapter 2. The scree plot revealed that, astonishingly, the same amount of total variance – 89% – was accounted for in the first three PCs, as was previously seen for the new spectrally homogeneous sample model in Chapter 3. The total variance spanning the first seven PCs was only slightly less than the Chapter 3 model (98%) at 97%, suggesting a similar spread of variables and respective magnitudes. This contrasts with the total variances calculated for the new spectrally heterogeneous samples discussed in Chapter 4, which were lower overall. This may be due to the much higher number of samples represented in this dataset, rather than the spectral variability necessarily.

As is pleasingly shown in Figure 6.22, the validation percentage variances track the calibration variances very closely, indicating the absence of extreme samples or outliers and confirming creation of a robust model.

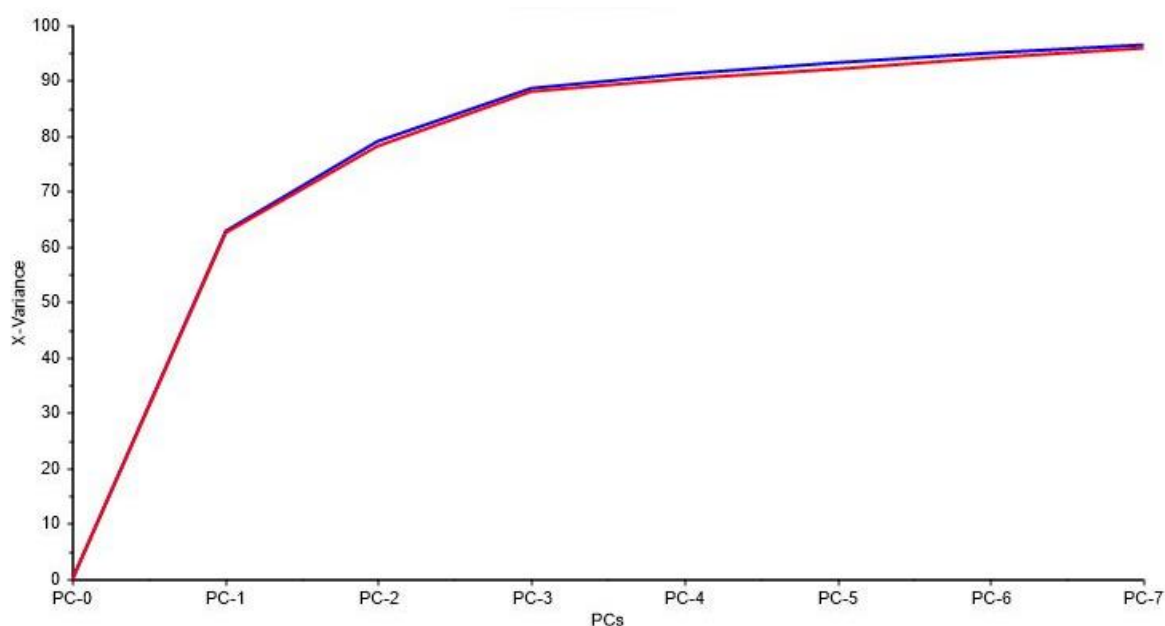


Figure 6.22: Explained variance plot for the donated cosmetic sample set ($n=120$), showing 89% of the model variance accounted for in the first three PCs and 97% of the model variance accounted for in the first seven PCs. The blue trace denotes the calibration variance; the red trace denotes the validation variance.

The 120 samples gave a total of 600 spectra. As a result, visualisation of 3-dimensional scores plots were cumbersome, so the standard 2-dimensional plots were used instead. Due to the complexity of the data and the many unknown variables previously discussed in the

preliminary considerations section (6.3.1), it was decided that only the first two PCs would be used to interpret scores and loadings. This is because it could not be guaranteed that any minor spectral discrepancies that would contribute to the higher PCs were inherent to the sample; they could equally be due to factors relating to product use.

Figure 6.23 shows the PCA scores plot of all donated cosmetic samples grouped by brand. Despite the similar explained variances to the Chapter 3 model, the distribution of samples is dissimilar (Figure 3.31) and there are no clearly defined groupings by brand. The scores along PC1 here, range from approximately -45 to 30, which is consistent with the PC1 scores from the Chapter 3 model. Yet, the scores along PC2 of -25 to 60 are distinctly different to the Chapter 3 model of -55 to 30.

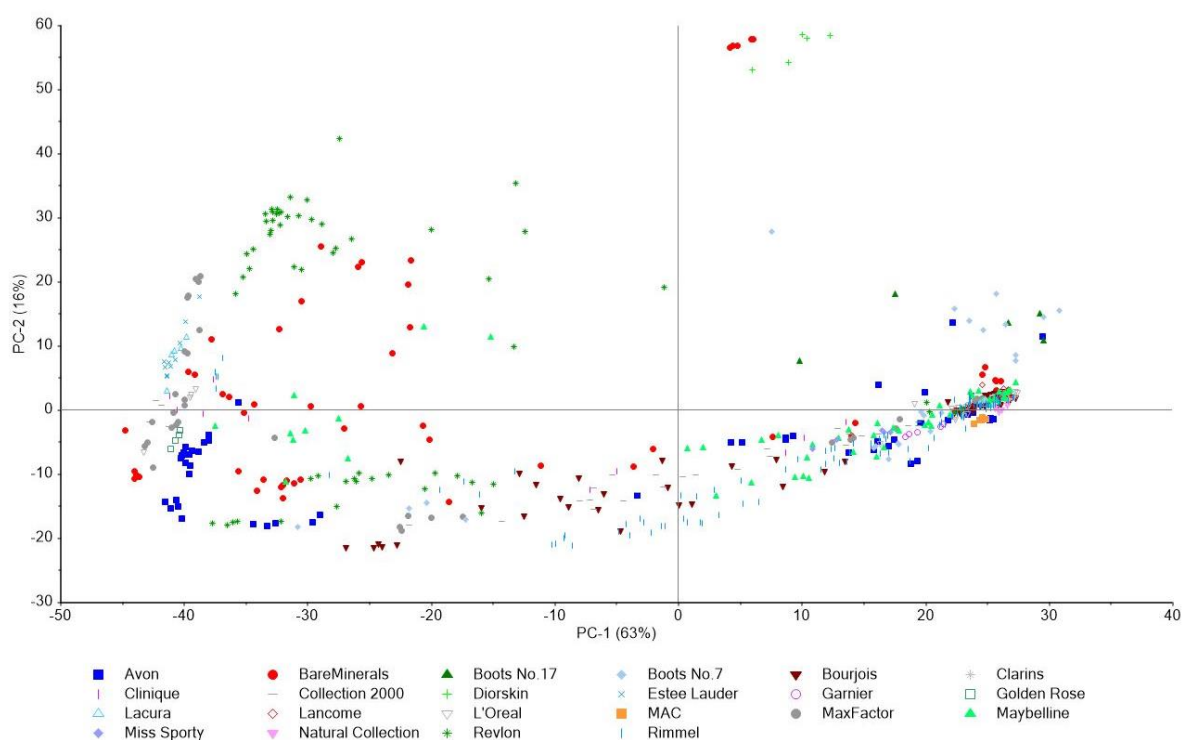


Figure 6.23: PCA scores plot (PC1 vs. PC2) of all the donated cosmetic samples, grouped by brand.

Inspection of the loadings plots for PC1 (Figure 6.24) reveal a startling likeness to those from the Chapter 3 model (Figure 3.32) and are almost exclusively based on anatase content. Nevertheless, the loadings for PC2 (Figure 6.25) are very different to those seen in the previous model (Figure 3.33). Figure 6.24 shows a strong negative correlation with the lattice peak, but positive correlations with the goethite and haematite peaks, and a doublet of peaks in the CH region, whereas the PC2 loadings for the new samples show a positive correlation with rutile, a strong negative correlation with the lecithin peak and a triplet of peaks in the CH region. It is evident that the minor doublet of peaks in the 1600 cm^{-1} region has inverted from

positive in Figure 3.33 to negative in Figure 6.24. These loadings plots confirm that the congruence of the explained variance values is coincidental and the products do not possess the same chemistry.

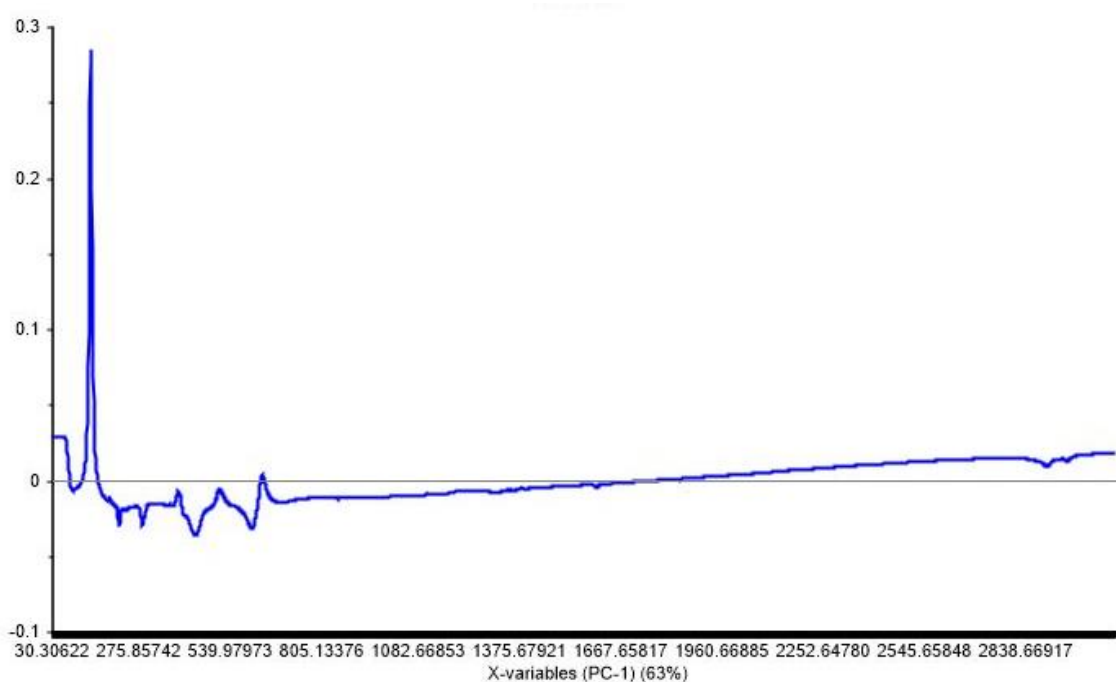


Figure 6.24: PCA loadings plot for PC1 of all the donated cosmetic samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading.

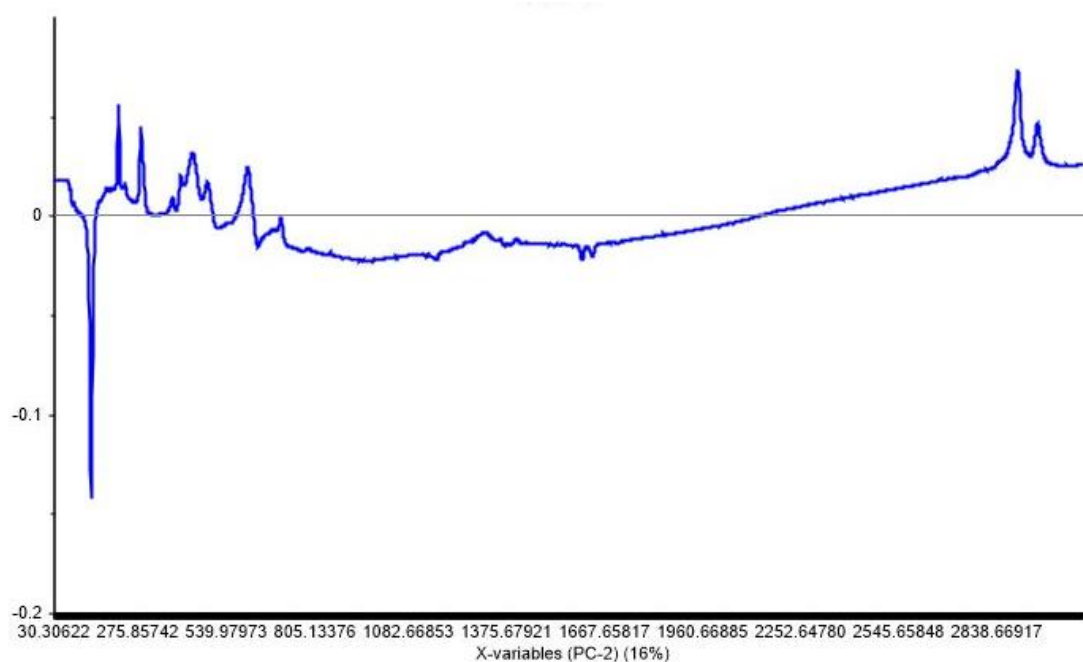


Figure 6.25: PCA loadings plot for PC2 of all the donated cosmetic samples. X-axis gives Raman shift (cm^{-1}), y-axis gives the loading.

Applying sample grouping by brand to the influence plot allows for the assessment of outliers and extreme samples. Figure 6.26 displays samples in all four quadrants of the influence plot: those appearing in the bottom left box are representative of the sample set; those in the top left box are poorly described by the model; those in the bottom right box have a high leverage and are highly influential to the model; and those in the top right box are potential outliers, exhibiting both a high residual and a high leverage¹³.

The potential outlying samples' (those in the top right quadrant of the plot) spectra were evaluated but, as was the case with previous PCA models, no samples were removed from the dataset as their measurements were deemed to be accurate¹³.

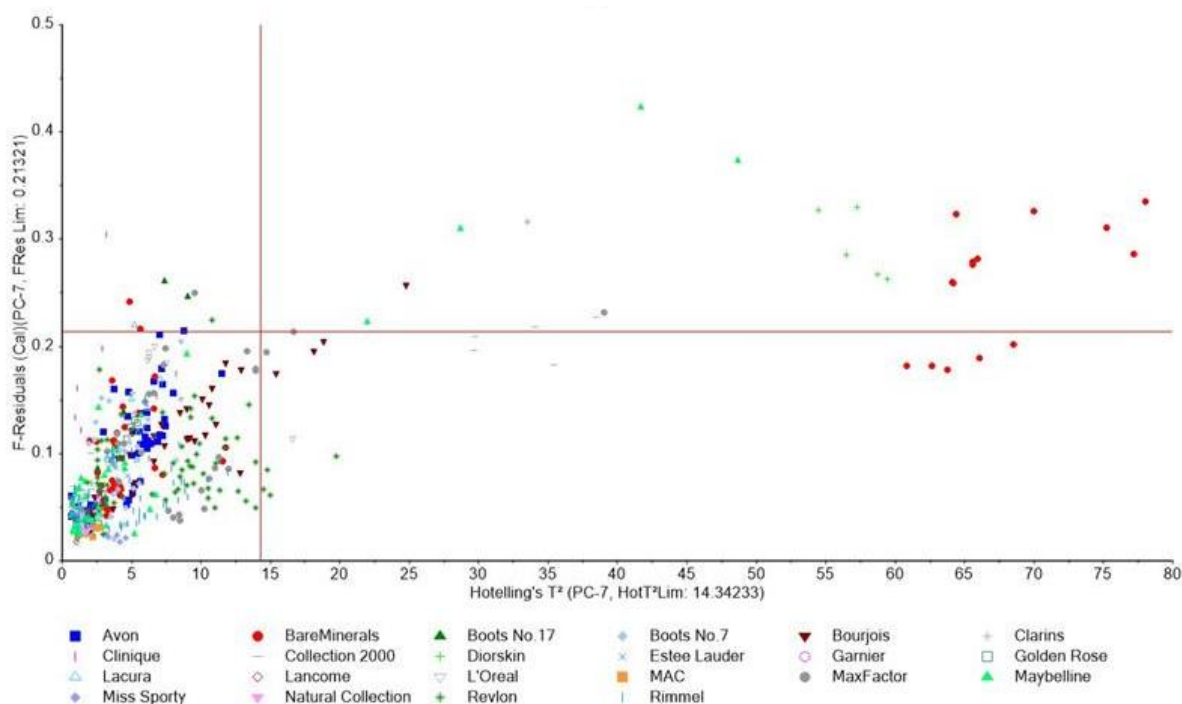


Figure 6.26: Influence plot (PC7) of all the donated cosmetic samples, grouped by brand.

The samples in the PCA scores were subsequently grouped by formulation (Figure 6.27) but again, no clearly defined classes were revealed. The only perceivable trend was all the cream-based products falling on the negative side of PC2, indicating a lack of spectral lattice peak and the presence of organic components. There did not appear to be any correlation between powdered samples and increased spectral heterogeneity, according to the proximity of the sample points, yet inspection of the influence plot (Figure 6.28) revealed that loose powders and pressed powders made up the majority of samples in the top right box, i.e. samples with both high residuals and leverages.

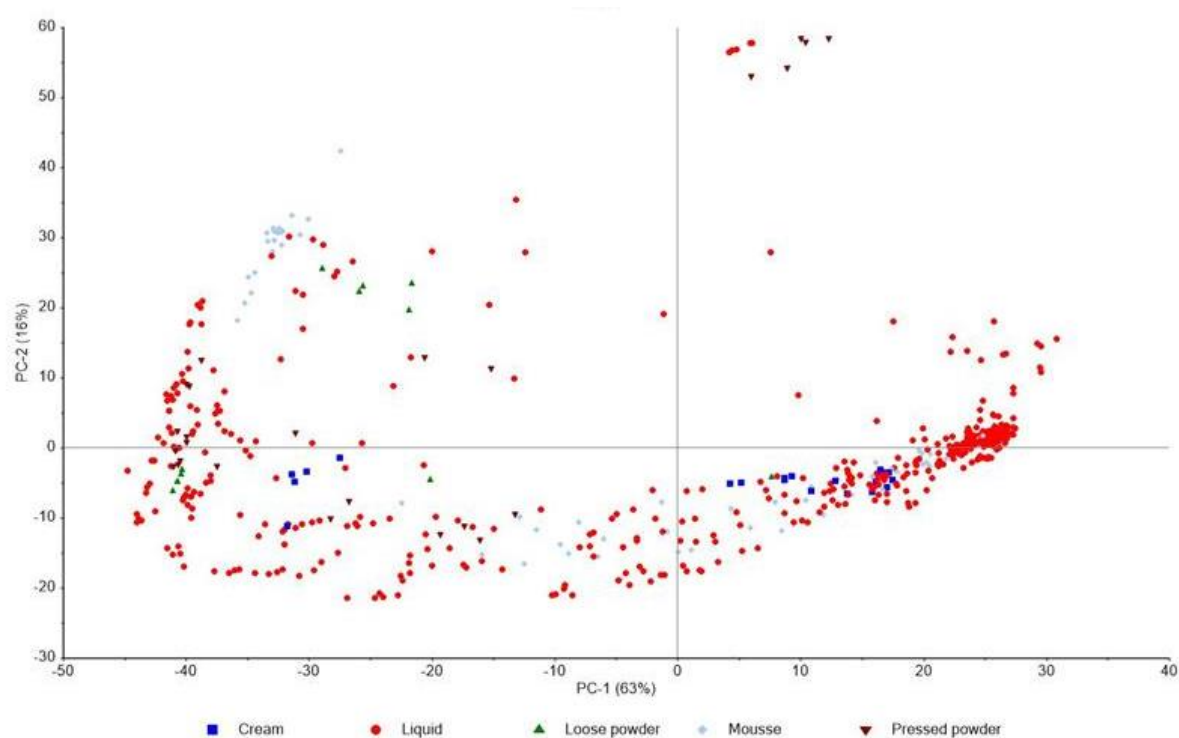


Figure 6.27: PCA scores plot (PC1 vs. PC2) of all the donated cosmetic samples, grouped by formulation.

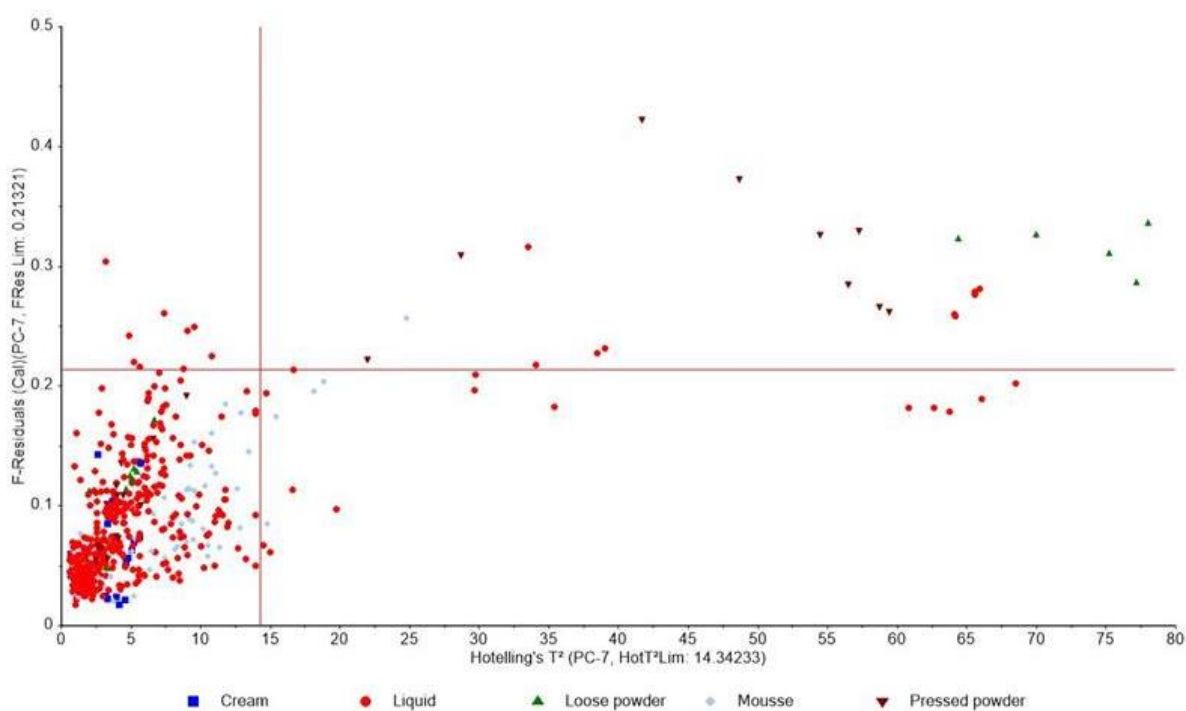


Figure 6.28: Influence plot (PC7) of all the donated cosmetic samples, grouped by formulation.

Product type was the final sample grouping applied to the model. Figure 6.29 now shows the emergence of class categories, with bronzers exclusively occupying space in the positive region along PC2, finishing powders only appearing in the negative space along PC2, and highlighters confined to positive scores along both PC1 and PC2. The foundations, concealers, tinted moisturisers, and primers tend to be spread evenly throughout all four quadrants of the scores plot, showing a variable but consistent chemical composition. Only the single primer sample situated at the top of the plot could be regarded as extreme in this case. Reference to the accompanying influence plot (Figure 6.30) confirms that this primer sample is indeed an extreme sample.

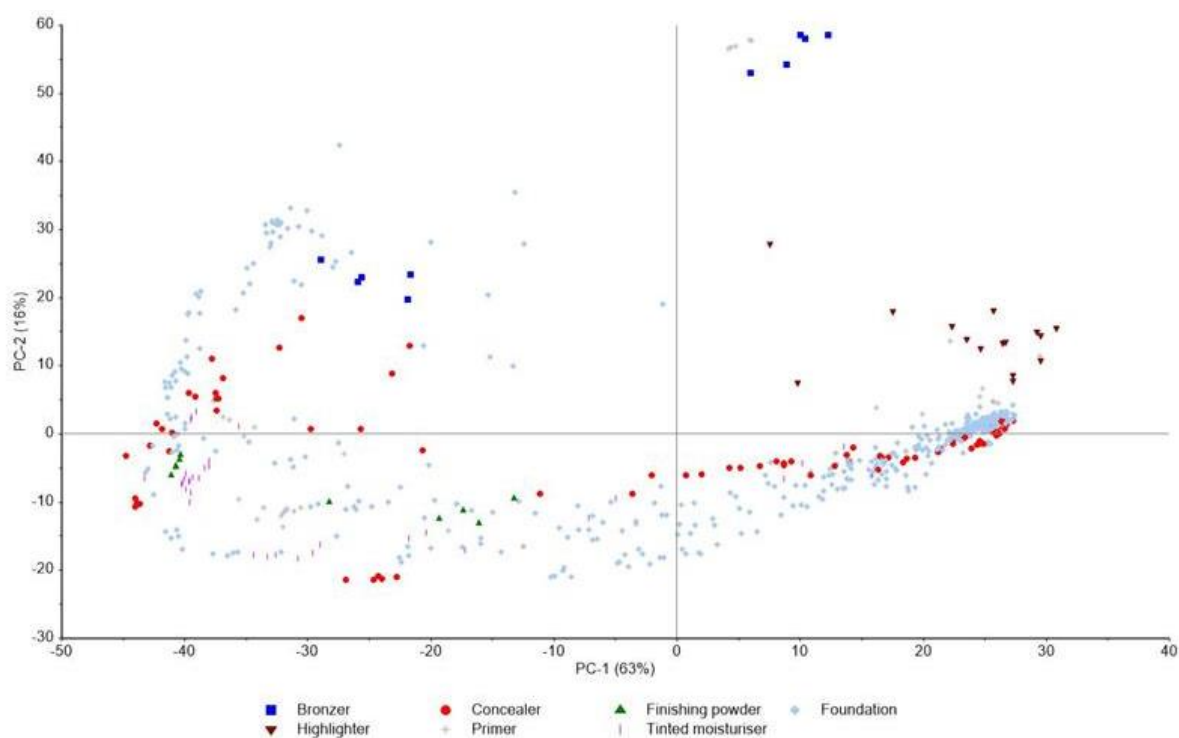


Figure 6.29: PCA scores plot (PC1 vs. PC2) of all the donated cosmetic samples, grouped by product type.

Further analysis of the influence plot reveals that both donated bronzers are also extreme samples with high leverage. This is to be expected given the very different nature and dark colour of these products compared to the general sample population. Removal of these samples from the PCA and performing a recalculation would significantly impact the scores and loadings, as the total variance across the dataset would be reduced¹⁴.

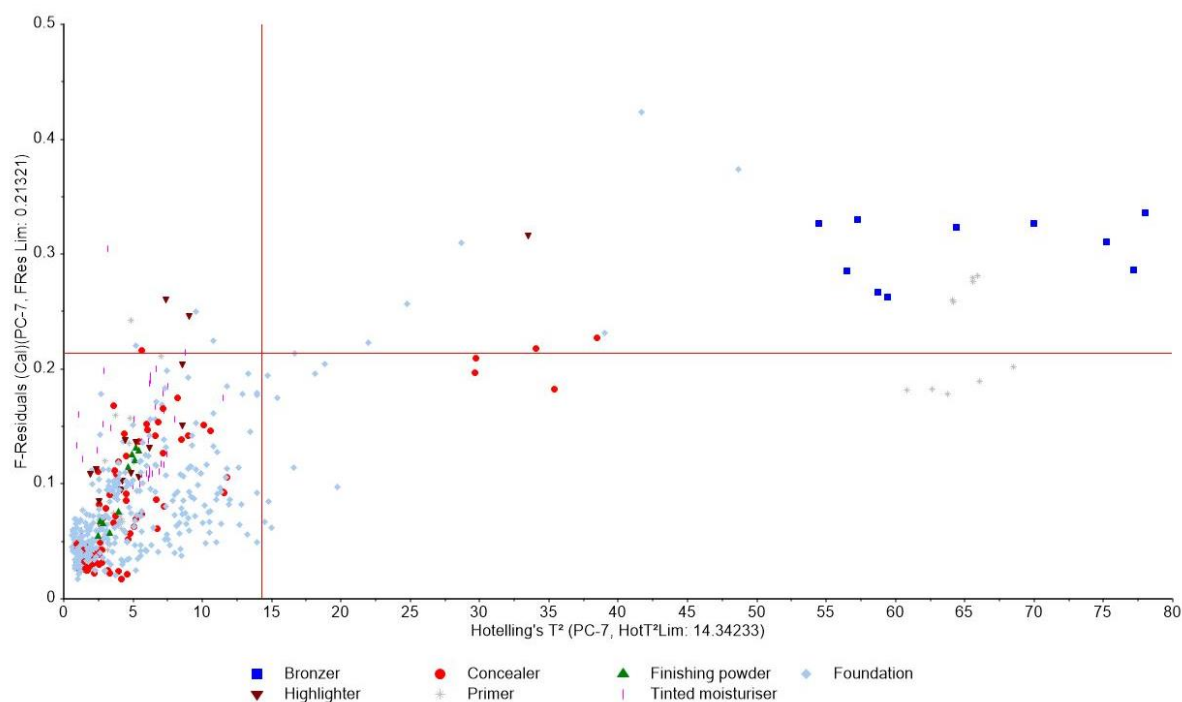


Figure 6.30: Influence plot (PC7) of all the donated cosmetic samples, grouped by product type.

6.3.5 Classification

Referring back to the discussions in Section 6.3.1 concerning differences in sample age, type, use, storage, and packaging, and issues surrounding potential sample dehydration, oxidation, UV irradiation, contamination, and degradation, it was decided that more work would need to be carried out to profile the spectral changes these variables induce.

In order to determine the origin of spectral discrepancies (i.e. homogeneity vs. heterogeneity) and whether they could be attributed to the sample itself rather than external factors, systematic studies would need to be completed that only changed one variable at a time. Then models could be created that accurately reflected the composition of the samples. As these studies have not been attempted in this Chapter, and the samples have not yet been categorised as intrinsically homogeneous or heterogeneous, it is unknown which of the two base models (Chapter 3 or Chapter 4) would best suit a PCA projection.

Furthermore, due to the lack of obvious sample grouping or patterns in the PCA scores plots examined thus far, there was no basis upon which to perform a supervised classification, such as a DA, as these require knowledge of defined classes^{13–19}.

A regression model, such as a partial least squares regression (PLSR) to predict sample behaviour under various circumstances may be a logical next step for integration into casework, and provides opportunities for future work.

6.4 Conclusions

This Chapter sought to apply the knowledge gained in Chapter 3 about chemical variability and the applicability of Raman to these types of cosmetics, with the exploration of spectral heterogeneity investigated in Chapter 4, and the assessment of ageing impact upon sample appearance and chemistry, to the analysis of a large set of “*real world*” samples.

These “*real world*” samples encompassed 120 expired testers, old donated samples, or discarded used products. The sample set included products from 78 different product lines, represented by 22 different brands, covering all price brackets from budget range through to the premium market, and a variety of shades. All seven product types were represented as were all five formulation types, giving an excellent range of variables.

Visual assessment of the samples revealed appearances consistent with those seen across Chapters 3 and 4; the presence of various microspheres, mica flakes and dispersed pigment particles were evident.

Raman spectroscopy demonstrated the donated samples comprised a mixture of spectrally homogeneous and heterogeneous products, although it was not known whether these spectral differences resulted from the ageing process or contamination, or were an intrinsic property of the samples themselves. Fortunately, comparisons could be drawn between certain donated samples and their new counterparts, analysed in Chapters 3, 4, and 5. Across multiple samples, from various product types it could be determined that baseline fluorescence and extra peaks in the spectral mid-region were more pronounced among the donated samples than their new counterparts. It is highly probable that these changes arose due to a combination of ageing effects and sample contamination, most likely derived from a biological source.

A PCA model was constructed that showed very similar explained variances to those of the Chapter 3 model. Calibration and validation variances tracked closely, indicating a robust model. Inspection of the scores, however, revealed that the sample populations were slightly different: the data swarm generally occupied the same space within the PC1 vs. PC2 scores plot, but the spread of the data points was different. Furthermore, the extreme values along PC2 were also different.

No clear trends and no clustering by brand or formulation were evident. However, there was tentative grouping seen when categorised by product type in that bronzers exclusively displayed positive values along PC2, whilst finishing powders exclusively had negative PC2 values. Highlighters were even more closely clustered, only occupying space in the positive regions of both PC1 and PC2. Nonetheless, both the bronzers in this dataset were identified

as outliers in the influence plots, so it was to be expected that they would retain extreme scores in one or more of the PCs.

No attempts at classification were made as there were too many unknown variables within the sample set, and a distinct lack of sample groupings, which would have rendered models ineffective. Regression modelling would be the next logical step for profiling the effect of ageing and/or contamination on the chemical variability of facial cosmetics in a forensic context.

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Chapter Seven: Conclusions

7.1 Key findings

The aim of this thesis was to determine the utility of facial cosmetics as a form of associative trace and whether Raman spectroscopy coupled with chemometrics was a well-suited technique for their analysis. Since Edmond Locard's demonstration of facial cosmetic microtrace value¹, limited research has been conducted and they remain an under-appreciated form of trace. Only five research papers detail the forensic analysis of facial cosmetics²⁻⁶ but none of these investigations use Raman spectroscopy. Thus, this research presents a novel application of Raman spectroscopy to the analysis of forensic cosmetic trace.

This thesis demonstrates the combination of visual microscopic and Raman spectroscopic characterisation of 297 facial cosmetic samples, with subsequent chemometric methods for objective spectral interpretation. It has been previously stated that assessment of cosmetic traces on a purely visual basis is not recommended, owing to subjective descriptions and the difficulties associated with analysing interference pigments^{2,7-11}. Therefore, the addition of chemical composition assessment via Raman spectroscopy circumvents these problems. Furthermore, the application of Raman microscopy is particularly well suited to the analysis of microtraces and is also preferential to advanced Raman techniques such as surface enhanced Raman spectroscopy (SERS)¹² as it involves less sample preparation and potential alteration. The application of chemometrics to Raman data allows for increased discrimination between samples, provides objective measures of similarity or dissimilarity, explores the chemistry of the samples and deduces the most influential factors driving separation. Principal component analysis (PCA) is a superlative statistical method to the commonly reported discriminatory power (DP), as DP does not describe the chemical variability within a sample set. Moreover, chemometric methods address the current need to express forensic sample comparisons in an unbiased way¹³⁻¹⁵.

Chapter 3 describes the microscopic and Raman analysis of 126 newly purchased samples that were assessed as being spectrally homogeneous. Microscopy afforded an initial level of visual discrimination but it transpired that visual assessment of sample homogeneity did not correlate with what was seen spectrally. Raman was found to be a very useful technique due to its sensitivity and selectivity, and despite concern in the literature¹⁶⁻²¹, fluorescence was not as much of an issue for these samples. It also proved more useful than ATR-FTIR for the analysis of mineral-based pigments, as demonstrated by the samples analysed here, due to the increased spectral range²²⁻²⁸. Spectrally homogeneous samples were dominated by only four of the seven product types assessed throughout this thesis: foundations, concealers, tinted moisturisers and finishing powders. All the newly purchased bronzers, highlighters and

primer samples fell into the spectrally heterogeneous category and formed part of Chapter 4. Chemometric treatment of the spectral data gave a quantitative means of differentiating between samples and revealed trends that were not immediately discernible by eye, such as the covariance between mid-range spectral peaks and those in the CH region. Assessment of the loadings showed the primary distinction between samples to be their titanium dioxide polymorph, followed by lecithin or castor oil content, as designated by PC1 and PC2 respectively. Plotting different score plot combinations of PCs against each other revealed various sample clusters, reinforcing the chemical complexity in the dataset. Whilst sample grouping by brand (manufacturer) did not reveal clusters, owing to the vast range of product types within any one brand, the data suggest that grouping instead by specific product line may be more helpful. Inspection of the influence plots permitted instant recognition of samples that were important to the model or required further verification, via their leverage and residual values. When grouping the influence plot by shade, the key role of pigment to the model was realised, yet this trend was not readily discernible by visual examination of the spectra. The large dataset represented significantly more samples than have been assessed in the literature²⁻⁶ (at least more than double) and encompassed a far more varied product range. Discriminant analysis (DA) was applied after PCA with mixed success and represents a future work opportunity. Nonetheless, the results contained in this Chapter contribute greatly to our understanding of the chemical variability amongst facial cosmetics and could assist with casework questioned (Q) versus known (K) comparisons, investigative leads, or sample eliminations.

The remaining 44 newly purchased samples that were assessed as being spectrally heterogeneous formed the basis of Chapter 4. It was found that the majority of these samples were powdered (loose or pressed), indicating these formulations tend to result in sampling heterogeneity, whereas liquid, cream and mousse formulations tended to yield more repeatable spectra. As stated in the previous paragraph, all the newly purchased bronzer, highlighter, and primer samples were assessed as spectrally heterogeneous and therefore did not feature in Chapter 3. In addition, the majority of the new finishing powders transpired to be heterogeneous. With the exception of primer (which is designed to be applied to the skin before all other decorative cosmetics), all these products are designed to be applied last in the cosmetic sequence. Bronzers and highlighters are contouring and brightening products that are designed to be applied over the base complexion products, and finishing powder (as the name would suggest) is designed to be used at the end of the routine. This raises a concern with regards to trace analysis in casework because if a cosmetic smear is transferred soon after the initial application (and therefore not had time to warm into the skin and mix with the base complexion products), it is highly likely that the first trace deposited will be comprise contouring and/or finishing types of cosmetic. Microscopic and Raman spectroscopic analyses

corroborated what was seen in Chapter 3: the visual assessment of a sample could not be used as a reliable indicator of its spectral heterogeneity. Indeed, one spectrally heterogeneous sample was surprisingly visually homogeneous. Significant differences in the level of spectral heterogeneity were seen across the sample set, further confirming their chemical complexity. The unexpected discovery that one of the foundations within the sample set was counterfeit showed the utility of these investigations not just in a crime scene investigation context but also for the verification of genuine consumer goods. PCA of the spectral data revealed some tentative trends but also highlighted that the subjective assessment of spectral heterogeneity was unhelpful as the PCA dimensionality reduction did not apply the same weightings to visual spectral differences. It was concluded that a better approach would have been to include all 170 samples in a single PCA and allow the PCs to determine spectral repeatability and sample heterogeneity. This was confirmed via a PCA projection classification, which indicated that the sample sets from Chapters 3 and 4 emanated from the same population. Nonetheless, assessment of the impact of sample heterogeneity was a valuable endeavour, as this variability is more likely to be replicated in casework where exhibits will neither be newly purchased, nor applied to glass slides.

The effects of ageing, both within product packaging after opening, and on a glass slide, were investigated for seven newly purchased samples in Chapter 5. High-resolution microscopic examination showed that the majority of sample ageing on a glass slide occurred within the first week. After 15 months of ageing, the most noticeable changes occurred amongst samples that were predominantly water-based. Other significant visual findings included drastic colour and texture changes, and the detachment of borosilicate glass pigments and silica microspheres from their respective matrices, which may have implications for casework. Sample aliquots taken from the original product packaging over the course of 15 months and analysed via Raman showed sample chemistry was largely unchanged despite the significant changes seen when samples were aged on a glass slide. This apparent product stability is encouraging as it means we can be relatively confident any chemical changes we see in casework traces will have arisen from various processes after product application to the skin, rather than degradation prior to use. Despite exhibiting the most significant visual changes, the products that mainly lost water content exhibited minimal spectral changes, presumably due to water's weak Raman scattering ability. Products with a higher organic content were more affected according to their Raman spectra, with the loss of the broad peak corresponding to lecithin being the most obvious change. Sample ageing on a glass slide also showed a substantial increase in Raman baseline fluorescence that did not respond well to photobleaching, indicating that older samples may exhibit this spectral feature. Inter-batch comparisons revealed that two of the product manufacturers had altered the titanium dioxide polymorph used in their formulations from anatase to rutile; the reason for this is unknown but

it is presumed to relate to anatase's photocatalytic activity and metastability. Four products showed consistent spectra indicating their compositions had been unchanged over the three/four-year period. PCA enabled a deeper analysis of the spectral data, reinforcing the benefit of adding chemometrics to trace evidence interpretation. For example, the spectral changes among the sample aliquots "*from bottle*" that were revealed in the scores plots but imperceptible by eye.

Several generous donations of old samples and expired shop testers resulted in the collection of 120 "*real world*" samples that were analysed in Chapter 6. This Chapter brought together all the knowledge gained across Chapters 3, 4, and 5 to enable interpretation of samples with an even more complex chemistry than was seen previously. Visual macroscopic and microscopic analyses did not show any obvious signs of ageing or contamination. However, Raman spectroscopy revealed extra peaks in the mid-spectral range of some samples and an increase in baseline fluorescence when compared to their newly purchased counterparts. These spectral changes indicated the effects of ageing and/or contamination, likely derived from biological sources. PCA of the 120 donated samples displayed a different data swarm to that seen in the Chapter 3 model, with no clear trends according to brand or formulation but tentative clusters when grouped by product type, especially for the highlighters. There was no indication to perform a classification due to the lack of definitive sample groupings, but this also represents an opportunity for future work. Owing to the level of spectral variation seen across these used samples, it is recommended that casework analyses employ a Q versus K approach (where possible), so that the trace can be compared directly back to its reference counterpart. This would be preferable to a database search or "*market survey*" approach as the chemistries of these products vary hugely after use and it is unlikely that a single model will be applicable across any given cosmetic type.

7.2 Limitations

Whilst it is unlikely that Raman spectroscopy will be able to "match" a crime scene sample to a reference library of new products due to the effects of changing formulations, ageing and contamination, it can provide significant insight into the chemical variability within a mixture.

Chemometric analysis of the "*market survey*" products (Chapter 3) enabled understanding of their chemical diversity and probed the underlying structure of the samples. However, it is unrealistic to create a classification model for operational use that is truly representative of the global market, particularly at any given point in time.

It was noted in Chapter 4 that spectral heterogeneity posed a problem for accurate sample association so an alternative analytical method may be more appropriate for these samples. A possible solution would be to utilise a lesser magnification such as the x10 microscope

objective for a larger field of view. Nonetheless, as this objective lens possesses a lower numerical aperture (NA) this will result in Raman signal reduction, which could be a problem for weakly scattering samples. An alternative to the random sampling method employed throughout this thesis is an automatic Raman mapping technique that takes replicate spectra across multiple spatial areas using a raster technique. However, it should be noted that these analyses are time-consuming, generate large volumes of spectral data and require expensive instrumentation with motorised stage capability and thus may not be appropriate in casework.

The inter-batch variation study carried out in Chapter 5 included a limited sample set, so conclusions cannot be made with confidence. Furthermore, no intra-batch variation studies were performed as the overall aim throughout this thesis was the assessment of chemical variability within a large data set: a “*market survey*”. Nevertheless, it can be presumed that if products are not exhibiting a noticeable change over three-four years, that intra-batch variations will also be minimal.

The notion of product mixing is highly relevant in casework as consumers rarely use one decorative cosmetic in isolation. Not only will using products in a particular sequence give rise to distinctive residues but the length of time those products are in contact with the skin prior to deposition will affect trace individualisation. The longer that cosmetic products are left on the skin, the longer they have to warm (via body temperature), and therefore become more fluid, mix with the wearer’s own eccrine and sebaceous secretions and combine into a truly unique trace. This is further complicated by differences in skin types, e.g. dry, dehydrated, oily, combination, etc. as these will affect the extent of product mixing. Unfortunately, time did not permit the investigation of these types of mixed product residues using the analytical technique devised in this thesis, either as a mixture of products on a glass microscope slide, or as a mixture on human skin, but further research is currently underway to address these variables.

Notwithstanding the large number of samples (297) analysed during the course of this thesis and the rich chemical data that has arisen from those analyses, no cosmetic population studies were undertaken. From the huge variety of decorative cosmetics available on the UK market it can be assumed that these products are prevalent in society, yet there remains no evidence to suggest which types are most common, which products are being used in combination (and therefore likely to appear as trace mixtures), and most importantly, how likely is it that these traces would be encountered in the environment, i.e. the background considerations. Given that these products can be extremely persistent, this further complicates consideration of background contributions.

Perhaps the most obvious limitation of this research is that all samples were analysed on clean glass microscope slides, which is not representative of casework trace origin. This decision was deliberately taken to mitigate as many potential variables as possible, and better understand the product chemistry without interfering substrates. Nonetheless, in order to integrate cosmetic analysis into casework, we need to investigate how these samples interact not only with human skin, but also on the supporting substrate after transfer. It is well known that loose fibres, for example, adhere to different substrates to varying degrees dependent upon their physical characteristics, i.e. there is a co-dependence of variables. It is envisaged the same will be true for cosmetic traces deposited on various substrates and again, provides an opportunity for future work.

7.3 Future directions

Given that this is the first Raman microscopic study of this category of cosmetic products, it is no surprise that there are many untapped opportunities for future work in this area.

With regards to the Raman spectroscopic analysis of facial cosmetics, further research could investigate the effects of changing laser wavelength source to see if a hybridisation of laser sources affords better discrimination of samples. As discussed in Section 7.2, Raman mapping would prove useful for the analysis of facial cosmetic samples, especially for better defining spectrally homogeneous and heterogeneous samples. As a non-destructive and non-contact technique, Raman spectroscopy can also be used in conjunction with other analytical techniques such as ATR-FTIR or NIR in a multi-modal sequence, for increased sample discrimination. It would be interesting to conduct colour block studies using Raman spectroscopy to determine how effective it might be at discriminating between cosmetic traces of any given colour. The Horiba LabRam spectrometer is a large and expensive benchtop instrument but investigations using handheld or portable Raman spectrometers may enable presumptive sample testing at crime scenes in the future.

The 297 different samples analysed throughout this thesis represent a significant body of knowledge, however, these cosmetic products are constantly evolving and being superseded. Therefore, further analyses of emerging products could be undertaken, as could newer variants or formulations of existing products, to profile potential ingredient changes. In this vein, it would be beneficial to work with cosmetic manufacturers towards a common goal. Currently there is no access to proprietary information but if companies were prepared to divulge their formulation compositions it might hasten profiling of these sorts of cosmetics so that databases and models can be created more efficiently. The assessment of global cosmetic chemical variation (but for two samples purchased in Poland) has not been carried out but also presents opportunity for future work. Analyses are already underway to profile

facial cosmetic samples from Australia and Indonesia. The next logical step is to extend these studies to include other decorative cosmetics such as blusher and eyeshadows, then to incorporate cosmetics such as skincare, sun care and body care, so that mixtures can be accounted for.

Concerning the treatment of the samples, many contextual factors need to be assessed. These encompass background levels, substrate interferences, matrix effects, and transfer and persistence considerations. A good starting point would be to perform target cosmetic studies to ascertain the probability of finding a specific trace on a random surface in the environment, akin to those performed previously for glitter particles¹⁰. Systematic ageing of a larger sample set, both from the bottle and on various substrates could be investigated, as could deliberate contamination or weathering of samples under different conditions/on different substrates to assess co-dependence of variables. Cosmetic product interactions with the skin could be assessed at various timed intervals and the residue deposited thereafter to ascertain which chemical components are lost to the skin and which are added to the resultant trace. These studies could be taken even further to assess how different skin types or ages dictate how cosmetics will interact with the skin matrix, or whether it matters how the cosmetic was applied (wet/dry), the method of application (brush, sponge, bare hands, etc.), the delay time from deposition of trace to its collection, or even the time delay between scene collection to laboratory analysis. The complexity can then be increased by analysing various product mixtures to assess the effects of product layering, as could be expected in casework samples. From there, activity level propositions could be investigated by simulations of operationally relevant specimens. The complexity could be increased even further by considering that Locard's Exchange Principle dictates evidence transfer is a two-way event, i.e. if two cosmetic-wearing individuals come into contact there will be reciprocal transfer, resulting in extremely variable and complicated resultant traces. Depending on the forces involved this may represent a partial or full transfer of cosmetic trace in one or both directions and modelling these interactions would be a formidable task.

Chemometric analysis could be extended by amalgamating all 297 samples into a single PCA model to serve as a valuable databasing tool for casework investigations. It would also be beneficial to see whether or not sample clustering improves when products are grouped by specific product line rather than by brand (manufacturer). There are many classification methods that were not explored during this thesis that may be better suited to the analysis of these samples than the PCA predictions or LDA/QDA classifications attempted. Casework style Q versus K comparisons could be investigated to assess the level of sample matching. Design of experiments (DOE) could be employed for instrumental parameter and sampling method optimisation.

The findings from the ageing experiments in Chapter 5 that borosilicate glass interference pigments and silica microspheres may detach from their product matrices after prolonged drying periods suggests that even in cases where the liquid cosmetic residues cannot be analysed or the matrix has degraded, that there is value in the solid particulates leftover. This has been demonstrated via analysis of cosmetic glitter particles^{10,11,29–37}, shimmer flakes^{38,39} and mascara⁴⁰ that may contain nylon and/or rayon fibres^{41–43}. This has significant relevance in casework as these particles, in addition to other inorganic pigments (such as mica, iron oxide, and bismuth oxychloride), may transfer more readily than the cosmetic matrix they originated from and have been shown to persist in the environment.

On a practical level it would be beneficial to work alongside crime scene investigators, forensic scientists, and pathologists to increase awareness of cosmetic prevalence, the nuances associated with cosmetic transfer and persistence (such as the solid particulates discussed above), and to encourage acceptance of their probative value as a form of trace. Working together, recovery protocols could be adapted to suit cosmetic analysis specifically^{44,45}, whether that be via swabbing, scalpel scraping or dry lifting, and analytical procedures established to extract the maximum amount of data from the samples. Ultimately, evidence that is not collected at the scene, is evidence that is lost. Every piece of evidence from a scene has the potential to provide the “*missing piece of the jigsaw*” and cosmetics are no exception.

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Appendix

Table A1: List of raw cosmetic components.

Cosmetic component		
Acrylates copolymer	Hydrolysed collagen protein	Rice starch
Allantoin	Iron oxide black	Rutile titanium dioxide
Aluminium oxide	Iron oxide brown	Salicylic acid
Anatase titanium dioxide	Iron oxide red	Shea butter
Ascorbic acid	Iron oxide yellow	Silica
Beeswax	Isopropyl myristate	Sodium hyaluronate
Benzylalcohol-DHA	Kaolinite	Squalane light
BHT	Lecithin	Stearic acid
Bismuth oxychloride	Limonene	Sulfur
Brookite titanium dioxide	Linalool	Talc
Calcium sulphate	Magnesium aluminium silicate	Tin oxide
Caprylyl glycol-EHG	Magnesium stearate	Ultramarine blue
Carnauba wax	Magnetite	Ultramarine pink
Castor oil	Mica beige	Urea
Cetyl alcohol	Mica bronze	Zinc oxide
Cetyl palmitate	Mica carmine red	
Chromium oxide green	Mica diamond cluster	
Citric acid	Niacinamide	
Cocoa butter	Octylmethoxycinnamate	
Coumarin	Panthenol	
Dimethicone 500	Paraben-DU	
DL-alpha Tocoherol	PEG-PPG-PEG	
Glyceryl stearate PEG 100 stearate	Petrolatum	
Goethite	Polymethylsilsesquioxane	
Haematite	Propylene glycol	

Table A2: List of all facial cosmetic samples used throughout Chapters 3, 4, and 6.

Brand	Range	Shade	Product Type	Formulation	Age
Astor	Lift Me Up Foundation	100	Foundation	Liquid	New
Autograph	Daily Defence Tinted Moisturiser	Medium Nude	Tinted Moisturiser	Liquid	New
Autograph	Lasting Miracle Finish Foundation	Nude	Foundation	Liquid	New
Avon	Bright Start Foundation	Nude	Foundation	Liquid	New
Avon	Ideal Flawless CC Cream	Ivory	Tinted Moisturiser	Liquid	New
Avon	Ideal Flawless Foundation	Nutmeg	Foundation	Liquid	New
Avon	Ideal Shade	Shell	Foundation	Liquid	Old
Avon	Face Perfecting Duo	Light	Tinted Moisturiser	Liquid	Old
Avon	Crème Compact Foundation	Almond	Foundation	Cream	Old
Avon	Anew Ultimate Sheer Tint	N/A	Tinted Moisturiser	Liquid	Old
Avon	Anew Reversalist Sheer Tint	N/A	Tinted Moisturiser	Liquid	Old
Avon	Pressed Powder	Beige	Finishing Powder	Pressed Powder	New
Avon	Personal Match Barely There	Light	Foundation	Liquid	Old
Avon	Soft Matte Mousse Makeup	Nude	Foundation	Mousse	Old
Avon	Face Perfecting Duo	Light	Concealer	Cream	Old
Avon	Invisible Light Illuminating Makeup Base	N/A	Primer	Liquid	Old
Avon	Solutions Truly Radiant Tint Release	N/A	Tinted Moisturiser	Liquid	Old
Bareminerals	Bareskin Serum Foundation	Bare Natural	Foundation	Liquid	New
Bareminerals	Ready Foundation	R210	Foundation	Pressed Powder	New
Bareminerals	Ready Foundation	R310	Foundation	Pressed Powder	New
Bareminerals	Blemish Remedy Concealer	Light	Concealer	Cream	New
Bareminerals	Bareskin Perfecting Veil	Light-Medium	Finishing Powder	Pressed Powder	New
Bareminerals	Original Foundation	Medium Beige	Foundation	Loose Powder	New
Bareminerals	SPF 30 Natural Sunscreen	Medium	Finishing Powder	Loose Powder	New
Bareminerals	All Over Face Colour	Turn on Gold	Highlighter	Loose Powder	New
Bareminerals	All Over Face Colour	Brilliant Rose	Highlighter	Loose Powder	New
Bareminerals	All Over Face Colour	Rose Gold Radiance	Highlighter	Loose Powder	New

Brand	Range	Shade	Product Type	Formulation	Age
Bareminerals	Bareskin Perfecting Veil	Illuminating	Finishing Powder	Pressed Powder	New
Bareminerals	Ready Colour Boost	Adrenaline Rush Light	Highlighter	Pressed Powder	New
Bareminerals	Ready Colour Boost	Adrenaline Rush Medium	Highlighter	Pressed Powder	New
Bareminerals	Ready Colour Boost	Adrenaline Rush Dark	Bronzer	Pressed Powder	New
Bareminerals	Stroke of Light Face Highlighter	N/A	Highlighter	Liquid	New
Bareminerals	Ready Bronzer	The Skinny Dip	Bronzer	Pressed Powder	New
Bareminerals	Ready Bronzer	The High Dive	Bronzer	Pressed Powder	New
Bareminerals	Ready Luminizer	The Long Weekend	Highlighter	Pressed Powder	New
Bareminerals	Ready All Over Face Colour	Glee	Highlighter	Pressed Powder	New
Bareminerals	Original Foundation	Fair Ivory	Foundation	Loose Powder	New
Bareminerals	Blemish Remedy Foundation	Clearly Pearl	Foundation	Loose Powder	New
Bareminerals	Blemish Remedy Foundation	Clearly Cream	Foundation	Loose Powder	New
Bareminerals	Under-Over Priming Finishing Powder	N/A	Finishing Powder	Loose Powder	New
Bareminerals	Multitasking Highlighter	Turn on Rose Gold	Highlighter	Loose Powder	New
Bareminerals	Bareskin Serum Concealer	Fair	Concealer	Liquid	New
Bareminerals	Barepro Liquid Foundation	Dawn 02	Foundation	Liquid	New
Bareminerals	Barepro Liquid Foundation	Natural 11	Foundation	Liquid	New
Bareminerals	Mineral Veil	Colour Correcting	Finishing Powder	Loose Powder	New
Bareminerals	Barepro Powder Foundation	Warm Light 07	Foundation	Pressed Powder	New
Bareminerals	All Over Face Colour	Glee Radiance	Highlighter	Loose Powder	New
Bareminerals	Complexion Rescue	04 Suede	Tinted Moisturiser	Liquid	New
Bareminerals	Complexion Rescue	Natural	Tinted Moisturiser	Liquid	New
Bareminerals	Complexion Rescue	Wheat	Tinted Moisturiser	Liquid	New
Bareminerals	Bareskin Serum Foundation	Bare Linen	Foundation	Liquid	New
Bareminerals	Complexion Rescue	Vanilla	Tinted Moisturiser	Liquid	New
Bareminerals	Original Foundation	Fairly Light	Foundation	Loose Powder	New
Bareminerals	Original Liquid Mineral Concealer	Light	Concealer	Liquid	New
Bareminerals	Mineral Veil	Original	Finishing Powder	Loose Powder	New
Bareminerals	Matte Foundation	Medium Beige	Foundation	Loose Powder	Old

Brand	Range	Shade	Product Type	Formulation	Age
Bareminerals	Matte Foundation	Fairly Light	Foundation	Loose Powder	New
Bareminerals	Multitasking Concealer	Bisque	Concealer	Loose Powder	New
Bareminerals	Multitasking Concealer	Summer Bisque	Concealer	Loose Powder	New
Bareminerals	Well Rested	N/A	Concealer	Loose Powder	New
Bareminerals	Well Rested Cream Colour Corrector	Neutralising	Concealer	Cream	New
Bareminerals	Original Foundation	Light	Foundation	Loose Powder	New
Bareminerals	Bareskin Serum Concealer	Light	Concealer	Liquid	New
Bareminerals	Bareskin Serum Foundation	Bare Ivory	Foundation	Liquid	New
Bareminerals	Bareskin Serum Foundation	Bare Satin	Foundation	Liquid	New
Bareminerals	Complexion Rescue	Opal	Tinted Moisturiser	Liquid	New
Bareminerals	Complexion Rescue	Buttercream	Tinted Moisturiser	Liquid	New
Bareminerals	BB Primer Cream	Light	Primer	Liquid	Old
Bareminerals	Pure Transformation Day Treatment	Medium	Foundation	Loose Powder	New
Bareminerals	Original Foundation	Fairly Medium	Foundation	Loose Powder	New
Bareminerals	Mineral Veil	Hydrating	Finishing Powder	Loose Powder	New
Bareminerals	Mineral Veil 5 in 1 BB Advanced Performance	Light	Finishing Powder	Loose Powder	New
Bareminerals	Ready Touch Up Veil	Tinted	Finishing Powder	Pressed Powder	New
Bareminerals	Stroke of Light	Luminous 1	Concealer	Liquid	Old
Bareminerals	Mineral Veil	Illuminating	Finishing Powder	Loose Powder	New
Bareminerals	Mineral Veil	Matte Glow	Finishing Powder	Loose Powder	New
Bareminerals	Bareskin Serum Concealer	Dark to Deep	Concealer	Liquid	Old
Bareminerals	Mineral Veil	Complexion Rescue	Finishing Powder	Loose Powder	New
Bareminerals	Mineral Veil	Original SPF 25	Finishing Powder	Loose Powder	New
Bareminerals	Mineral Veil	Brightening Pearl Natural Light	Finishing Powder	Loose Powder	New
Bareminerals	Mineral Veil	Diamond Light	Finishing Powder	Loose Powder	New
Bareminerals	Well Rested Cream	Clear	Concealer	Liquid	Old
Bareminerals	All Over Face Colour	Warmth	Bronzer	Loose Powder	Old
Bareminerals	Stroke of Light	Luminous 2	Concealer	Liquid	Old
Bareminerals	Primetime Primer	Original	Primer	Liquid	Old

Brand	Range	Shade	Product Type	Formulation	Age
Bareminerals	Biolumcent Mineral Brightening Treatment	N/A	Primer	Liquid	Old
Bareminerals	All Over Face Colour	Clear Radiance	Highlighter	Loose Powder	New
Bareminerals	Blemish Remedy Treatment Concealer	Original	Concealer	Loose Powder	New
Bareminerals	Primetime Primer	Neutralising	Primer	Liquid	Old
Bareminerals	Prep Step Moisture Set Prep Cream	N/A	Primer	Liquid	Old
Bareminerals	All Over Face Colour	Pure Radiance	Highlighter	Loose Powder	New
Bareminerals	All Over Face Colour	Champagne Diamond Radiance	Highlighter	Loose Powder	New
Bareminerals	Bareskin Serum Bronzer	Bare Glow	Bronzer	Liquid	New
Barry M	Flawless Light Reflecting Concealer	2 Medium	Concealer	Liquid	New
Body Collection England	Concealer	Medium	Concealer	Liquid	New
Body Shop	Stick Concealer	04	Concealer	Cream	New
Bourjois	123 Perfect Foundation	53 Beige Clair	Foundation	Liquid	New
Bourjois	Healthy Mix	52 Vanille	Foundation	Liquid	New
Bourjois	Mineral Matte	81	Foundation	Mousse	Old
Bourjois	Mineral Matte	82	Foundation	Mousse	Old
Bourjois	Mineral Matte	83	Foundation	Mousse	Old
Bourjois	Mineral Matte	85	Foundation	Mousse	Old
Bourjois	Comme Apres 10h de Sommeil	71 Abricote	Foundation	Liquid	Old
Bourjois	Comme Apres 10h de Sommeil	72 Rose	Foundation	Liquid	Old
Bourjois	Comme Apres 10h de Sommeil	73 Beige	Foundation	Liquid	Old
Bourjois	Healthy Mix	51 Vanille Clair	Foundation	Liquid	Old
Bourjois	Healthy Mix	52 Vanille	Foundation	Liquid	Old
Bourjois	Healthy Mix	53 Beige Clair	Foundation	Liquid	Old
Bourjois	Healthy Mix	54 Beige	Foundation	Liquid	Old
Bourjois	Healthy Mix	55 Beige Fonce	Foundation	Liquid	Old
Bourjois	Healthy Mix	56 Hale Clair	Foundation	Liquid	Old
Bourjois	Healthy Mix Correcting Concealer	51	Concealer	Liquid	Old
Bourjois	Anticernes Pinceau	82 Beige Rose	Concealer	Liquid	Old
Bourjois	123 Perfect Foundation	53 Light Beige	Foundation	Liquid	Old

Brand	Range	Shade	Product Type	Formulation	Age
C2000	Perfecting Minerals	Ivory 1	Foundation	Loose Powder	New
C2000	Perfecting Minerals	Honey 2	Foundation	Loose Powder	New
C2000	Perfecting Minerals	Natural Beige 3	Foundation	Loose Powder	New
C2000	Perfecting Minerals	Biscuit 4	Foundation	Loose Powder	New
C2000	Lasting Perfection	Ivory 2	Foundation	Liquid	Old
C2000	Lasting Perfection	Biscuit 3	Foundation	Liquid	Old
C2000	Lasting Perfection	Natural Beige 4	Foundation	Liquid	Old
C2000	Lasting Perfection	Honey 5	Foundation	Liquid	Old
C2000	Lasting Perfection	Golden 6	Foundation	Liquid	Old
C2000	Lasting Perfection Concealer	Deep 4	Concealer	Liquid	Old
C2000	Cover Up Stick	12 Buff	Concealer	Cream	New
Chanel	Vitalumiere	50 Naturel	Foundation	Liquid	New
Clarins	Teint Eclat Lissant	3.5 Sandy Beige	Foundation	Liquid	Old
Clinique	Repair Wear Laser Focus Foundation	03	Foundation	Liquid	New
Clinique	Moisture Surge CC Cream	Medium	Tinted Moisturiser	Liquid	Old
Clinique	Supermoisture Makeup	04 Ivory	Foundation	Liquid	Old
Collection	Cover & Go Foundation	Ivory 1	Foundation	Liquid	New
Collection	Cover & Go Concealer	Ivory 1	Concealer	Cream	New
Collection	Cover & Go Foundation	Light 2	Foundation	Liquid	New
Collection	Cover & Go Concealer	Light 2	Concealer	Cream	New
Collection	Cover & Go Foundation	Fair 3	Foundation	Liquid	New
Collection	Cover & Go Concealer	Fair 3	Concealer	Cream	New
Collection	Cover & Go Foundation	Natural 4	Foundation	Liquid	New
Collection	Cover & Go Concealer	Natural 4	Concealer	Cream	New
Collection	Cover & Go Foundation	Medium 5	Foundation	Liquid	New
Collection	Cover & Go Concealer	Medium 5	Concealer	Cream	New
Collection	Colour Match Foundation	Ivory 2	Foundation	Liquid	New
Dermacol	Full Makeup Cover	211	Foundation	Cream	New
Dermalogica	Concealing Spot Treatment	N/A	Concealer	Liquid	New

Brand	Range	Shade	Product Type	Formulation	Age
Diego Dalla Palma	Zero Dark Circles Concealer	110	Concealer	Liquid	New
Diorskin	Star	020 Light Beige	Foundation	Liquid	New
Diorskin	Nude Makeup	030 Medium Beige	Foundation	Liquid	New
Diorskin	Terra Bella Sun Powder	003 Bronze Tan	Bronzer	Pressed Powder	Old
Elemis	Glow Priming Moisturiser	N/A	Tinted Moisturiser	Liquid	New
Estee Lauder	Double Wear	Tawny	Foundation	Liquid	New
Estee Lauder	Individualist	Beech	Foundation	Liquid	New
Estee Lauder	Double Wear	Pure Beige	Foundation	Liquid	Old
Estee Lauder	Double Wear	Ivory Beige	Foundation	Liquid	Old
Fantastic Collection	True Match	F01 Light	Foundation	Liquid	New
Fantastic Collection	True Match	F02 Medium	Foundation	Liquid	New
Fantastic Collection	True Match	F03 Dark	Foundation	Liquid	New
Fashionista	Catwalk 360 Pressed Powder	Taupe	Finishing Powder	Pressed Powder	New
Garnier	Anti-dark Circles Roll-on Concealer	N/A	Concealer	Liquid	Old
Giorgio Armani	Luminous Silk Foundation	8.25	Foundation	Liquid	New
Golden Rose	Translucent Finishing Powder	N/A	Finishing Powder	Loose Powder	Old
Golden Rose	Mineral Makeup Liquid Foundation	N/A	Foundation	Liquid	New
JML	Mineral Magic Compact	N/A	Foundation	Pressed Powder	New
Laboratoire Actiderm	Mineral Makeup	Bastille	Foundation	Liquid	New
Lacura	CC Stick Concealer	Peach	Concealer	Cream	New
Lacura	Liquid Makeup	10 Transparent	Foundation	Liquid	Old
Lacura	Foundation	10 Transparent	Foundation	Liquid	Old
Lacura	BB Cream	Medium Tone	Tinted Moisturiser	Liquid	New
Lancome	Color Ideal	010 Beige Porcelaine	Foundation	Liquid	New
Lancome	Color Ideal	01 Beige Albatre	Foundation	Liquid	New
Lancome	Photogenic	05 Cognac	Foundation	Liquid	Old
Lancome	Teint Idole Ultra	03 Beige Diaphane	Foundation	Liquid	Old
L'Oreal	Infallible 24h Matte	13 Rose Beige	Foundation	Liquid	New
L'Oreal	Nude Magique Cushion Foundation	07 Golden Beige	Foundation	Liquid	New

Brand	Range	Shade	Product Type	Formulation	Age
L'Oreal	Visible Lift	220 Sand Beige	Foundation	Liquid	Old
L'Oreal	Infallible 24h Matte	13 Rose Beige	Foundation	Liquid	Old
L'Oreal	Touche Magique Illuminating Concealer	Medium	Concealer	Liquid	Old
L'Oreal	Matte Morphose	140 Creamy Natural	Foundation	Mousse	Old
L'Oreal	Nude Magique CC Cream	Anti-dullness	Tinted Moisturiser	Liquid	Old
M&S	Luminescent Tinted Moisturiser	Medium	Tinted Moisturiser	Liquid	New
MAC	Mineralise Skin Finish	Soft & Gentle	Highlighter	Pressed Powder	New
MAC	Studio Sculpt	NC25	Foundation	Liquid	New
MAC	Select Cover Up	NW20	Concealer	Liquid	Old
Makeup Gallery	Gorgeous Flawless	Beige 4	Foundation	Liquid	New
Maxfactor	Miracle Match	Natural 50	Foundation	Liquid	New
Maxfactor	Pan Stik	12 True Beige	Foundation	Cream	New
Maxfactor	Radiant Lift	040 Light Ivory	Foundation	Liquid	New
Maxfactor	Facefinity 3 in 1	050 Natural	Foundation	Liquid	New
Maxfactor	Seamless	Ivory 02	Foundation	Liquid	Old
Maxfactor	Lasting Performance	106 Natural Beige	Foundation	Liquid	Old
Maxfactor	Facefinity	03 Natural	Foundation	Pressed Powder	Old
Maxfactor	Facefinity	02 Ivory	Foundation	Pressed Powder	Old
Maxfactor	Miracle Match	Natural 50	Foundation	Liquid	Old
Maxfactor	Flawless Perfection	45 Warm Almond	Foundation	Liquid	Old
Maxfactor	Facefinity 3 in 1	100 Suntan	Foundation	Liquid	Old
Maybelline	Fit Me Concealer	15 Fair	Concealer	Liquid	New
Maybelline	Super Stay 24hr	48 Sun Beige	Foundation	Liquid	New
Maybelline	Dream Satin	30 Sand Sable	Foundation	Liquid	Old
Maybelline	Super Stay 24h	48 Sun Beige	Foundation	Liquid	Old
Maybelline	Pure Liquid Minerals	040 Fawn	Foundation	Liquid	New
Maybelline	Dream Matte Mousse	010	Foundation	Mousse	Old
Maybelline	Dream Matte Mousse	040	Foundation	Mousse	Old
Maybelline	Express Makeup 3 in 1	Beige 032	Foundation	Cream	Old

Brand	Range	Shade	Product Type	Formulation	Age
Maybelline	Pure Stay	30 Nude	Foundation	Pressed Powder	Old
Maybelline	Fit Me Concealer	15 Fair	Concealer	Liquid	Old
Maybelline	Dream Satin	4 Light Porcelain	Foundation	Liquid	New
Maybelline	Pure Liquid Minerals	010 Ivory	Foundation	Liquid	New
Maybelline	Dream Matte Mousse	005 Porcelain	Foundation	Mousse	Old
Maybelline	Instant Anti-age The Eraser	021 Nude	Foundation	Liquid	Old
Maybelline	Fit Me Fluid	135 Creamy Natural	Foundation	Liquid	New
Maybelline	Pure Liquid Minerals	030 Sand	Foundation	Liquid	New
Maybelline	Super Stay Better Skin Concealer	02 Light	Concealer	Liquid	New
Maybelline	Dream Lumi Touch Concealer	01 Ivory	Concealer	Liquid	Old
Maybelline	Super Stay 24hr	05 Light Beige	Foundation	Liquid	Old
Maybelline	Super Stay 24hr	10 Ivory	Foundation	Liquid	New
Miss Sporty	Mini Me Concealer	003 Dark	Concealer	Cream	New
Miss Sporty	So Energetic Natural Radiance	03 Dark	Foundation	Liquid	New
Miss Sporty	Cover Stick	1	Concealer	Cream	New
Miss Sporty	So Clear Anti-spot Stick Concealer	N/A	Concealer	Cream	Old
Natural Collection	Cover Up Cream	Fair	Concealer	Liquid	Old
Neutrogena	Flawless Finish	30 Blushing Ivory	Foundation	Liquid	New
No.17	Skin Glow Face Illuminator	Pink Flash	Highlighter	Liquid	Old
No.7	Beautiful Skin BB Cream	Dry-Very Dry	Tinted Moisturiser	Liquid	Old
No.7	Triple Protection Tinted Moisturiser	Fair	Tinted Moisturiser	Liquid	New
No.7	Highlights Illuminating Lotion	N/A	Highlighter	Liquid	Old
No.7	Beautifully Matte Mousse	Cool Ivory	Foundation	Mousse	Old
No.7	Instant Radiance Medium Coverage	Cool Ivory	Foundation	Liquid	Old
No.7	Beautiful Skin BB Cream	Normal-Oily	Tinted Moisturiser	Liquid	New
No.7	Stay Perfect Superlight	Cool Ivory	Foundation	Liquid	New
No.7	Skin Illuminator	Peach	Highlighter	Liquid	Old
Ocuz	Compact Powder	P03	Finishing Powder	Pressed Powder	New
Olay	Total Effects Touch of Foundation	Medium	Tinted Moisturiser	Liquid	New

Brand	Range	Shade	Product Type	Formulation	Age
Olay	Complete Care Touch of Foundation	Fair	Tinted Moisturiser	Liquid	New
Olivia Hale	Foundation	Café Au Lait	Foundation	Liquid	New
Oriflame	Love Nature Corrective Stick	Beige	Concealer	Cream	New
Osiris	Avise BB Cream	Light	Tinted Moisturiser	Liquid	New
Phoera	Soft Matte Long Wear Foundation	Nude 102	Foundation	Liquid	New
Pixi	Colour Correct Powder Foundation	No. 2 Nude	Foundation	Pressed Powder	New
Pixi	Glowy Gossamer Duo	Subtle Sunrise (Gold)	Highlighter	Pressed Powder	New
Pixi	Glowy Gossamer Duo	Subtle Sunrise (Rose)	Highlighter	Pressed Powder	New
Pixi	Pat Away Concealing Base	No. 3 Warm	Concealer	Liquid	New
Pixi	Quick Fix Powder	Translucid	Finishing Powder	Loose Powder	New
Pixi	Beauty Balm	No. 2 Nude	Foundation	Liquid	New
Primark	My Perfect Colour Matte Foundation	Amber Honey	Foundation	Liquid	New
Pur	Disappearing Ink Concealer	Light	Concealer	Liquid	New
Revlon	Colorstay Normal-Dry	150 Buff	Foundation	Liquid	New
Revlon	Even Out	02 Nude Beige	Foundation	Liquid	Old
Revlon	Beyond Natural	210 Light	Foundation	Liquid	Old
Revlon	Beyond Natural	220 Light/Medium	Foundation	Liquid	Old
Revlon	Beyond Natural	230 Medium	Foundation	Liquid	Old
Revlon	Colorstay Mineral Mousse	030 Light	Foundation	Mousse	Old
Revlon	Colorstay Mineral Mousse	040 Light-Medium	Foundation	Mousse	Old
Revlon	Colorstay Mineral Mousse	060 Medium	Foundation	Mousse	Old
Revlon	Colorstay Mineral Mousse	080 Deep	Foundation	Mousse	Old
Revlon	Colorstay Normal-Dry	150 Buff	Foundation	Liquid	Old
Revlon	Photoready Makeup	003 Shell	Foundation	Liquid	Old
Revlon	Photoready Makeup	004 Nude	Foundation	Liquid	Old
Revlon	Photoready Makeup	005 Natural Beige	Foundation	Liquid	Old
Rimmel	Fix and Protect Makeup Primer	N/A	Primer	Liquid	New
Rimmel	Fresher Skin	100 Ivory	Foundation	Liquid	New
Rimmel	Lasting Finish	200 Soft Beige	Foundation	Liquid	New

Brand	Range	Shade	Product Type	Formulation	Age
Rimmel	Lasting Matte	010 Warm Porcelain	Foundation	Mousse	New
Rimmel	Match Perfection	200 Soft Beige	Foundation	Liquid	New
Rimmel	Match Perfection Concealer	010 Ivory	Concealer	Liquid	New
Rimmel	Stay Matte	200 Soft Beige	Foundation	Mousse	New
Rimmel	Stay Matte	200 Soft Beige	Foundation	Liquid	Old
Rimmel	Recover	Soft Beige	Foundation	Liquid	Old
Rimmel	Hydrasense	Ivory	Foundation	Liquid	Old
Rimmel	Renew & Lift	100	Foundation	Liquid	Old
Rimmel	Renew & Lift	103	Foundation	Liquid	Old
Rimmel	Renew & Lift	200	Foundation	Liquid	Old
Rimmel	Lasting Finish	200	Foundation	Liquid	Old
Rimmel	Lasting Finish	201	Foundation	Liquid	Old
Rimmel	Stay Matte Pressed Powder	001 Transparent	Finishing Powder	Pressed Powder	Old
Rimmel	Lasting Finish	100 Ivory	Foundation	Liquid	New
Rimmel	Match Perfection Concealer	010 Ivory	Concealer	Liquid	Old
Rimmel	Clear Complexion Cover Stick	004 Natural Beige	Concealer	Cream	New
Rimmel	Hydrasense	010 Light Porcelain	Foundation	Liquid	Old
Rimmel	Match Perfection	100	Foundation	Liquid	Old
Rimmel	Match Perfection	103	Foundation	Liquid	Old
Rimmel	Match Perfection	200	Foundation	Liquid	Old
Rimmel	Match Perfection	201	Foundation	Liquid	Old
Rimmel	Match Perfection	400	Foundation	Liquid	Old
Rimmel	Stay Matte	100	Foundation	Liquid	Old
Rimmel	Stay Matte	103	Foundation	Liquid	Old
Rimmel	Stay Matte	201	Foundation	Liquid	Old
Rimmel	Stay Matte	300	Foundation	Liquid	Old
Rimmel	Lasting Finish	203	Foundation	Liquid	Old
Rosie by Autograph	Amazing Skin Secret Concealer	Shade 1	Concealer	Liquid	New
Tarte	Amazonian Clay Foundation	20N Light Neutral	Foundation	Mousse	New

Brand	Range	Shade	Product Type	Formulation	Age
The Ordinary	Serum Foundation	1.2N Light Neutral	Foundation	Liquid	New
Vita Liberata	Body Blur Instant HD Skin Finish	Medium	Tinted Moisturiser	Liquid	New

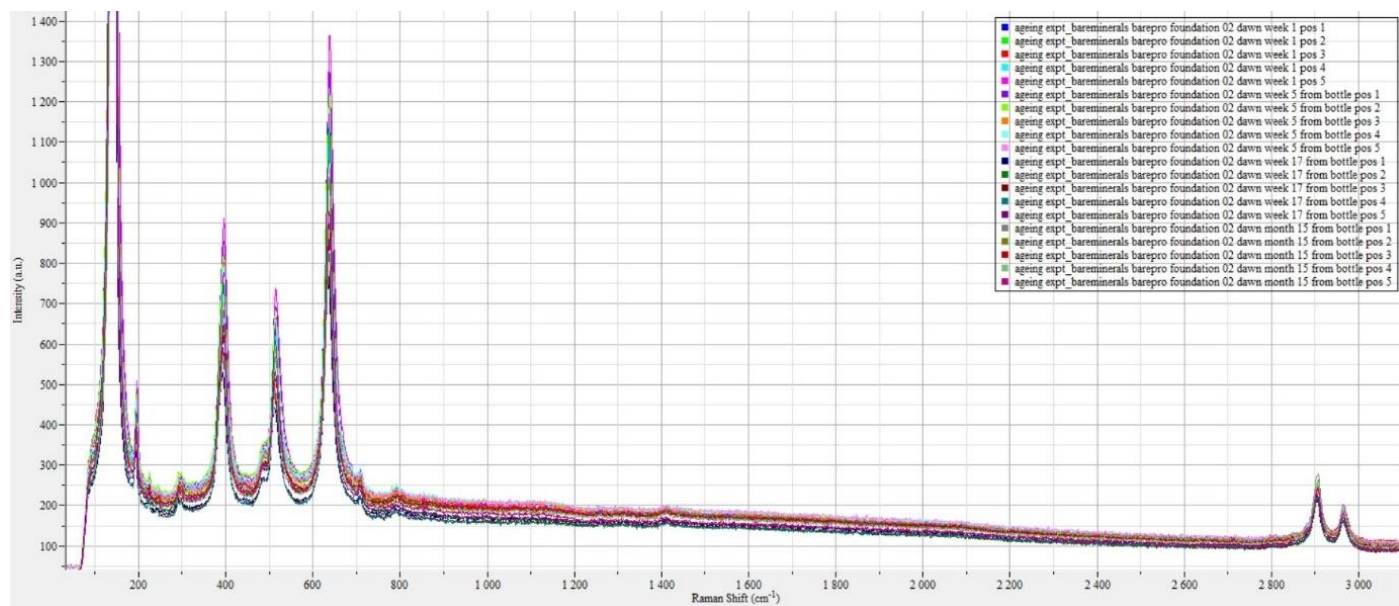


Figure A1: Raman spectra (unprocessed) of BareMinerals BarePro Foundation upon product opening, at week 5, at week 17 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.

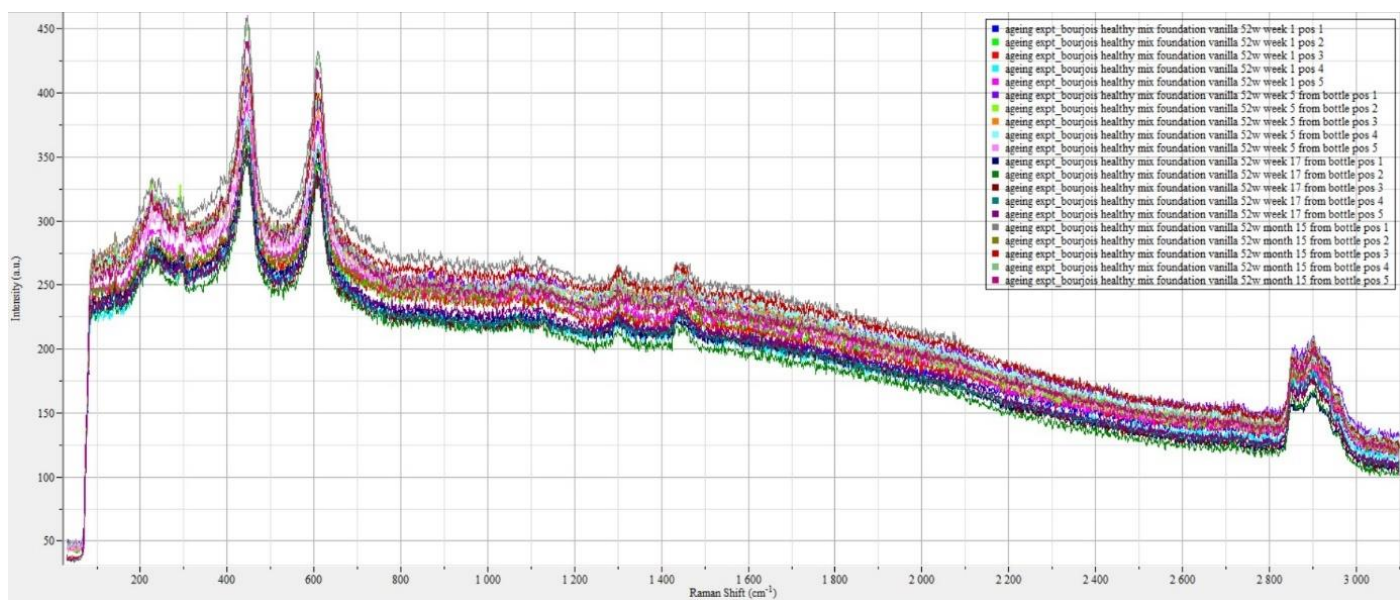


Figure A2: Raman spectra (unprocessed) of Bourjois Healthy Mix Foundation upon product opening, at week 5, at week 17 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.

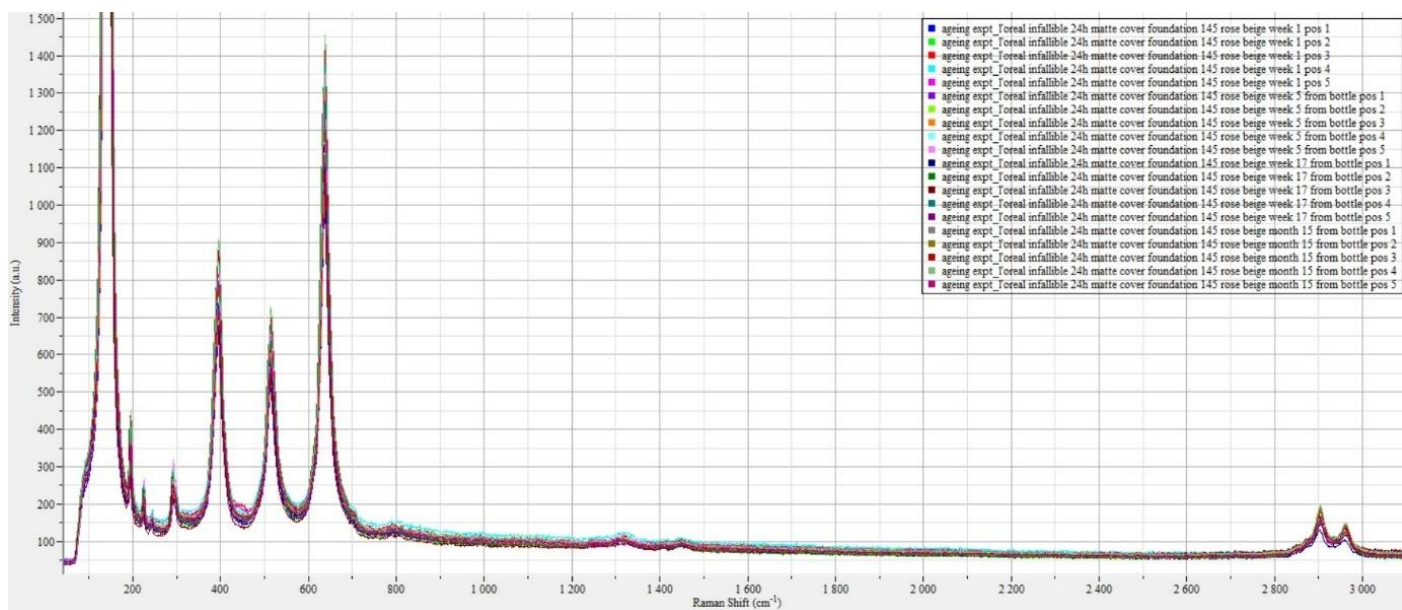


Figure A3: Raman spectra (unprocessed) of L'Oreal Infallible Foundation upon product opening, at week 5, at week 17 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.

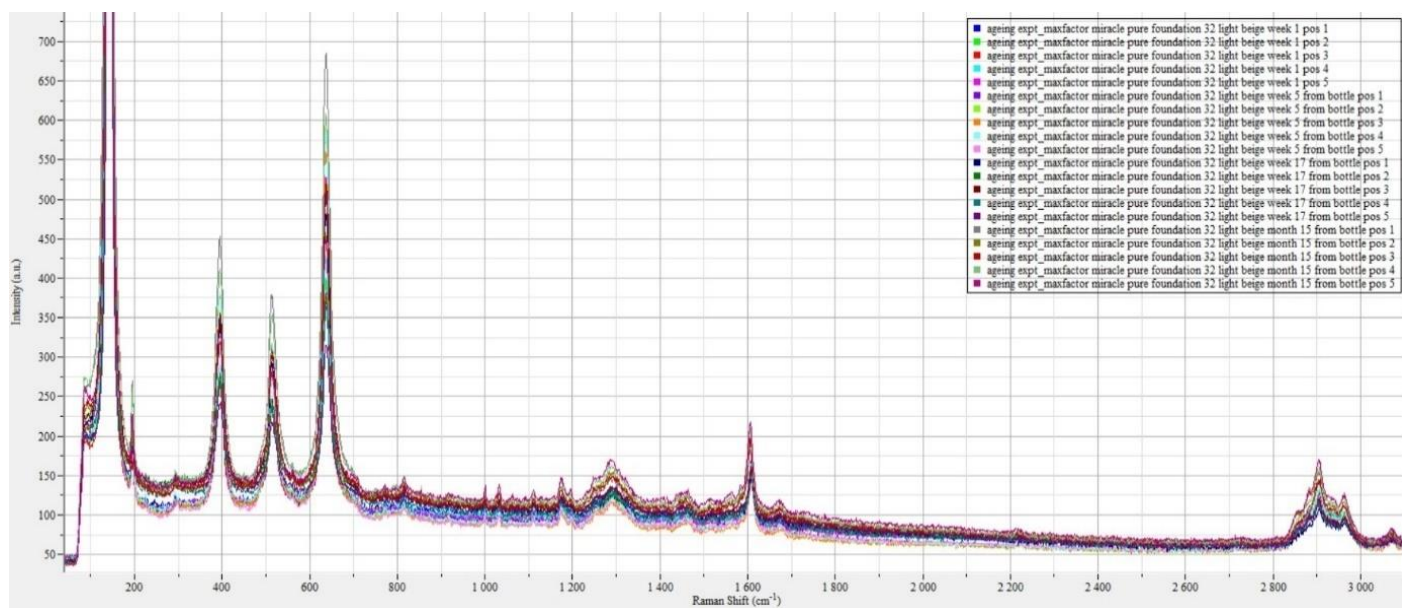


Figure A4: Raman spectra (unprocessed) of Max Factor Miracle Pure Foundation upon product opening, at week 5, at week 17 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.

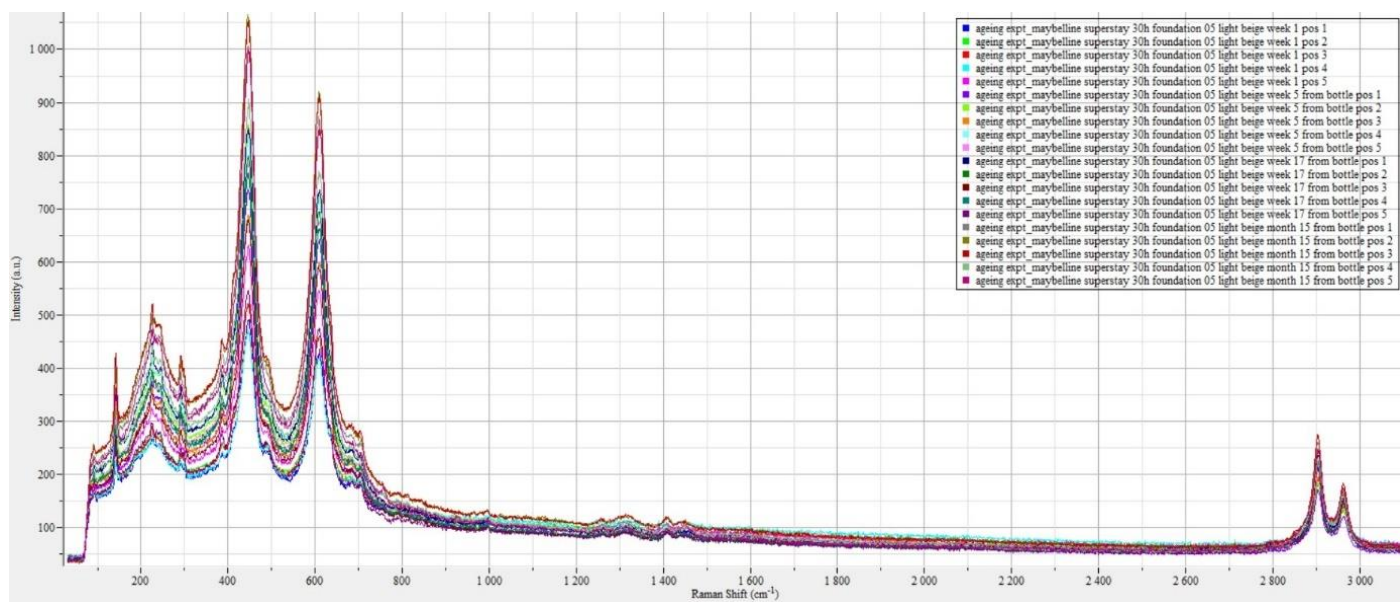


Figure A5: Raman spectra (unprocessed) of Maybelline Superstay Foundation upon product opening, at week 5, at week 17 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.

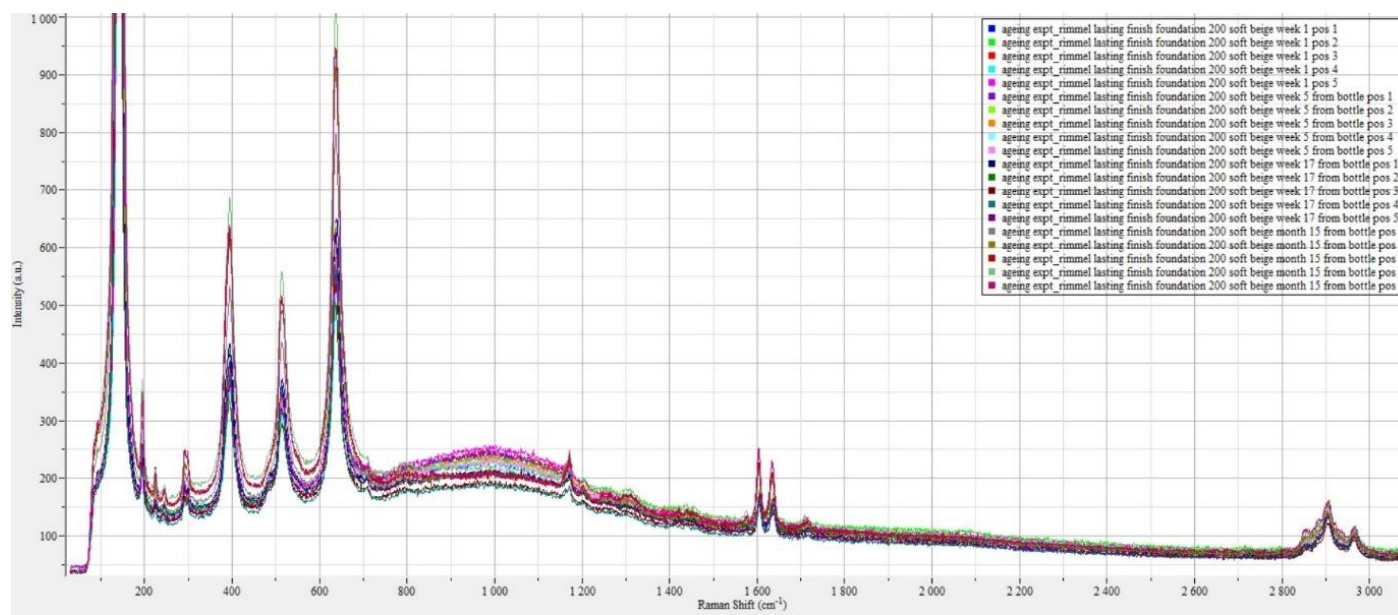


Figure A6: Raman spectra (unprocessed) of Rimmel Lasting Finish Foundation upon product opening, at week 5, at week 17 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.

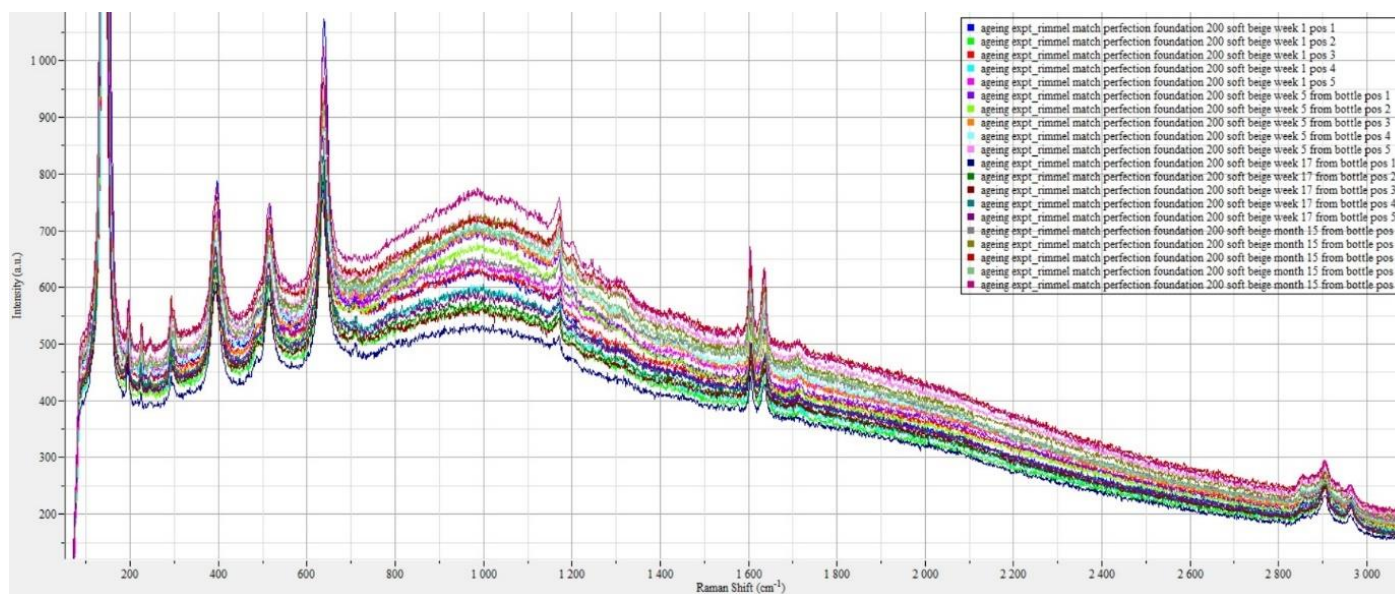


Figure A7: Raman spectra (unprocessed) of Rimmel Match Perfection Foundation upon product opening, at week 5, at week 17 and at month 15, exhibiting spectral homogeneity and minimal change across the entire time interval.