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and amino acids to successfully stabilize a range of proteins, for applications in both therapeutic formulation and biocatalysis. Proteins were shown to retain their enzymatic activity, even after storage at increased humidity for prolonged periods. The procedure for the preparation of PCMCs is essentially a very simple co-precipitation crystallization procedure. An aqueous protein solution is mixed with a concentrated solution of excipient. This combined protein aqueous mixture is then dispensed drop-wise into a highly mixed water-soluble anti-solvent, whereupon the protein and excipient instantly co-precipitate. Depending on the protein payload, the co-precipitated PCMC particle is typically micron-sized, with the protein molecules located on the surface of the excipient crystal. Producing milligram quantities of PCMCs can easily be performed with basic laboratory apparatus although a larger scale production system was required to provide pre-clinical quantities of material. Simply increasing the size of the batch apparatus proved unfruitful because the mixing rheology differs on larger scale apparatus. Consequently a continuous flow co-precipitation was envisaged. Using two pumping units and a specially designed mixing flow cell a continuous flow co-precipitation system was constructed. This allowed protein/excipient solution and anti-solvent to be continuously co-precipitated, producing an outflow of PCMC suspension. The PCMCs could be harvested directly from this suspension. Using this strategy, grams of PCMCs could be produced per hour of production. Initial studies using a continuous flow strategy have produced PCMCs with good retention of protein activity, plus excellent particle characteristics. Typically particles are $<5\ \mu\text{m}$, which makes them especially amenable to the pulmonary delivery of therapeutic peptides, proteins, nucleic acids and other water-soluble bioactive molecules.

Kreiner, M., et al (2001) *Chem. Comm.* **12**: 1096–1097

036

Use of small-angle neutron scattering (SANS) and surface tension to better understand the mechanism of membrane and surfactant micelle interaction of endosomolytic polyamidoamines

Z. Khayat[†], R. Duncan^{*}, N. Lavignac^{*}, P. Griffiths[†] and A. Paul[†]

^{*}Centre for Polymer Therapeutics, Welsh School of Pharmacy, King Edward's VII Avenue, Cardiff CF10 3XF and [†]School of Chemistry, Cardiff University, PO Box 912, Cardiff CF10 3TB, UK. E-mail: Khayat@cf.ac.uk

Polyamidoamines (PAAs) are a family of synthetic, water-soluble, linear polymers, prepared by hydrogen-transfer polyaddition of aliphatic amines or bis-secondary amines to bis-acrylamides (Ferruti et al 2000). They display pH-dependent breakage of model membranes (e.g. red blood cells (RBCs)) and can disrupt the intracellular endosomal (endosomolytic) and lysosomal membrane to mediate intracellular delivery of genes and proteins (Richardson et al 2001). However, their mechanism of membrane perturbation is still poorly understood. Recent studies using SANS and pulsed-gradient spin-echo NMR have shown that the size of the PAA ISA23Cl polymer coil changes with pH (Griffiths et al 2004). With decreasing pH the ISA23 radius of gyration increases to a maximum ($R_g \sim 8\ \text{nm}$) at $\sim\text{pH } 3$, before subsequently decreasing, until at very low pH, the coil collapses ($R_g < 2\ \text{nm}$). The aim of this study was to investigate the interaction between PAA and surfaces providing representative model membranes using techniques commonly employed in synthetic surfactant research. To investigate the polymer-membrane interaction, RBCs, surfactant micelles and liposomes were chosen as models and ISA23Cl as the PAA. Combinations of anionic (SDS and phosphatidylcholine) and non-ionic surfactants (C_{14}BNMG and C_{12}E_4), were used to prepare the 'surfaces'. Surface tension technique was used to study the onset of the interaction between these model surfactant micelles (10 mM to 0.008 mM) and ISA23Cl (0.2 wt%) as a function of pH. Maximum bubble pressure was used to measure the surface tension. Scattering and spectroscopy techniques probe the structure and dynamics of the complex directly. SANS has been used to quantify the dimensions of the surfactant micelles, polymer-surfactant and polymer-membrane complexes. The neutron scattering of the liposome (1 mM) in the absence and presence of ISA23Cl (1 wt%) at pH 7.4 and 5.5 was measured. The SANS measurements were performed on LOQ diffractometers at the ISIS Spallation Neutron Source, Oxfordshire (UK). The liposomes (phosphatidylcholine: phosphatidylethanolamine, ratio 5:2) were prepared using freeze/thaw extrusion method. ISA23Cl demonstrated pH-dependent haemolytic activity. At pH 7.4 and 6.5 no haemolytic activity was seen (0.7 and 1.1%, haemoglobin release, respectively), but at pH 5.5 a significant haemolysis was observed (18% haemoglobin release). The surface tension (mNm^{-1}) versus $\log[\text{total concentration of surfactant}]$ curve does not change in shape following the addition of ISA23Cl, at pH 8, but a clear change is observed at pH 4, which is due to the interaction occurring between ISA23Cl and the surfactant micelle. And similar conclusions can be gained from electron paramagnetic resonance (EPR) studies. The scattering of the liposome at pH 7.4 is not affected by the addition of

ISA23Cl, but at pH 5.5 a clear change in scattering is observed, the size of the liposome seems to increase in size at this pH. From the surface tension studies it is clear that ISA23Cl was found to interact with the surface at low pH when the polymer and the surface have opposite charge but not when both bear similar charge. And this is an agreement with SANS data, which demonstrates polymer-liposome interaction at a low pH, again when ISA23Cl and liposome are oppositely charged.

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037

Amphiphilic doxorubicin conjugates alter the intracellular distribution and activity of the drug

S. B. Siddique, A. G. Schätzlein^{*}, C. G. Wilson, P. Gellert[†], S. Das[†] and I. F. Uchegbu

Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, ^{*}CRD Department of Medical Oncology, University of Glasgow, Glasgow and [†]AstraZeneca UK, Alderly Park, Macclesfield, UK. E-mail: SoryiaSiddique@hotmail.com

Efficacy of chemotherapy is often limited by the narrow therapeutic index of anti-cancer drugs. Hence, various strategies are being employed to alleviate the side effects of these drugs, by modifying drug pharmacokinetics and biodistribution. Amongst the various strategies employed, the exploitation of the EPR effects has been reported, which takes advantage of the leaky tumour vessel endothelium. To potentially utilize the EPR effect for targeting and simultaneously explore the potential of modified intracellular trafficking to overcome P-glycoprotein (P-gp) related multi-drug resistance, we have synthesised amphiphilic conjugates of doxorubicin. Novel amphiphilic SDox and UDox conjugates were synthesised by the covalent attachment of DSPE-PEG to doxorubicin and DSPE-PEG-NH₂ to Cis Aconitic-doxorubicin. These novel polymers were able to form particulates. The acid liability of the unstable conjugate was determined. In-vitro investigations involved determining the potential modulation of the P-gp pump by employing the parental cell line, which does not express P-gp and is sensitive to doxorubicin and the resistant cell line (A2780AD) which exhibits P-gp mediated doxorubicin resistance. This involved cytotoxicity studies, uptake and intracellular trafficking of SDox and UDox conjugate for the A2780 and A2780AD cells. Tumouricidal efficacy of UDox and doxorubicin was determined in nude mice bearing A2780 tumour. Biodistribution studies of UDox and doxorubicin were attempted. HPLC method was developed to analyse in-vivo doxorubicin metabolites. SDox and UDox both had an affinity for cellular membranes, for the A2780 and the A2780 AD cell line. The conjugates appear to initially accumulate in the cell membrane and within small spherical compartments within the cell indicating endocytosis uptake, potentially by passing the P-gp pump. The conjugates were similar in cytotoxicity for the parental and resistant cell line. The UDox showed an increase in dose tolerance in comparison with doxorubicin and similar in tumour growth delay to doxorubicin.

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The effect of NaCl on the formation of theophylline microballoons in an o/w emulsion

M. Kouchak and A. Badriani

Department of Pharmaceutics, School of Pharmacy, Jundishapur Medical Sciences University, Ahwaz, Iran. E-mail: koochekm@yahoo.com

Emulsion solvent diffusion is a method to prepare microballoons as floating controlled-release systems in the stomach (Kawashima et al 1992). The major problem of o/w emulsification technique is the low encapsulation efficiency of moderately water-soluble drugs such as theophylline, caffeine and salicylic acid. The drug can diffuse from the organic dispersed phase into the aqueous continuous phase, which results in poor entrapment (Watts & Davies 1990). In this study, the influence of variation in continuous phase composition of emulsion on the physical characteristics of resultant theophylline microspheres was investigated. Theophylline, ethyl cellulose and butylphthalate were dissolved in dichloromethane/alcohol mixture, added to 0.1N HCl containing different amount of polysorbate 80, polyvinyl alcohol, NaCl (20%) or theophylline (saturated). The mixture was stirred at 600 rev min⁻¹ for 3 h. Resultant microspheres were separated from solution by filtration. Floating behaviour of microspheres was studied in the solution of HCl 0.1 N containing 0.02% polysorbate 80. The solution was stirred at 100 rev min⁻¹ for 12 h and the buoyant beads were counted every hour. Size distribution of prepared microspheres was measured by the sieve analysis method. To assess the drug loading, microballoons were dissolved in ethanol and