# Drug loading of foldable commercial intraocular lenses using supercritical impregnation

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A B S T R A C T

The drug delivery through intraocular lenses (IOLs) allows the combination of cataract surgery act and postoperative treatment in a single procedure. In order to prepare such systems, "clean" supercritical $CO_2$ processes are studied for loading commercial IOLs with ophthalmic drugs. Ciprofloxacin (CIP, an antibiotic) and dexamethasone 21-phosphate disodium (DXP, an anti-inflammatory drug) were impregnated into foldable IOLs made from poly-2-hydroxyethyl methacrylate (P-HEMA). A first pre-treatment step was conducted in order to remove absorbed conditioning physiological solution. Supercritical impregnations were then performed by varying the experimental conditions. In order to obtain transparent IOLs and avoid the appearance of undesirable foaming, it was necessary to couple slow pressurization and depressurization phases during supercritical treatments. The impregnation yields were determined through drug release studies. For both drugs, release studies showdeep and reproducible impregnation for different diopters.

For the system P-HEMA/CIP, a series of impregnations was performed to delimit the experimental range at two pressures (80 and 200 bar) in the presence or absence of ethanol as a co-solvent for two diopters (+5.0 D and +21.0 D). Increase in pressure in the absence of a co-solvent resulted in improved CIP impregnation. The addition of ethanol (5 mol%) produced impregnation yields comparable to those obtained at 200 bar without co-solvent. A response surface methodology based on experimental designs was used to study the influence of operating conditions on impregnation of IOLs (+21.0 D) in the absence of co-solvent. Two input variables with 5 levels each were considered; the pressure (80–200 bar) and the impregnation duration (30–240 min). CIP impregnation yields ranging between 0.92 and 3.83 $\mu g_{CIP}/mg_{IOL}$ were obtained from these experiments and response surface indicated the pressure as a key factor in the process.

The DXP impregnation in P-HEMA was higher than CIP at all the tested conditions (8.50–14.53 $\mu g_{DXP}/mg_{IOL}$). Furthermore, unlike CIP, highest DXP impregnation yields were obtained in the presence of ethanol as a co-solvent (5 mol%). NMR spectroscopy was performed to confirm complete removal of ethanol in the co-solvent-treated IOLs.

*Chemical compounds studied in this article:*
Poly (2-hydroxyethyl methacrylate) (PubChem CID: 13360)
Dexamethasone 21-phosphate disodium (PubChem CID: 16961)
Ciprofloxacin (PubChem CID: 2764)
Carbon dioxide (PubChem CID: 280)
Ethanol (PubChem CID: 702)
Disodium hydrogenophosphate (PubChem CID: 24203)
Monobasic potassium phosphate (PubChem CID: 516951)

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Foldable P-HEMA intraocular lenses
Response surface methodology
Drug release study

## 1. Introduction

Cataract is the most common cause of blindness and severe visual impairment worldwide, and its surgery is the most frequently performed ocular procedure. The number of patients with cataract is continuously increasing (Eperon et al., 2013), and currently about 2 million people have their cataractous lenses removed and replaced with an intraocular lens (IOL) each year (Eperon et al., 2008). The surgery involves implantation of an artificial intraocular lens to replace opacified (damaged) natural crystalline lens (Eperon et al., 2013). It is considered safe, however, postoperative infections including endophtalmitis (Parsons et al., 2005; Barry et al., 2006), (rare but potentially devastating condition) and posterior capsular opacification (less serious but common) (Wright et al., 1988; Miyake et al., 2000; Simone and

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**Nomenclature**

| | |
|---|---|
| $scCO_2$ | Supercritical $CO_2$ |
| IOL | Intraocular lens |
| P-HEMA | Poly(2-hydroxyethyl methacrylate) |
| CIP | Ciprofloxacin |
| DXP | Dexamethasone 21-phosphate disodium |
| API | Active pharmaceutical ingredient |
| Ethanol | EtOH |
| $T$ | Temperature |
| $P$ | Pressure |
| $t$ | Duration |
| $Dep_{rate}$ | Depressurization rate |
| min | Minute |
| °C | Celsius degree |
| D | Diopter |
| RSM | Response surface methodology |
| ANOVA | ANalysis Of Variance |
| bi | Coefficient of the model |
| Signif. | Significance (%) |
| ATR-FTIR | Attenuated total reflectance-Fourier transform infra-red |
| DSC | Differential scanning calorimeter |
| $m_{imp}$ | Impregnated mass |
| $m_{CIP\ imp}$ | Impregnated mass of CIP |
| $m_{DXP\ imp}$ | Impregnated mass of DXP |
| $y_{imp}$ | Impregnation yield |
| $t_{imp}$ | Impregnation duration |
| $t_{release}$ | Release duration |
| $T_g$ | Glass transition temperature |
| $M_t$ | Cumulative amount of drug released at time $t$ |
| $M_\infty$ | Cumulative amount of drug released at infinite time |
| $k$ | Kinetic constant |
| $n$ | Release exponent representing release mechanism |
| $m_{0IOL}$ | Initial mass of dry IOL |

Whitacre, 2001; Yorio et al., 2008) are known to regularly occur in patients.

To prevent short- and long-term complications, a concentrated solution of anti-inflammatory or antibiotic drugs is injected (subconjunctival, topical, intracameral or intravitreal) in the eye after cataract surgery (Parsons et al., 2005). However, the efficacy of this treatment is limited either due to poor drug bioavailability across the blood-ocular barriers (McGhee et al., 2002) or serious side effects (Eperon et al., 2008).

Significant advances have been made in developing new treatments for the prevention of ocular risks following cataract surgery. The advent of new technologies opens the door to new controlled drug delivery systems to prevent postoperative complications. The ability of these systems to deliver drugs at predetermined rates for predefined periods of time in the specific targeted site have been used to overcome the shortcomings of conventional techniques. Most of these proposed ophthalmic drug delivery systems are polymer-based and are either of a reservoir or a matrix type (Yorio et al., 2008).

The development of drug incorporated IOLs allows the combination of the cataract surgery and postoperative treatment in a single procedure (Anderson et al., 2009). It can provide a prolonged intraocular release of anti-inflammatory and antibiotic agents after surgery leading to improved efficacy, reduced toxicity, and better patient compliance (Uhrich et al., 1999).

Several manufacturing processes have been developed to produce polymeric (biocompatible or biodegradable) drug delivery systems including molecular imprinting (Alvarez-Lorenzo and Concheiro, 2004; Venkatesh et al., 2007), ion ligands binding (Uchida et al., 2003; Sato et al., 2005), soaking into liquid (Karlgard et al., 2003; Aqil and Gupta, 2012) among others (Li et al., 2007). Nevertheless, these conventional techniques have some disadvantages such as; high processing temperatures that can deteriorate thermosensitive Active Pharmaceutical Ingredients (API), or the use of organic solvents that must be removed through numerous purification steps to meet FDA's requirements (Champeau et al., 2015a).

To overcome the above-mentioned limitations, the supercritical fluid assisted impregnation has proven to be an alternative green process for pharmaceutical products (Pasquali and Bettini, 2008). The activity of the drug molecules can be preserved notably because supercritical carbon dioxide ($scCO_2$) processing is performed at moderate temperatures (Sun, 2002).

The sorption of $scCO_2$ in a large number of natural and synthetic polymers permits impregnation of hydrophobic molecules without or with a minimal use of a co-solvent. The major advantages of supercritical impregnation include; tunable solute loading and impregnation depth by slight changes in processing conditions. Moreover, residual organic solvent free end-products are obtained since $scCO_2$ is released spontaneously as a gas during depressurization (De Souza et al., 2014).

Supercritical impregnation techniques have been successfully applied to polymer processing among other applications to develop drug delivery systems (Üzer et al., 2006 López-Periago et al., 2009; Kikic and Vecchione, 2003; Masmoudi et al., 2011). Drug impregnation of biocompatible or biodegradable polymers requires the use of a vector phase to solubilize and carry the drug component within the impregnation support (López-Periago et al., 2009). Using $scCO_2$ as impregnation carrier is advantageous since it

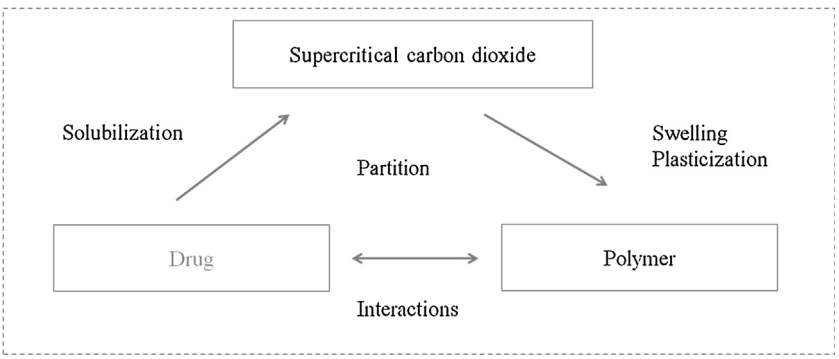


**Fig. 1.** Interactions governing supercritical impregnation.

swells the polymer matrix, thus increasing the free volume among polymer molecules. This enhances the mass transfer of the fluid phase containing the drug within the polymer matrix (Kazarian and Martirosyan, 2002). Supercritical impregnation often leads to molecular dispersion of the drug within the polymer (with a homogeneous distribution) that is also known to improve the dissolution kinetics of the drug (Yu et al., 2011). Moreover, this can be carried out either in a batch mode or in a continuous mode using one or two autoclaves.

In scientific literature, the works of Sand (1986) and Berens et al. (1992) are often used as the first references for the application of the supercritical fluid impregnation in polymeric materials. Alessi et al. (1998) and Kikic and Sist (1998) had then showed the possibility of using supercritical impregnation in the development of polymeric drug delivery systems.

In general, the supercritical impregnation process involves the following three steps:

1. The solubilization of the solute in the $scCO_2$ and swelling of the polymer by $CO_2$ sorption.
2. Drug partition of the solute between the $CO_2$ rich phase and the polymer.
3. $CO_2$ release and entrapment of the solutes within the polymer.

Different interactions are involved in $scCO_2$ impregnation process as presented in Fig. 1.

The supercritical impregnation can take advantage from the sorption of $scCO_2$ in polymers leading to their swelling and plasticization (Kikic and Vecchione, 2003). It is possible to tune the degree of polymer swelling by modifying the sorption degree while varying $scCO_2$ density. The process of diffusion of the drug within $CO_2$-swollen polymer can therefore be controlled to obtain desired amount of drug into the polymer support (Kazarian et al., 1998). The ability of $CO_2$ to interact with the functional groups in the molecule polymer (Kazarian, 2000) results in enhanced segmental and chain mobility and an increase in interchain distance (Kazarian and Martirosyan, 2002) leading to the depression in glass transition temperature ($T_g$) (from 0.5 to 2.8 °C/bar, *e.g.*, 1.2 °C/bar for PMMA (Tomasko et al., 2003)).

Berens et al. (1992) demonstrated that impregnation of dimethyl phthalate into polymers such as PMMA, PolyVinyl Chloride (PVC), PolyCarbonatye (PC) and PolyVinyl Alcohol (PVA) can be enhanced in $scCO_2$-swollen polymers. Similarly, Kazarian et al. (1997, 1999) showed that $CO_2$ acts as a kind of 'molecular lubricant', making it easier for polymeric chains to slip over one another, thus accelerating the solute diffusion.

Various physico-chemical properties including solubility of drug in supercritical phase are crucial for the development of drug delivery systems using $scCO_2$. The solubility controls the amount of drug component that can be carried by the fluid phase and has been already reviewed in the literature (Škerget et al., 2011; Gupta and Shim, 2006). Solutes with high solubility in $scCO_2$ can be easily delivered within the polymeric matrix. Meanwhile, studies show that the impregnation of low $CO_2$-philic API could also be achieved if it has a strong affinity for the polymer leading to favorable partitioning toward the polymer matrix (Kazarian et al., 1998). Furthermore, higher drug inclusion can be achieved by the addition of small quantities of polar co-solvent to the $scCO_2$ phase to improve the solubility of solid compound in the media. A favorable partitioning of the drug toward polymer is also important for satisfactory impregnation of given API along with its sufficient solubility in the media. Kazarian and Martirosyan (2002),Kazarian (2004) and Lora and Kikic (1999) proposed two main mechanisms of drug impregnation of polymers using supercritical fluids.

**Table 1**
Bibliographical conditions and results of $scCO_2$ impregnation applied for ocular applications.

| Author | Polymer | API | $P$ (bar) | $T$ (°C) | Co-solvent | $t_{imp}$ (h) | $Dep_{rate}$[a] (bar min$^{-1}$) | Mode[b] | Drug loading (wt%) |
|---|---|---|---|---|---|---|---|---|---|
| Braga et al. (2008) | Chitosane derivatives (CMC, BC and SCC) | Flurbiprofen | 90–130 | 30, 40 and 50 | 5 mol% EtOH | 1 | 6 | B | 1.7–14.7 |
| | | Timolol maleate | 90–140 | 30, 40 and 50 | | 1 | 12 | B | 8.6–85 |
| Duarte et al. (2008) | P(MMA-EHA-EGDMA) | Flurbiprofen | 100–180 | 35–40 | / | 3–5 | Slowly | S-C and B | 0.18–0.82 |
| Natu et al. (2008) | PCL and PCL/POE and PCL/PEVA blends | Timolol maleate | 110–200 | 40 | With/without water and ethanol | 2 | 5 | B | 0–3.3 |
| Costa et al. (2010a) | Acrylic-based hydrogel (Nelifecon A, | Timolol maleate | 90–160 | 40 | Without or with EtOH (2–5 mol%) | 1 | 0.1–0.2 | B | 0.3–1.3 |
| | Hilafilcon B, Methafilcon A and Omafilcon A) | Flurobiprofen | 90–160 | 40 | Without or with EtOH (2–5 mol%) | 1 | 0.1–0.2 | B | 1–6 |
| Costa et al. (2010b) | Silicon based hydrogel (Balafilcon A) | Timolol maleate | 170 | 40 | EtOH and water at 5, 10 and 15 mol% | 1.5 | 0.6 | B | 1.04–1.81 |
| | | Acetazolamide | 170 | 40 | | 1.5 | 0.6 | B | 0.15 |
| Masmoudi et al. (2011) | PMMA | Cefuroxim sodium | 80–200 | 35–60 | Without or with EtOH (5 mol%) | 2 | 2 | B | 0.016–0.063 |
| Yañez et al. (2011) | Acrylic based hydrogel P(HEMA/BEM) | Flurbiprofen | 120 | 40 | Water-swollen hydrogel | 14 | Slow | B | 1–55 |
| | | | | | | 5 | | S-C | |
| Braga et al. (2011) | Silicon based hydrogel (Balafilcon) | Acetazolamide | 150–200 | 40–50 | EtOH 5 mol% | 1 | 0.6–1.5 | B | 0.52–1.97 |
| | | | | | Soft commercialized contact lens | | | | |
| González-Chomón et al. (2012) | Acrylic based hydrogel P(HEMA/BEM) | Norfloxacin | 150–300 | 40 | Water-swollen gel | 14 | 1 | B | 0.01–0.16 |
| Yokozaki et al. (2015) | Acrylic-based hydrogel (Hilafilcon B) | Salicylic acid | 90–150 | 30, 40 and 45 | Water | 2 | 0.06 | B | 29.6–54.5 |

[a] $Dep_{rate}$: depressurization rate (bar min$^{-1}$).
[b] B: batch; S-C: semi-continuous.

The mechanism based on the partition coefficient relies on the affinity of a solute in a fluid phase toward the polymer due to the specific interactions such as van der Waals, *etc*. This approach can explain the impregnation of compounds with low solubility in $scCO_2$ (Kazarian et al., 1998). In this mechanism, the solute is believed to be molecularly dispersed within the polymer and the process is expected to complete when an equilibrium concentration is achieved in the matrix (Kazarian, 2004). The second mechanism involves deposition of a solute into the polymeric matrix when $CO_2$ leaves swollen polymer during depressurization (Berens et al., 1992). During the impregnation process, the $scCO_2$ solubilizes the drug and swells the polymer. The solute enriched fluid phase is allowed to diffuse inside the matrix for a predetermined period followed by a depressurization step. The solubility of the drug in $scCO_2$ suddenly decreases and simultaneous $CO_2$ expulsion from the polymer results in solute entrapment inside the matrix at this stage. This approach is especially effective for solutes with high solubility in $scCO_2$ (Kazarian, 2004; Lora and Kikic, 1999).

One of the major advantages of supercritical impregnation is the possibility of adjusting the impregnation efficacy by 'tuning' the properties of $scCO_2$. Different operational parameters of the process can be varied such as the pressure, temperature, impregnation duration, process mode (batch or semi-continuous), $CO_2$ flow rate in a semi-continuous mode, solute concentration in the supercritical phase, the use (nature and amounts) of a co-solvent as well as the depressurization rate.

The supercritical impregnation for ocular applications has been widely discussed in the scientific literature and known to result in enhanced drug loading and controlled delivery of an API. Table 1 reviews the publications on $scCO_2$ impregnation applied for the development of polymer-based therapeutic ophthalmic articles.

Supercritical impregnation was shown to be more efficient and tunable than conventional soaking methods (Braga et al., 2011; González-Chomón et al., 2012) while requiring shorter processing durations. The supercritical processing mode also has influence on the impregnation efficiency. For example, batch processes are known to provide higher impregnation yields in short durations with homogeneous distribution of a drug within polymer compared to semi continuous process (Duarte et al., 2008). Furthermore, processing with $scCO_2$ is proven to retain critical functional properties such as glass transition temperature, transmittance, oxygen permeability (Costa et al., 2010b; Yañez et al., 2011; Braga et al., 2011; González-Chomón et al., 2012) and contact angle of contact lenses (Costa et al., 2010b; Yañez et al., 2011). The transparency of ocular articles like contact or intraocular lenses is very important, hence, foaming phenomenon has to be imperatively avoided could simply be achieved by slow depressurizations (Masmoudi et al., 2011).

The aim of the present work was to study the impregnation of dexamethasone 21-phosphate disodium (DXP) and ciprofloxacin (CIP) in intraocular lenses (IOLs) using $scCO_2$. DXP ($C_{22}H_{28}FNa_2O_8P$), a synthetic adrenal corticosteroid with potent anti-inflammatory properties is used in eye, ear and systemic formulations. CIP ($C_{17}H_{18}FN_3O_3$) is a synthetic antibiotic of second-generation fluoroquinolone and commonly prescribed for the treatment of eye infections including corneal ulcers (Pellegrino et al., 2008). Commercially available foldable IOLs made of P-HEMA were used in this work. The ability of these lenses to absorb water assists oxygen supply to cornea and requires only 2–3 mm incision in comparison to 10–12 mm for non-foldable lenses (Aquavella and Rao, 1987; Florkey et al., 2003). A number of parameters such as pressure, temperature, pressurization/depressurization rate, co-solvent requirement and impregnation duration were investigated to achieve optimum and homogeneous distribution of both drugs in P-HEMA IOLs.

A pretreatment step was necessary to dry P-HEMA lenses and to obtain a reproducible initial state for drug impregnation. The IOLs were dried using two different modes; in an oven and with $scCO_2$. Thermal analysis was performed to verify the presence of water and to establish appropriate drying method. For all experiments thereafter, IOLs were initially dried in an oven at 90 °C before the impregnation process.

Ethanol was chosen as a co-solvent and employed at 5% (molar) in order to increase drugs solubility in the $scCO_2$ and to enhance the polymer swelling (Bertucco and Vetter, 2001). It can significantly improve the polarity of the fluid phase since it has a complet miscibility in $scCO_2$. Ethanol is also a 'class 3' solvent according to the FDA which marks it as comparatively safe for human health (Champeau et al., 2015a). NMR analyses on $scCO_2$-treated IOLs was performed to determine the presence of residual solvent.

*In vitro* drug release studies in simulated aqueous humor were conducted on all drug-incorporated lenses. The drug release was quantified using UV–vis spectroscopy and impregnation yields were calculated for both CIP and DXP.

## 2. Materials and methods

### 2.1. Materials

Supercritical impregnations were performed on Tecsoft foldable acrylic intraocular lenses (FLEX IOLs); supplied by 'The Fred Hollows Intraocular Lens' (Nepal). The IOLs of three diopters (+5.0 D, +21.0 D and 32.0 D) used in this work are commercially available and manufactured from the derivatives of poly(2-hydroxyethyl methacrylate) (P-HEMA). The P-HEMA IOLs are supplied soaked in a physiological solution. The properties of the IOLs as reported by the supplier are summarized in Table 2 and a photograph of the IOL as well as the chemical formula of the polymer is presented in Fig. 2.

All other chemicals used in this work are listed in Table 3 and the skeletal formulas of both drugs are presented in Fig. 3.

### 2.2. Methods

#### 2.2.1. IOLs pretreatment

The P-HEMA IOLs are supplied pre-soaked in a physiological solution. They absorb a certain quantity of this solution due to their hydrophilic nature which makes them flexible under ambient pressure and temperature. These wet IOLs were dried following two different methods; in an oven and using supercritical $CO_2$.

The drying in the oven was performed at two temperatures (40 and 90 °C) for 10 days.

The drying of IOLs with $scCO_2$ was carried out in a batch mode under 80 bar for 30 min and 140 bar 135 min. Other parameters *i.e.*, temperature (35 °C), pressurization rate ($CO_2$ flow rate of 250 g $h^{-1}$) and depressurization rate (2 bar $min^{-1}$) were kept constant for these experiments.

#### 2.2.2. IOLs impregnation

A schematic diagram of the experimental high-pressure set-up is shown in Fig. 4. It is mainly composed of a 125 ml high-pressure

**Table 2**
Properties of foldable IOLs.

| P-HEMA (FLEX) | | | |
|---|---|---|---|
| Dioptric power (D) | +5.0 | +21.0 | +32.0 |
| Optical diameter (mm) | 6 | 5.9 | 5.8 |
| Overall diameter (mm) | 13.5 | 13 | 12.5 |
| Convexity | Biconvex | Biconvex | Biconvex |

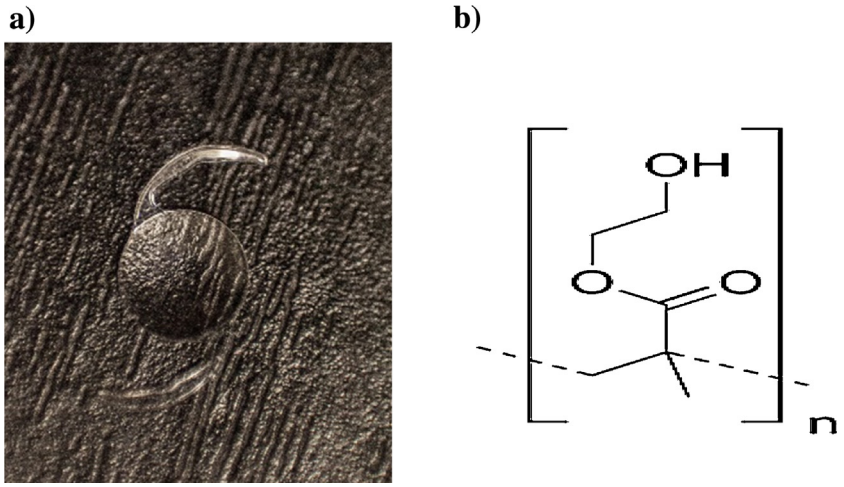


**Fig. 2.** P-HEMA (a) aspect of non treated IOL and (b) skeletal formula.

**Table 3**
Chemical compounds references.

| Chemical | Supplier | Purity% | CAS | Batch No. |
|---|---|---|---|---|
| Dexamethasone 21-phosphate disodium | Sigma–Aldrich (France) | 98 | 50-02-2 | MKBS2101V |
| Ciprofloxacin | Sigma–Aldrich (France) | 98 | 85721-33-1 | BCBM7969V |
| Carbon dioxide | Air liquid (France) | 99.7 | | |
| Ethanol | Groupe MERIDIS (France) | 99.8 | | 210.05.14 |
| Monobasic potassium phosphate | Sigma–Aldrich (France) | 99.0 | 7778-77-0 | BCBM7799V |
| Disodium hydrogenphosphate | Sigma–Aldrich (France) | 99.0 | 75588-79-4 | A0320538 |

cell (Top Industrie S. A., France) and a high-pressure liquid $CO_2$ pump (Milton Roy, France). The autoclave is positioned on a magnetic stirrer to ensure fast solubilization and homogenization of the API and immersed in a thermostat bath to regulate its temperature.

Supercritical impregnations were carried out in a batch mode where IOLs (2 per batch) were placed on an aluminum support inside the high-pressure cell to separate them from the stirrer bar. A known quantity of the API was introduced in the autoclave and was protected by a frit filter to prevent any contamination of the IOLs surface. For the impregnation with a co-solvent, a predefined quantity of ethanol was first placed in the bottom of the autoclave and IOLs support was positioned carefully to prevent any contact with the lenses. The high-pressure vessel was closed and heated to 35 °C and then filled with $CO_2$. For this purpose, $CO_2$ was first liquefied through a cooling unit and then pressurized and supplied to the system after heating by a feed pump until desired pressure was reached. The fluid phase containing API was allowed to diffuse within the IOLs for a pre-determined impregnation duration. The system was then slowly depressurized (2 bar $min^{-1}$) in order not to damage the IOLs and to avoid foaming (Masmoudi et al., 2011).

For impregnations carried out using a co-solvent; a supplementary $CO_2$ washing step (1 h) was carried out before depressurization to remove ethanol and avoid its condensation inside the autoclave.

*2.2.3. Experimental design and response surface methodology*

Response surface methodology (RSM) consists of a group of mathematical and statistical techniques that can be used to define the relationships between the response and independent variables. In RSM, an empirical mathematical model is postulated and a suitable experimental design is performed to estimate required coefficients. This model, once validated can be used to predict the response in the whole experimental domain with good precision (Baş and Boyacı, 2007).

For supercritical impregnation of P-HEMA IOLs, a two factors central composite design with 9 individual design points in a spherical domain was adopted (Table 4). The variables studied were pressure (in bar, $x_1$) and impregnation duration (in min, $x_2$). Other variables of the process; temperature (35 °C) and depressurization rate (2 bar $min^{-1}$) were kept constant. The experiments of the planned design were carried out in the absence of a co-

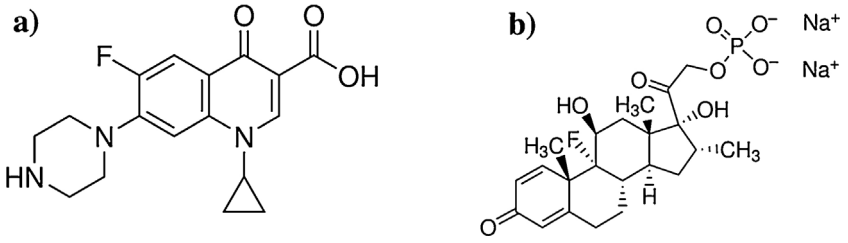


**Fig. 3.** Structural formula of (a) ciprofloxacin and (b) dexamethasone 21-phosphate disodium.

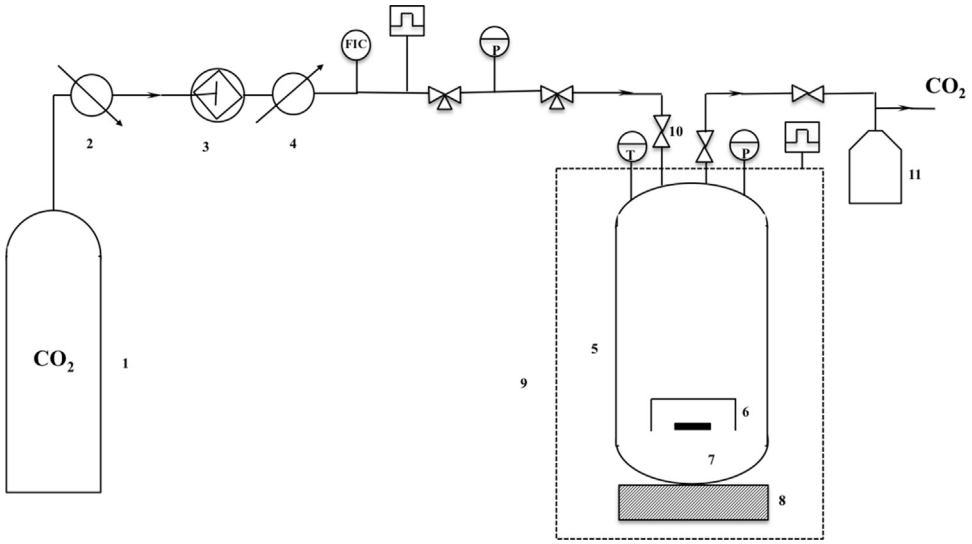


**Fig. 4.** Supercritical impregnation set-up: (1) $CO_2$ cylinder, (2) cooling bath, (3) high pressure liquid pump, (4) heating bath, (5) high pressure cell, (6) support, (7) magnetic bar, (8) magnetic stirrer, (9) thermostat bath, (10) depressurization valve, (11) solvent trap.

solvent. Response or dependent output variable ($Y$) studied was the impregnated amount quantified through release studies. Experiments in the given domain were repeated to verify the validity of the stated model.

A second order polynomial model as presented in Eq. (1) was postulated to capture the possible nonlinear effects and curvatures in the studied domain:

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{j=1}^{k} b_{ii} x_i^2 + \sum \sum_{i<j} b_{ij} x_i x_j \quad (1)$$

where $x_j$ ($j$ = 1,2, . . . $k$) is the undimensional variables and $b_0$, $b_i$, $b_{ii}$ and $b_{ij}$ are regression coefficient for intercept, linear, quadratic and synergic, respectively. The coefficients were estimated using multilinear regression from the results of studied responses. The calculations were performed with the Nemrod-W software (LPRAI, Marseille, France) developed for building and processing experimental design.

For validation of the model suitability, several techniques were used *i.e.*, residual analysis, ANOVA (ANalysis Of VAriance) and prediction error sum of squares residuals (especially the coefficient of determination, $R^2$). After validation, this model was used to calculate the response all over the domain.

*2.2.4. Differential scanning calorimeter (DSC) analysis*

Thermal analyses of various pretreated and impregnated IOLs were performed in a TA Q2000 DSC. Samples were accurately weighed (18–20 mg) into aluminum pans and thermograms were obtained over a temperature range of 50–300 °C. Each sample was exposed to heat-cool-heat cycle with a heating or cooling rate of 10 °C/min. DSC analysis was performed on samples before and after pretreatment and drug impregnation to identify possible changes in the thermal properties of IOLs. The DSC measurements were duplicated.

**Table 4**
Variables (factors) used for central composite design.

| Independent variables | Symbols | Codes-variable levels | | |
|---|---|---|---|---|
| | | −1 | 0 | +1 |
| $P$ (bar) | $X_1$ | 80 | 140 | 200 |
| $t_{imp}$ (min) | $X_2$ | 30 | 135 | 240 |

*2.2.5. Nuclear magnetic resonance (NMR) analysis*

Solid-state NMR spectroscopy was used for quantifying residual solvent content in the impregnated IOLs. $^{13}C$ NMR spectra were obtained using a Bruker Avance 400 MHz spectrometer consisting Bruker double-channel probe operating at a resonance frequency of 106 MHz. 20 mg of each sample was placed into zirconium dioxide rotors with 4 mm outer diameter. The rotors were equipped with two PTFE spacers and spun at a Magic Angle Spinning rate of 10 kHz.

The cross polarization (CP) technique (Schaefer and Stejskal, 1976) was applied with a ramped 1H-pulse starting at 100% and decreasing until 50% during the contact time of 2 ms to circumvent Hartmann–Hahn mismatches (Cook et al., 1997; Peersen et al., 1993). A dipolar decoupling GT8 pulse sequence (Gerbaud et al., 2003) was applied during the acquisition time to improve the resolution. In order to obtain a good signal-to-noise ratio in the $^{13}C$ CPMAS experiment, 8 K scans were accumulated at room temperature using a delay of 3 s. The $^{13}C$ chemical shifts were referenced to tetramethylsilane and calibrated with the glycine carbonyl signal set at 176.5 ppm.

*2.2.6. Drug release study*

*In vitro* drug release studies were carried out in simulated aqueous humor (pH of 7.2). It was prepared by mixing 9.08 g $L^{-1}$ of a monobasic potassium phosphate ($KH_2PO_4$) and 9.47 g $L^{-1}$ of a disodium hydrogenophosphate ($Na_2HPO_4$) solutions in a volume ratio of 0.285/0.715 respectively.

Prior to release studies, IOLs were washed with 5 ml of simulated aqueous humor for 3 min under stirring to remove any drug deposited at the surface. The rinsing solution was analysed spectrophometrically for CIP and DXP content at 248 and 277 nm respectively using Jenway 6715 UV/Vis. Drug concentrations in washing solution was found to be too low for UV–vis analysis.

Release studies were conducted by immersing impregnated IOLs in 5 ml of simulated aqueous humor (pH of 7.2) under stirring in a closed vessel at 37 °C. An aliquot of 0.4 ml was collected every day for 60 days and CIP or DXP release was quantified at 248 nm or 277 nm respectively (Jenway 6715 UV/Vis). Aliquots were then returned to the release vessel to maintain the initial volume. The drug release from both IOLs impregnated in the same batch was studied separately in order to verify the homogeneity and reproducibility of the process.

*2.2.7. Modeling of drugs release from P-HEMA IOLs*

There are a large number of articles on drug release modeling from matrix systems starting from the pioneering work of Higuchi (1967) to recent detailed models of Galdi and Lamberti (2012). Ritger and Peppas (1987a) suggested an empirical equation which can be used to analyze low drug release (below 60%) from non-swellable polymeric delivery systems:

$$\frac{M_t}{M_\infty} = kt^n \quad (2)$$

where $M_t$ and $M_\infty$ represent the cumulative drug released at times $t$ and infinity respectively. $k$ is a kinetic constant that incorporates structural/geometric characteristic of a delivery system (polymer + drug) and $n$ is designated as an exponent representing the release mechanism.

Eq. (2) can also be used for the analysis of controlled release systems based on moderately swelling polymers (*e.g.*, systems based on hydroxypropyl methyl cellulose, poly(vinyl alcohol), poly (2-hydroxyethyl methacrylate), *etc.*). Therefore, this equation was used to model drug release from IOLs as overall swelling of these systems was less than 25%. In this work, the release constant $k$ and release exponent parameter $n$ were fitted using Eq. (2) to the first 60% release of the impregnated drugs from IOLs.

*2.2.8. Impregnation yields*

The impregnated amounts were determined through release studies and defined as cumulative mass release of the drug after reaching to a constant value. The impregnation yield was calculated according to the following equation (Eq. (3)).

$$y_{imp} = \frac{m_{imp}}{m_{0IOL}} \quad (3)$$

where $m_{imp}$ is mass of impregnated API and $m_{0IOL}$ is initial mass of dry IOL.

## 3. Results and discussion

### 3.1. IOLs foaming

Foaming due to sorption and release of $scCO_2$ from a polymeric matrix is very common. Hence, it was important to understand the effect of pressurization and depressurization rates on the optical properties of P-HEMA IOLs.

In our experimental conditions, rate of pressurization was observed to be a significantly important factor in controlling the integrity of IOLs. In order to elucidate this phenomenon, influence of the pressurization rate of $scCO_2$ at three different flow rates of slow (250 g $h^{-1}$), intermediate (650 g $h^{-1}$) and fast pressurization (>900 g $h^{-1}$) was studied in a batch mode at fixed pressure (200 bar), temperature (35 °C) and duration (2 h). Based on previous studies on rigid PMMA IOLs, the depressurization was carried out under controlled rate of 2 bar $min^{-1}$ (Masmoudi et al., 2011).

The $scCO_2$ treatment of IOLs with pressurization rates of 650 and 900 g $h^{-1}$ resulted in foaming and loss in the optical properties of the IOLs as illustrated in Fig. 5.

Similar loss in optical properties was also observed for samples treated at lower pressure (80 bar) and slower depressurization rate (0.7 bar $min^{-1}$) with or without a co-solvent for pressurization rates of 650 and 900 g $h^{-1}$.

For the considered application of IOLs, foaming even with few bubbles has to be imperatively avoided. This was achieved by controlled pressurization at 250 g $h^{-1}$ coupled with a depressurization rate of 2 bar $min^{-1}$. Samples treated at abovementioned conditions resulted in transparent IOLs at both 80 and 200 bar and in the presence or absence of a co-solvent (Fig. 6).

Generally in the literature, foaming in polymers is reported to occur during depressurization phase. This is due to the quick desorption of gas from a matrix during rapid depressurization. This fast release of $CO_2$ induces nucleation and growth of bubbles until the pressure reaches a point where foamed structure freezes (Kazarian, 2004). The number and size of the created bubbles depend on the pressure, temperature and impregnation duration in addition to the depressurization rate (Goel and Beckman, 1994; Reverchon and Cardea, 2007; Xu et al., 2007).

In our work, even in slow depressurization conditions, foaming was observed for rapid pressurization phases which suggests that a brisk $CO_2$ sorption in P-HEMA lenses promotes sudden swelling and subsequent deformation. All $scCO_2$ treatments of IOLs (pre-treatments as well as impregnations) were performed at a $CO_2$ flow rate of 250 g $h^{-1}$ from hereon to avoid foaming and loss of optical properties during processing.

### 3.2. IOLs pretreatment

Since the hydrophilic P-HEMA IOLs were supplied pre-conditioned in a physiological solution, a preliminary drying step was necessary to extract absorbed water and to obtain reproducible initial conditions for impregnation. At first, the influence of drying mode (oven and $scCO_2$) was studied to understand the suitability of

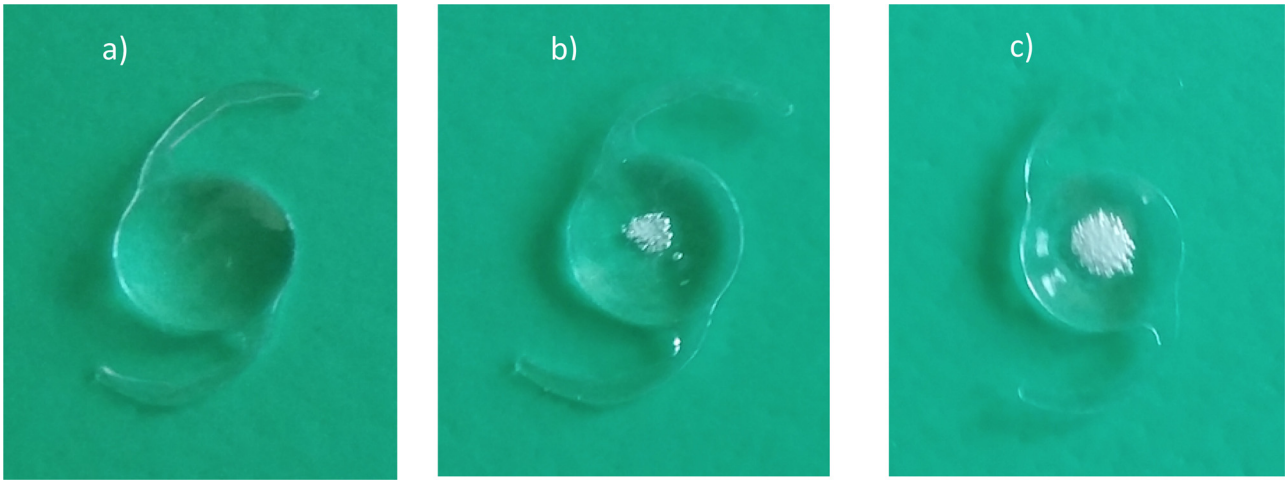


**Fig. 5.** Influence of the pressurization rate on the visual aspect of some IOLs; (a) non treated IOL; (b) IOL treated with $scCO_2$ at 200 bar with a pressurization rate of 650 g $h^{-1}$; (c) IOL treated at 200 bar with a rapid pressurization rate of 900 g $h^{-1}$.

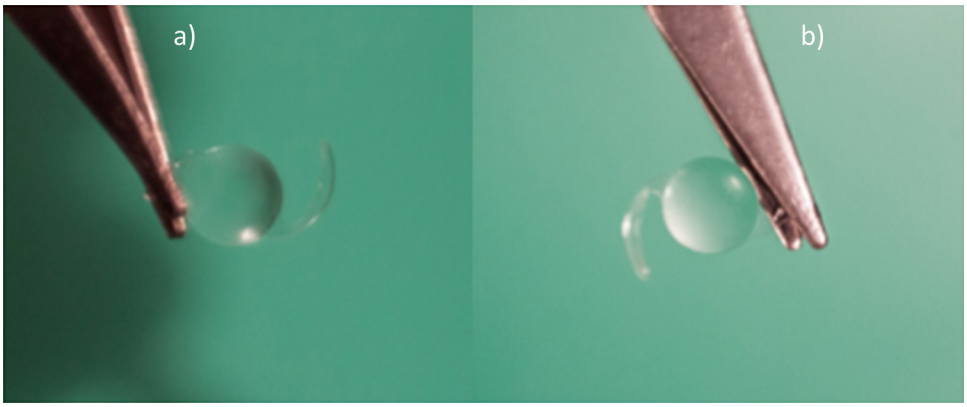


**Fig. 6.** Influence of the pressurization rate (250 g $h^{-1}$) on the visual aspect of IOLs treated with $scCO_2$; (a) in the presence of ethanol (5%); (b) in the absence of co-solvent.

these methods and to determine required temperature and pressure for efficient drying.

The IOLs were dried in an oven at two temperatures (40 and 90 °C) and DSC analyses were performed to determine the $T_g$ of IOLs after this procedure. DSC analyses of the IOLs dried in an oven at 40 °C and 90 °C showed a $T_g$ of 113 °C and 121 °C, respectively (pre-treatments as well as impregnations). This can be attributed to the efficient removal of water from IOLs at higher temperature. Absorbed water in a polymeric matrix acts as a plasticizer which leads to decreased $T_g$ as evident by the samples dried at 40 °C. The temperature of 90 °C was required for the removal of water efficiently from P-HEMA IOLs.

DSC analyses of the IOLs dried with $scCO_2$ at (80 bar, 30 min) and (140 bar, 135 min) show a $T_g$ of respectively 119 and 121 °C, respectively (Table 5). Once again, the increase of $T_g$ with increase in the pressure and drying time can be explained by the efficient removal of water from P-HEMA IOLs.

A $T_g$ of 121 °C for IOLs dried by both oven (90 °C) and $scCO_2$ (140 bar, 35 °C for 135 min) methods along with the same weight loss (0.21 $g_{water}/g_{dried\ IOL}$) indicated removal of most of the water at these conditions.

### 3.3. Supercritical impregnation of IOLs

#### 3.3.1. Ciprofloxacin impregnation

*3.3.1.1. Preliminary impregnations.* IOLs were dried at 90 °C in an oven before preliminary impregnation experiments. The drug impregnations were performed with or without co-solvent at 80 and 200 bar on IOLs with two different diopters (+5.0 D and +21.0 D).

The impregnation results presented in Table 6 are expressed in term of the impregnated mass of ciprofloxacin in IOL ($m_{cip\ imp}$) and the impregnation yield ($y_{imp}$).

In the absence of co-solvent, a pressure increase from 80 to 200 bar led to improved CIP impregnation. The drug impregnation for diopter +5.0 D increased from 0.92 to 3.45 $\mu g_{drug}/mg_{IOL}$. Similarly, an increase from 0.95 to 2.86 $\mu g_{drug}/mg_{IOL}$ for diopter +21.0 D. This improvement in CIP loading can be attributed to the concurrent increase in the solubility of the drug and polymer swelling due to $CO_2$ sorption (Yu et al., 2011; Braga et al., 2008; Guney and Akgerman, 2002; Champeau et al., 2015b). The addition of ethanol as a co-solvent in the procedure resulted in significant increase in drug impregnation at 80 than 200 bar. It is well known that co-solvent such as ethanol promotes the solubility of polar drugs in $CO_2$ by enhancing the overall polarity of the fluid phase. Furthermore, the $CO_2$ sorption and swelling/plasticizing can also increase with the addition of a co-solvent if polymer/$CO_2$/co-solvent interactions are favored (Costa et al., 2010b; Masmoudi et al., 2011). An absence of any further drug impregnation at higher pressure in the presence of ethanol could be either due to the saturation of IOLs or lack of improvement in polymer/drug interactions.

Interestingly, impregnation yields were comparable for samples processed with ethanol (at 80 and 200 bar) and only $scCO_2$ at 200 bar (without ethanol). Hence, CIP loading was performed without the use of co-solvent from hereon which is also preferable for the intended application

*In vitro* drug release from impregnated IOLs was conducted for 60 days for both diopters (+5.0 and +21.0 D). The release kinetics from CIP loaded IOLs is illustrated in Fig. 7 (+5.0 D) and which suggests in-depth (absence of surface adsorption) and homogeneous impregnation of the drug in IOLs.

Release profiles discussed above were fitted with Eq. (2) and the fitting parameters obtained for both diopters (+5.0 and +21.0 D) are summarized in Table 6.

For all the experiments, the exponent value ($n$) is ranging between 0.5 and 1.0 which suggests that the drug release is occurred by an anomalous transport type (*i.e.*, the superimposition of Fickian controlled and swelling controlled release) (Ritger and Peppas, 1987a,b).

The release rate constant ($k$) decreased as the impregnation pressure increased or when a co-solvent was used. This indicates that the affinity between P-HEMA IOLs and CIP increases with the increase in pressure or the addition of a co-solvent (Yañez et al., 2011). A regression coefficient of higher than 96% indicates that the model currently used fits well with drug release profiles. Release exponents were similar for IOLs impregnated in different conditions for both diopters.

*3.3.1.2. Experimental design.* Following the results of the first series of experiments, it could be concluded that drug loadings were significantly influenced by the change in pressure in the absence of a co-solvent. Moreover, increase in pressure in the presence of co-solvent had minimal influence on the impregnation yield. Therefore, a response surface methodology based on experimental design was used to study the influence of

**Table 5**
$T_g$ of P-HEMA IOLs drying in an oven and with $scCO_2$.

| Drying mode | Drying conditions | $T_g$ (°C) ± 0.3 |
|---|---|---|
| Oven | 40 °C | 113 |
| Oven | 90 °C | 121 |
| $ScCO_2$ | 80 bar and 35 °C, 30 min | 119 |
| $ScCO_2$ | 140 bar and 35 °C, 135 min | 121 |

**Table 6**
Impregnation rates and kinetics parameters of IOLs of +5.0 D and +21.0 D diopters (at 35 °C and 2 h), determined by drug release studies.

| | +5.0 D | | | | | | | +21.0 D | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $P$ (bar) | $m_{0IOL}$[a] (mg) | $m_{CIP\ imp}$ (μg) | $y_{imp}$ (μg$_{drug}$/mg$_{IOL}$) | $t_{release}$ (days) | Kinetics parameters | | | $m_{0IOL}$[a] (mg) | $m_{CIP\ imp}$ (μg) | $y_{imp}$ (μg$_{drug}$/mg$_{IOL}$) | $t_{release}$ (days) | Kinetics parameters | | |
| | | | | | $n$ | $k$ | $R^2$ | | | | | $n$ | $k$ | $R^2$ |
| Without co-solvent | | | | | | | | | | | | | | |
| 80 | 11.9 | 11 ± 0.2 | 0.9 ± 0.1 | ≈20 | 0.835 | 0.072 | 0.995 | 20.1 | 20 ± 0.2 | 0.9 ± 0.1 | ≈20 | 0.748 | 0.076 | 0.972 |
| 200 | 11.3 | 39 ± 0.5 | 3.4 ± 0.5 | ≈40 | 0.907 | 0.043 | 0.962 | 20.6 | 59 ± 0.5 | 2.9 ± 0.3 | ≈40 | 0.810 | 0.058 | 0.975 |
| With co-solvent | | | | | | | | | | | | | | |
| 80 | 11.5 | 44 ± 0.6 | 3.8 ± 0.6 | ≈40 | 0.866 | 0.052 | 0.987 | 20.5 | 61 ± 0.5 | 3.0 ± 0.3 | ≈40 | 0.842 | 0.052 | 0.997 |
| 200 | 11.4 | 47 ± 0.6 | 4.1 ± 0.6 | ≈40 | 0.884 | 0.050 | 0.994 | 20.3 | 65 ± 0.5 | 3.2 ± 0.3 | ≈40 | 0.794 | 0.065 | 0.965 |

[a] Initial mass of the dry IOL before impregnation.

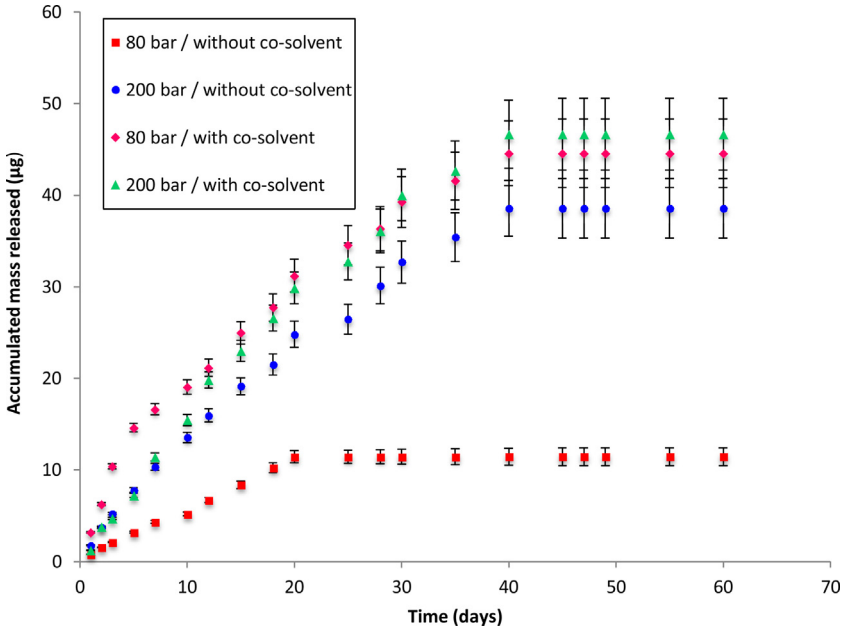


**Fig. 7.** Accumulated drug release from IOLs (+5.0 D) impregnated at 35 °C with the pressurization rate of 250 g h$^{-1}$, impregnation duration of 2 h and depressurization rate of 2 bar min$^{-1}$.

operating conditions on the impregnation of CIP on +21.0 D IOLs. Two entry values with 5 levels each were considered; pressure (80–200 bar) and impregnation duration (30–240 min).

The operational conditions and results from these experiments are summarized in Table 7. All impregnations were carried out at 35 °C with a $CO_2$ flow rate of 250 g h$^{-1}$ and a depressurization rate of 2 bar min$^{-1}$ without the use of co-solvent.

For the different experimental conditions, impregnated amounts ranged between 21 and 67 μg which suggests that the studied factors have noteworthy influence on the response surface. Fig. 9 presents release kinetics of CIP impregnated samples prepared at different pressures and durations.

The drug release was dependent on the mass of impregnated CIP in IOLs at various conditions. For example, a sustained release

**Table 7**
Experimental design conditions, impregnation rates and kinetics parameters of IOLs (+21.0 D) impregnated at 35 °C.

| $P$ (bar) | $t_{imp}$ ($x_1$) (min) | $m_{0IOL}$ (mg) | $m_{CIP\ imp}$ (μg) | $y_{imp}$ (μg$_{drug}$/mg$_{IOL}$) | $t_{release}$ (days) | Kinetics parameters | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | $n$ | $k$ | $R^2$ |
| 100 | 60 | 19.0 | 26 ± 0.2 | 1.4 ± 0.1 | ≈18 | 0.555 | 0.133 | 0.977 |
| 100 | 210 | 18.6 | 30 ± 0.3 | 1.6 ± 0.1 | ≈40 | 0.837 | 0.039 | 0.987 |
| 180 | 60 | 18.9 | 39 ± 0.3 | 2.1 ± 0.2 | ≈30 | 0.685 | 0.090 | 0.981 |
| 180 | 210 | 19.5 | 53 ± 0.4 | 2.7 ± 0.2 | ≈40 | 0.772 | 0.058 | 0.991 |
| 140 | 30 | 19.2 | 22 ± 0.1 | 1.1 ± 0.1 | ≈18 | 0.727 | 0.061 | 0.985 |
| 140 | 240 | 20.0 | 41 ± 0.3 | 2.1 ±0.1 | ≈45 | 0.982 | 0.031 | 0.991 |
| 80 | 135 | 19.1 | 21 ± 0.2 | 1.1 ± 0.1 | ≈10 | 0.859 | 0.307 | 0.992 |
| 200 | 135 | 19.5 | 67 ± 0.4 | 3.4 ± 0.2 | ≈45 | 0.839 | 0.053 | 0.989 |
| 140 | 135 | 19.0 | 33 ± 0.2 | 1.7 ± 0.1 | ≈20 | 0.894 | 0.123 | 0.972 |
| 140 | 135 | 18.8 | 35 ± 0.1 | 1.7 ± 0.1 | ≈20 | 0.555 | 0.133 | 0.977 |
| 140 | 135 | 18.8 | 31 ± 0.1 | 1.6 ± 0.1 | ≈20 | 0.837 | 0.039 | 0.987 |

ranging from 10 to 30 days was obtained for IOLs impregnated at 100 bar/60 min, 180 bar/60 min, 140 bar/30 min, 80 bar/135 min and 140 bar/135 min. Whereas, samples with higher impregnation yields (impregnated at 100 bar/210 min, 180 bar/210 min, 140 bar/240 min and 200 bar/135 min) showed drug release for significantly longer durations (40–45 days).

Fig. 10 presents examples of samples prepared at 140 and 180 bar to explain the effect of impregnation duration on release kinetics. It is interesting to note that the drug release is always higher and extended for samples prepared with longer impregnation durations at a given pressure even when slopes do not appear significantly dissimilar. Therefore, it can be concluded that increasing the impregnation time allows a more in-depth diffusion of the drug facilitated by the improved swelling of the polymer.

Release profiles were fitted to Eq. (2) to obtain release exponents for impregnated IOLs similar to the preliminary experiments. The fitting parameters obtained for IOL samples are summarized in Fig. 7.

Similar to preliminary experiments, release exponents ranging between 0.5 and 1 confirm drug release to occur by an anomalous transport type (*i.e.*, the superimposition of Fickian controlled and swelling controlled release). The impregnated amounts obtained from release profiles were used to calculate an impregnation model by multilinear regression. The model coefficients are presented in Eq. (4) and a regression coefficient ($R^2$) of 0.945 suggests that this model can be considered as reliable tool to predict changes in impregnated amounts within the studied operating conditions.

$$y = 0.032 + 0.012x_1 + 0.005x_2 + 0.006x_{11}^2 - 0.001x_{22}^2 + 0.002x_{12} \quad (4)$$

Fig. 11 shows the impregnated amount predicted by the RSM model as a function of pressure and impregnation duration.

A high variation of response in terms of drug loading in IOLs with the pressure increase is evident in Fig. 11. This is expected due to improved drug solubility and swelling/plasticization of polymers in $scCO_2$ at higher pressures (Champeau et al., 2015a,b; Yu et al., 2011; Duarte et al., 2008; Ritger and Peppas, 1987b; Costa et al., 2010a). The effect of impregnation duration is minimal at low pressures but significant at pressures above 140 bar. A direct

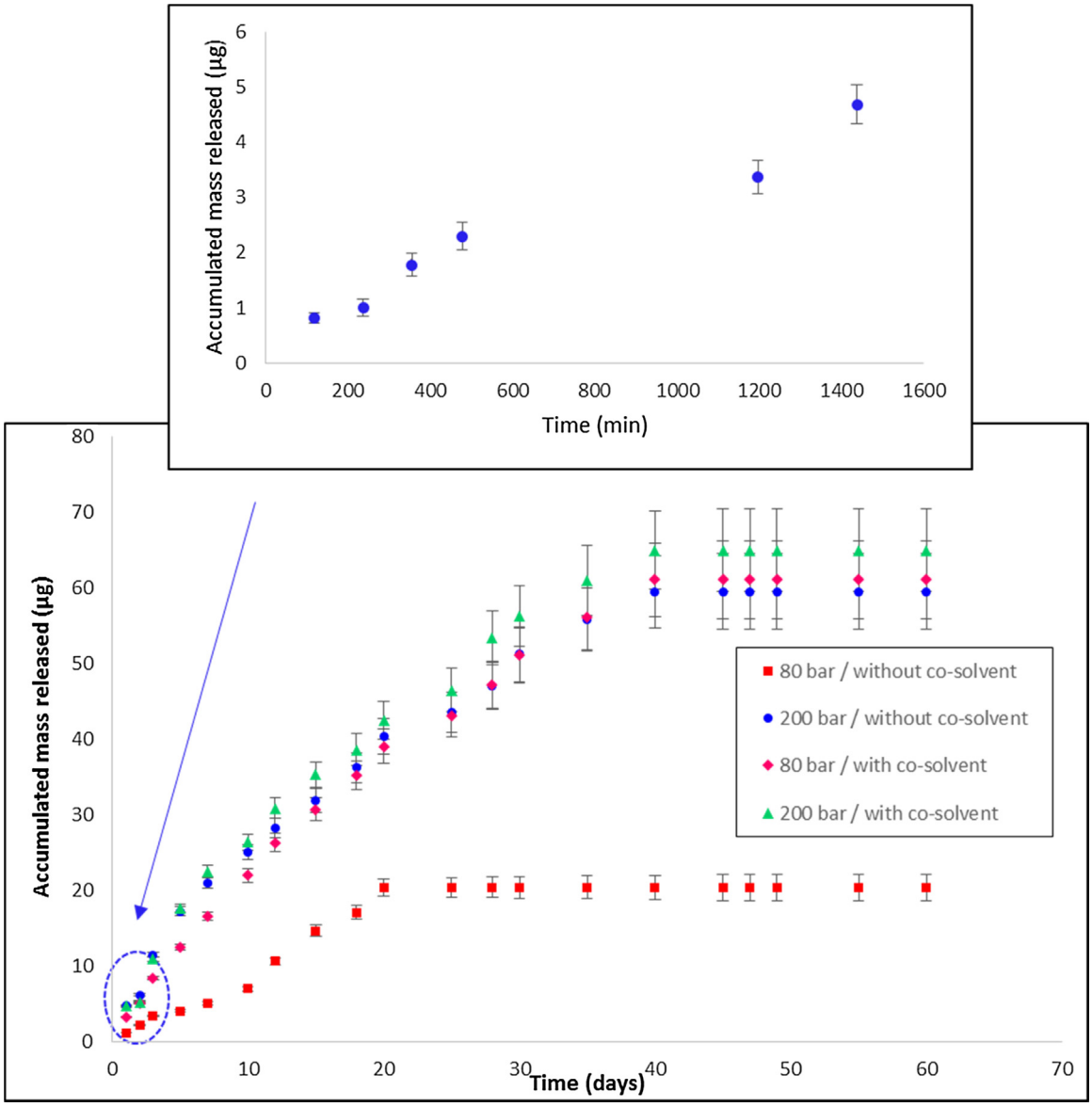


**Fig. 8.** Accumulated drug release from IOLs (+21.0 D) impregnated at 35 °C with the pressurization rate of 250 g $h^{-1}$, impregnation duration of 2 h and depressurization rate of 2 bar $min^{-1}$.

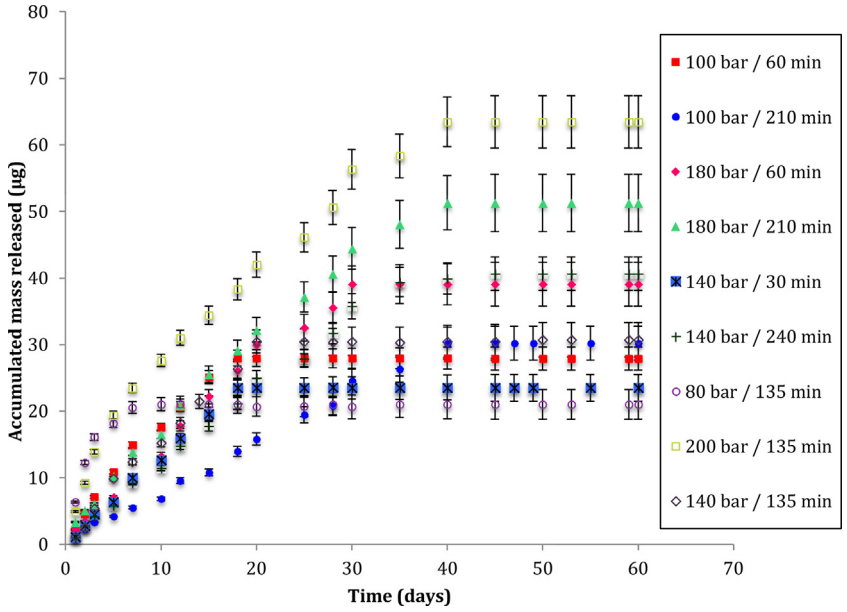


**Fig. 9.** Accumulated drug release from IOLs (+21.0 D) impregnated, using experimental design, at 35 °C, pressurization rate of 250 g $h^{-1}$, without co-solvent and depressurization rate of 2 bar $min^{-1}$.

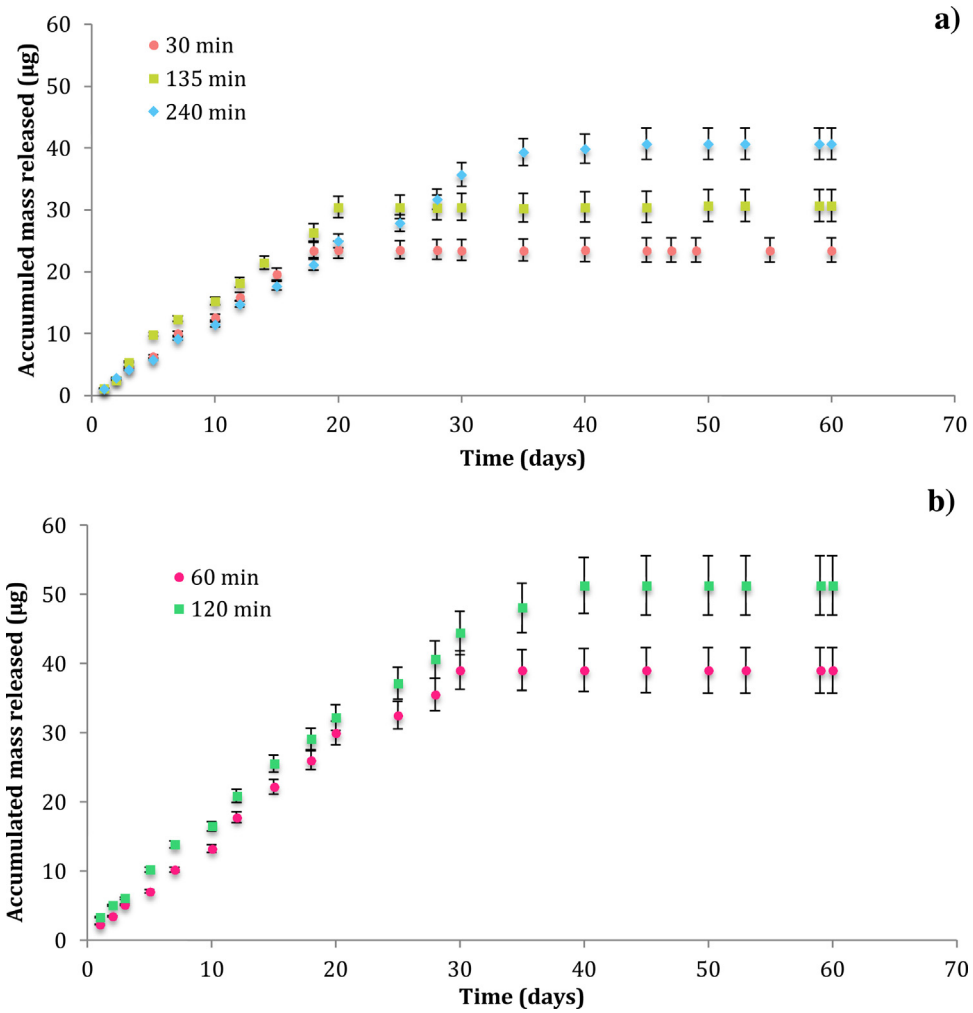


**Fig. 10.** Accumulated drug release from IOLs (+21.0 D) impregnated, using experimental design, at (a) 140 bar, (b) 180 bar.

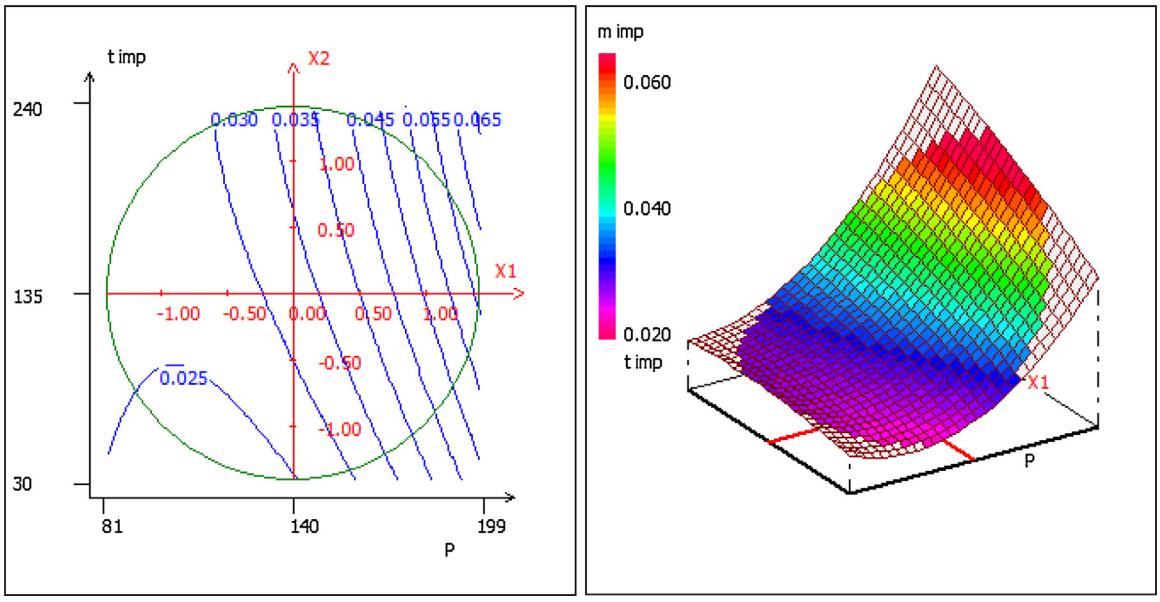


**Fig. 11.** A two-dimensional contour plot and a three-dimensional response surface of impregnated mass (mg) illustrating optimal conditions for the supercritical impregnation of P-HEMA IOLs with CIP.

impact of increase in processing time on drug impregnation at higher pressures is apparent in Fig. 11. This could also be attributed to higher $CO_2$ sorption resulting in polymer swelling and improved dissolution of CIP. In other words, it could be suggested that thermodynamic equilibrium was not reached at low impregnation durations.

The lowest drug loading of 21 μg was obtained on samples processed at 80 bar for 135 min whilst highest drug loading (67 μg) was achieved at 200 bar for the same duration. 80 and 200 bar were the lowest and highest pressures studied in this work and response surface clearly shows high pressure as the key factor for efficient drug impregnation in IOLs.

### 3.3.2. Dexamethasone 21-phosphate disodium impregnation

It is well known that the efficiency of the supercritical impregnation is not only dependent on the employed operational conditions but also on the physico-chemical interactions between all involved substances in the process. The supercritical impregnation of DXP on P-HEMA IOLs was carried out at various conditions similar to CIP. The influence of pressure (80 and 200 bar) and use of a co-solvent (5 mol% ethanol) on drug impregnation was studied for diopters +21.0 and +32.0 D. Other parameters *i.e.*, temperature (35 °C), impregnation duration (2 h), pressurization (250 g $h^{-1}$) and depressurization rates (2 bar $min^{-1}$) were kept constant for all experiments.

The experimental conditions and impregnation yields for DXP loading are summarized in Fig. 8.

Impregnation yields obtained at the different experimental conditions were comparable and reproducible for both diopters. The increase in pressure from 80 to 200 bar had no significant effect on the amount of DXP impregnated in IOLs. However, higher impregnation yield was obtained upon the addition of ethanol in the processing media. The increase in pressure for samples processed with co-solvent showed minimal improvement in the drug loading.

These results strongly suggest that the use of co-solvent is favorable toward DXP impregnation. However, residual solvent can be a concern for ocular implants. Hence, NMR analyses were carried out on DXP impregnated (without and with washing step before depressurization) IOLs to determine the presence of

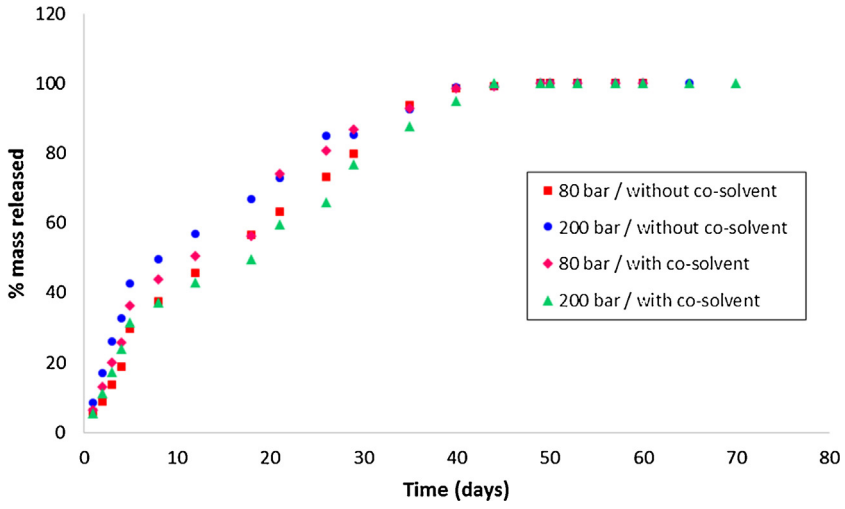


**Fig. 12.** Drug released (%) from IOLs (+21.0 D) impregnated at 35 °C, pressurization rate of 250 g $h^{-1}$, impregnation duration of 2 h and depressurization rate of 2 bar $min^{-1}$.

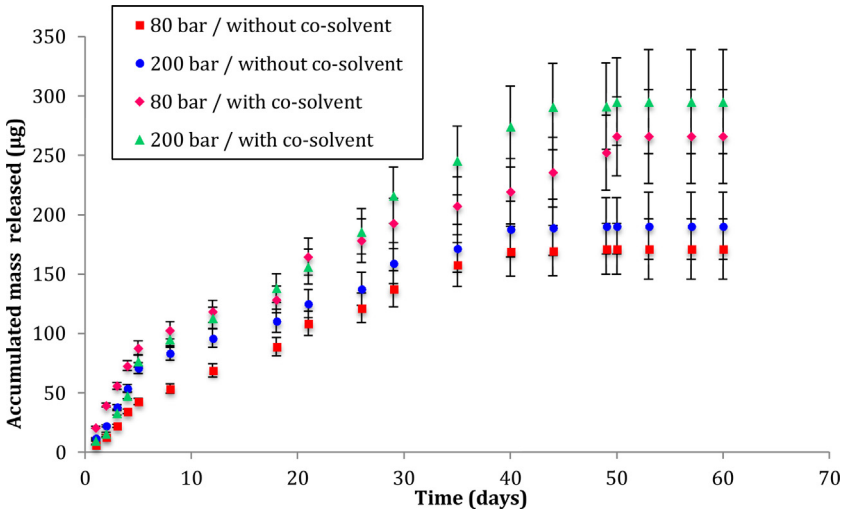


**Fig. 13.** Accumulated drug release from IOLs (+32.0 D) impregnated at 35 °C, pressurization rate of 250 g h$^{-1}$, impregnation duration of 2 h and depressurization rate of 2 bar min$^{-1}$.

**Table 8**
Impregnation rates and kinetics parameters of IOLs of +21.0 D and +32.0 D diopters (at 35 °C and 2 h), determined by drug release studies (40 days of release).

| $P$ (bar) | +21.0 D | | | | | | +32.0 D | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $m_{0IOL}$[a] (mg) | $m_{DXP\ imp}$ (µg) | $y_{imp}$ ($\mu g_{drug}/mg_{IOL}$) | Kinetics parameters | | | $m_{0IOL}$[a] (mg) | $m_{DXP\ imp}$ (µg) | $y_{imp}$ ($\mu g_{drug}/mg_{IOL}$) | Kinetics parameters | | |
| | | | | $n$ | $k$ | $R^2$ | | | | $n$ | $k$ | $R^2$ |
| Without co-solvent | | | | | | | | | | | | |
| 80 | 19.4 | 165 ± 24 | 8.5 ± 1.3 | 0.842 | 0.058 | 0.967 | 20.1 | 171 ± 25 | 8.5 ± 1.3 | 0.919 | 0.044 | 0.962 |
| 200 | 20.0 | 182 ± 27 | 9.1 ± 1.4 | 0.781 | 0.099 | 0.954 | 20.5 | 191 ± 28 | 9.3 ± 1.4 | 0.790 | 0.077 | 0.931 |
| With co-solvent | | | | | | | | | | | | |
| 80 | 19.0 | 247 ± 37 | 13.1 ± 2.0 | 0.763 | 0.080 | 0.942 | 20.2 | 266 ± 39 | 13.2 ± 2.0 | 0.791 | 0.098 | 0.934 |
| 200 | 19.5 | 270 ± 40 | 13.8 ± 2.1 | 0.733 | 0.072 | 0.946 | 20.3 | 295 ± 43 | 14.5 ± 2.2 | 0.929 | 0.038 | 0.944 |

[a] Initial mass of the dry IOL before impregnation.

ethanol. Ethanol peaks disappears when a supplementary $CO_2$ washing step is performed indicating a residual solvent content to be lower than 0.01 wt% of ethanol in the IOLs.

The DXP release from impregnated IOLs is presented in Fig. 12 (in function of the % drug released which is defined as a ratio between different cumulative released amounts and the final constant one obtained while reaching the release plateau) and Fig. 13 (in function of the cumulative drug released) for both +21.0 and +32.0 D diopters, respectively.

Samples prepared at both pressures and with or without co-solvent exhibited same release profile without any burst release. Similar to CIP samples, this indicates in-depth and homogenous impregnation of DXP within P-HEMA IOLs.

Release profiles of DXP were fitted to Eq. (2) and the release parameters are presented in Table 8.

The regression coefficients of more than 93% was obtained for both diopters prepared at discussed conditions. Furthermore, similar release exponents for the impregnated IOLs suggest DXP release to also occur by an anomalous transport type.

## 4. Conclusion

IOLs have proven significance in the field of therapeutics (Aqil and Gupta, 2012), their development is an upcoming route for ocular drug delivery. This work was aimed at developing drug impregnated foldable intraocular lenses (P-HEMA) in order to combine cataract surgery and postoperative treatment in a single procedure. Two commonly used drugs, ciprofloxacin and dexamethasone 21-phosphate disodium to prevent cataract postoperative complications were studied in this work.

The supercritical impregnation was carried out in a batch mode and the impregnated yields were determined by drug release studies. This work underlines the importance of coupling slow pressurization and depressurization during supercritical treatment of P-HEMA IOLs in order to avoid the appearance of undesirable foaming. A pressurization rate of 250 g h$^{-1}$ accompanied by depressurization at 2 bar min$^{-1}$ was optimum to maintain the optical properties of IOLs. A pretreatment step to remove adsorbed fluid on P-HEMA lenses was carried out using two different methods; oven and $scCO_2$. DSC analyses of IOLs dried in an oven at 90 °C and with $scCO_2$ at 40 °C and 140 bar for 135 min showed same $T_g$ (121 °C) confirming complete removal of water.

CIP and DXP impregnation was performed at a range of pressures and temperatures in $scCO_2$ to study the effect of these parameters on impregnation yield. For P-HEMA/CIP system, supercritical impregnations were initially carried out at pressures 80 and 200 bar in the presence or absence of ethanol as a co-solvent for two diopters (+5.0 D and +21.0 D). Drug loading enhancement with the pressure was observed in the absence of co-solvent for both diopters. Whereas, addition of co-solvent had no further improvement in the impregnation yield of CIP. Following the results of the first series of experiments and according to the aimed application, a response surface methodology based on experimental designs was applied to study the

influence of operating conditions on impregnation in the absence of a co-solvent. Two input variables were considered; pressure (80–200 bar) and impregnation duration (30–240 min). The CIP impregnation ranging between 1.10 and 3.44 μg/mg was obtained from these experiments. The response surface indicated pressure to be the governing factor in impregnation where increase in pressure promoted drug loading. The effect of impregnation duration on CIP loading was only evident at relatively high pressures (>140 bar).

Similarly, influence of pressure (80 and 200 bar) and co-solvent was also studied for the P-HEMA/DXP system. Unlike CIP, use of ethanol (5 mol%) as a co-solvent improves DXP impregnation in IOLs. The DXP loading involved addition of an extra washing step to ensure complete removal of ethanol from P-HEMA IOLs. The NMR analysis was performed on drug impregnated lenses which did not show any presence of residual solvent.

The highest impregnation yields for DXP and CIP in P-HEMA lenses were 14.53 and 4.12 $\mu g/mg_{IOL}$ respectively. This indicates higher affinity of DXP for P-HEMA IOLs than CIP. This study provides important information on the impregnation of two commonly used drugs in the complications related to cataract surgery on P-HEMA IOLs which could be used to carry out simultaneous loading of both drugs in the future.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2016.01.016.

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