SUPERCRITICAL DEVELOPMENT OF SUSTAINED DRUG DELIVERY INTRAOCULAR LENSES

Y. MASMOUDI[1*], S. ABDELLI[1], O. FORZANO[2], J-M. ANDRE[2], E. BADENS[1]

[1]Laboratoire de Mécanique, Modélisation & Procédés Propres (UMR CNRS 6181), Université Paul Cézanne Aix Marseille, Europôle de l'Arbois, Bâtiment Laennec, hall C, BP 80, 13545 Aix-en-Provence cedex 4 – France

[2]Service d'Ophtalmologie de l'Hôpital de la Timone,

264 rue Saint Pierre, 13385 Marseille cedex 5 - France

*email address : yasmine.masmoudi@univ-cezanne.fr – Fax : (+33) 4 42 90 85 15

ABSTRACT

Controlled drug delivery systems (DDS) are receiving an increasing interest since they enhance the drug efficacy by a target action in the body and a sustained release profile. Most of these DDS are polymer based where the polymer is biocompatible and sometimes biodegradable and acts as a reservoir or a matrix for the drug.
Supercritical technologies have been demonstrated to be a clean and effective alternative to traditional methods of drug and polymer processing. Recent developments are focusing on the elaboration of DDS. For that purpose, classical supercritical processes of particle generation (RESS, SAS, PGSS, …) can be applied to form polymer/drug capsules or coprecipitates. Supercritical fluids can also be used as an impregnation vehicle of the drug within the polymeric matrix.

The present work deals with supercritical impregnation of intraocular lenses (IOLs) to prevent postoperative endophthalmitis in cataract surgery. Commercially available rigid IOLs made from derivative of Poly (Methyl MethAcrylate) (PMMA) were impregnated with cefuroxime sodium, an antibiotic, and dexamethasone, an anti-inflammatory drug, through a discontinuous process.
The impregnation efficacy was determined in term of impregnation yield as well as in term of in-vitro drug release.
The influence of some experimental operating conditions was studied by varying the pressure (8 and 20 MPa), the temperature (308 and 333 K), or by adding a cosolvent (ethanol). The influence of the IOLs dioptre was also studied (+21.0 D, +30.0 D).
At rapid depressurization rates, a non desired foaming phenomenon was observed in most of the experimental conditions. This phenomenon was avoided by carrying out slow depressurizations (0.2 MPa/min).

INTRODUCTION

Cataract surgery consists in replacing the opacified natural crystalline lens with a synthetic intraocular lens (IOL). It is generally safe but risk of postoperative endophthalmitis has to be considered [1]. In order to prevent such infectious risks, a solution used nowadays consists in injecting in the eye a concentrated solution of the anti-infectious drug at the end of surgery [2]. However, even if the drug is delivered closer to the potential infection area, its removal is rapid. A relevant solution to overcome those drawbacks could be the use of controlled drug delivery systems (DDS) placed inside the eye. Indeed, a slow release of the drug close to the infection area may happen during a certain period of time. Moreover, if the DDS is the impregnated IOL, this solution does not require an additional act of the surgeon.
DDS are receiving an increasing interest since they allow a targeted and sustained action in the body. Most of them are polymer-based where the polymer is biocompatible biocompatible and sometimes biodegradable and acts as a reservoir or a matrix for the drug [3]. In this last case, the medical molecule can be incorporated inside the polymeric matrix through an impregnation route.

One of the main limitations of conventional impregnation process is the presence of residual solvent, generally organic, in the final substrate which can lead to some toxic effects.
The use of organic solvents can be limited or even avoided through the application of supercritical technologies. Other advantages of those technologies result from the specific properties of supercritical fluids such as a low viscosity, a low surface tension as well as a high density and diffusivity higher than liquids, leading to faster and more homogeneous impregnation. Furthermore, when applied to polymers, compressed fluid can act as a swelling and/or plasticizing agent promoting therefore the impregnation process [4], [5].
Two main mechanisms are generally involved and concurrent in supercritical impregnations of polymers **[6**], **[7**].
The first concerns the deposition of the solute into the polymeric matrix during the depressurisation phase. The solubility of the solute in the supercritical phase plays a key role in this mechanism. In fact, the supercritical fluid/solute mixture diffuses inside the existing pores of the polymeric matrix. During depressurization, CO_2 quits the polymeric matrix. A part of the solute is re-precipitated/re-crystallized and therefore trapped within the polymer matrix.
The second mechanism, usually named impregnation with molecular dispersion, is related to the dissolution of the CO2/solute mixture into the polymeric matrix promoting the swelling/plasticization phenomena. During the depressurisation phase, the solute precipitates within the existing pores (deposition phenomenon) as well as within the previously swollen polymer (molecular dispersion phenomenon). The partition of the solute between the polymer and the fluid phase plays a key role in this last phenomenon. It is based on physico-chemical interactions that can take place between the compounds involved in the process. Affinity between the solute and the polymer controls the solubility of the solute in the polymer and favours the partition towards the polymeric matrix whereas affinity between the solute and the supercritical fluid controls the solute solubility in the high pressure phase and favours the partition towards this mobile phase. The dense fluid /polymer interactions influence also the impregnation mechanism, since they control the solubility of the fluid in the polymer, and therefore its swelling/plasticizing effect.

The presence of cosolvents or any additive/substance implies physico-chemical interactions with the polymeric phase as well as with the high pressure fluid and can influence the impregnation phase.
Supercritical impregnation of polymers has been successfully applied for the elaboration of several DDS [8], [9] and recently for ophthalmic applications **[7**], [10-[17]. In the present work, we are studying the supercritical impregnation of commercially available polymeric IOLs with drugs frequently used to prevent cataract postoperative infectious complications; cefuroxime sodium, an antibiotic and dexamethasone, an anti-inflammatory drug. The objective is to combine cataract surgery and postoperative treatment in a single procedure, by inserting an already impregnated IOL during eye surgery. The great challenge of this study is to load the IOL with the drug without modifying its transparency and its optical power.

MATERIALS AND METHODS

1- CHEMICALS

1.1- Impregnation support

The studied impregnation supports are commercially available rigid IOLs currently used for cataract treatments. They are supplied by "the Fred Hollows Intraoculars Lens" (Nepal) and are made from derivative of Poly (Methyl MethAcrylate) (PMMA). Two dioptres were used within this study; +21.0 D and +31.0 D.

1.2- Drug

Two salts were used as active pharmaceutical ingredients (APi); Cefuroxime sodium ($C_{16}H_{15}N_4NaO_8S$), an antibiotic and Dexamethasone 21-phosphate disodium ($C_{22}H_{28}FNa_2O_8P$) an anti-inflammatory drug. They are both supplied by Sigma Aldrich (France). Their skeletal formulae are presented in Figure 1. They are solid under ambient conditions with a molar mass respectively of 446.4 $g.mol^{-1}$ and 516.4 $g.mol^{-1}$.
In the rest of the study, cefuroxime sodium will be designed by cefuroxime and Dexamethasone 21-phosphate disodium by dexamethasone.

a) b)

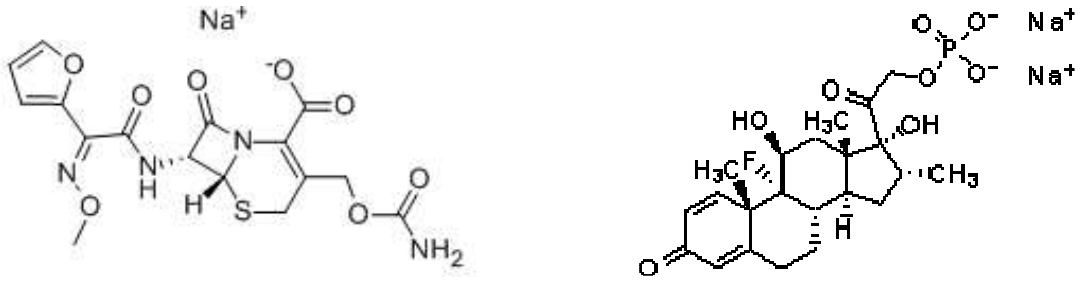


Figure 1: Skeletal formula of a) cefuroxime sodium and b) dexamethasone 21-phosphate disodium

1.3- Solvents

The supercritical fluid used within this study is carbon dioxide (purity > 99.7%). It is supplied by Air Liquide (France).
Ethanol (purity > 99.8%) was used as a cosolvent and was supplied by Carlo Erba (Italy).

2- Experimental set-up

2.1 – Supercritical impregnation

The supercritical impregnation set-up is represented in Figure 2. It is mainly composed of a 125 cm^3 high pressure cell (Top Industrie S. A., France). A detailed description of the discontinuous impregnation process is described elsewhere [16], [17].

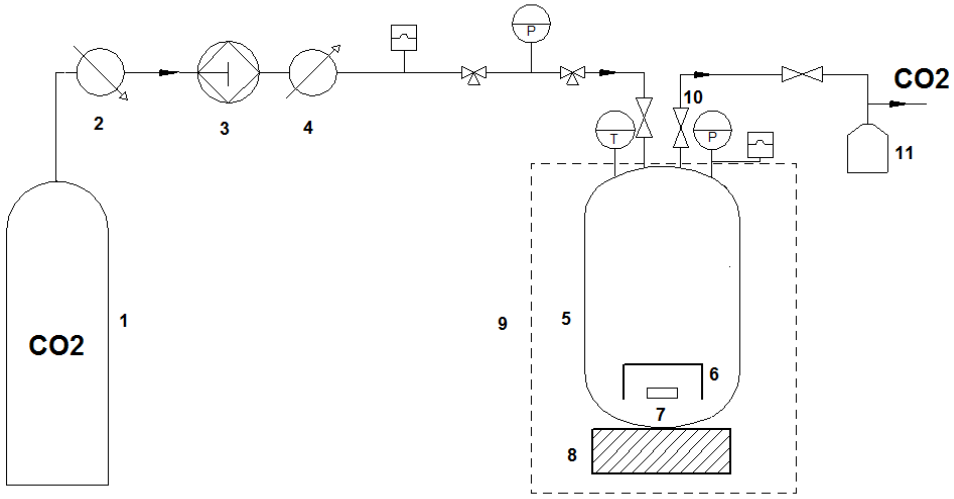


Figure 2 : Supercritical impregnation set-up: (1) CO_2 bottle, (2) Cooling bath, (3) Liquid pump, (4) Heating bath, (5) High pressure cell, (6) Support, (7) Magnetic bar, (8) Magnetic stirrer, (9) Thermostated bath, (10) Depressurisation valve, (11) Solvent trap.

2.2 – Impregnation yield

The impregnation yield (Y_{imp}) is defined as the mass ratio of the impregnated drug in the IOLs and the non impregnated IOLs. The amounts of the impregnated drug were determined gravimetrically by double weighing the IOLs before and after supercritical impregnations (Mettler Toledo AK 160 balance, with a precision of 10^{-4} g). To limit the precision error, the mass of the 5 IOLs was taken into account for each impregnation batch.
The impregnation yield is therefore calculated as follows:

$$Y_{imp}\ (mg_{drug} / mg_{implant}) = \frac{mass\ of\ \ impregnated\ \ IOLs - mass\ of\ \ non\ impregnated\ \ IOLs}{mass\ of\ \ non\ impregnated\ \ IOLs} \qquad (1)$$

For the different experiments and because of the CO_2 desorption from the polymeric matrix at the end of the supercritical impregnation, the mass of the impregnated IOLs showed a regular decrease before levelling off. Thus, the stabilised mass was considered for determining the amounts of impregnated drug as well as the impregnation yields.

RESULTS

The influence of some experimental conditions on the amount of impregnated APi was studied for the two considered drugs. For experiments carried out with cefuroxime, the influence of the pressure (8 and 20 MPa) and the use of a cosolvent (molar fraction of 5 % of

ethanol) as well as that of the depressurisation rate were studied for the dioptre +21.0 D. A more detailed study on this drug is already published [17].

On the base of the obtained results on cefuroxime, and in order to avoid the damaging of the optical properties of the IOLs by the appearance of a foaming phenomenon, slow rate depressurisations were followed on experiments carried out with dexamethasone.
The influence of the variation of the pressure (8 and 20 MPa) as well as that of the use of a cosolvent (5 mol.% of ethanol) were studied for two different dioptres +21.0 D and +30.0 D.
For all the experiments, the impregnation duration was set to 2 hours.

Cefuroxime impregnation

Rapid depressurisations

Studies on cefuroxime were carried out on IOLs with a dipotre of +21.0 D. First, the depressurisation phases were carried out rapidly (in few minutes). The experimental conditions are sumed up in Table 1.

Table 1: Gravimetric impregnation yields of cefuroxime and aspects of IOLS from rapid depressurised batches

Label	P (MPa)	T (K)	Foaming	$Y_{imp} \pm 0.002$ (g_{drug}/g_{IOL})
Rapid depressurisation – Without cosolvent				
Cef_1	8	308	No	0.001
Cef_2			No	0.001
Cef_3	8	333	No	0.004
Cef_4			No	0.003
Cef_5			No	0.003
Cef_6	20	333	Yes	0.029
Rapid depressurisation - With cosolvent				
Cef_7	8	308	Yes	0.059
Cef_8	8	308	Yes	0.057
Cef_9	20	333	Yes	0.063

The reproducibility of the results was verified for one of the more favorable impregnation conditions (8 MPa and 308 K in the presence of cosolvent : Cef_7 and Cef_8) and the result variation was in the range of the error precision.

In rapid depressurisation conditions, it can be observed that at 8 MPa, very low impregnation yields were obtained (≤ 0.004 g_{drug}/g_{IOL}). Increasing the pressure leads to an increase in the impregnation yields (0.029 at 20 MPa and 333 K). Nevertheless, in these conditions a foaming phenomenon was observed (Figure 3). When adding a cosolvent, the impregnation yields vary between 0.057 and 0.063. Once again foamed IOLs were obtained.

The apparition of the foaming phenomenon results from the swelling/plasticising effect of the CO2 on the polymeric matrix coupled with the rapid depressurisations [18]-[20]. Indeed, under supercritical conditions, the sorption of CO_2 in polymers is enhanced leading to their swelling and to the reduction of their glass transition temperature (Tg). A rapid depressurisation to atmospheric pressure results in a CO_2 supersaturation within the polymeric matrix, causing the nucleation and growth of gas bubbles in the polymers.
An increase in pressure or the addition of a cosolvent favours the CO_2 solubilisation in the polymer. Thus, the amount of CO_2 in the polymeric matrix is higher leading to an increase in the supersaturation during the depressurisation phase.

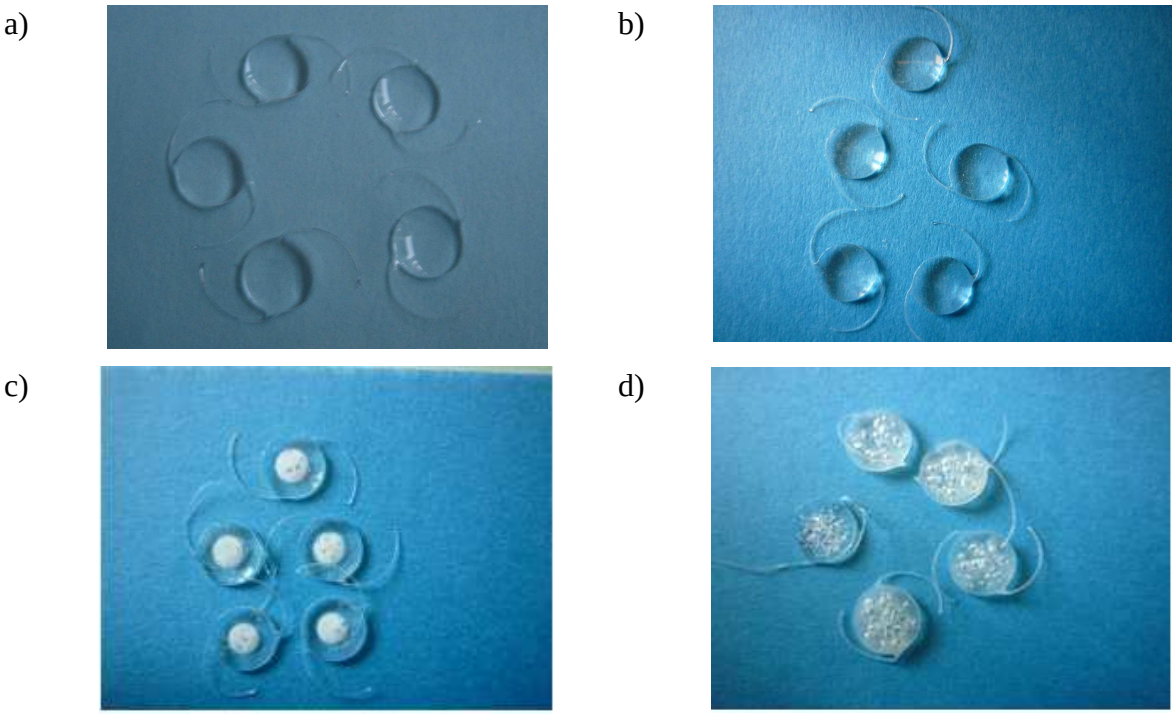


Figure 3 : Aspect of some IOLs a) Non impregnated; b) Impregnated at 8 MPa and 308 K (Cef_2); c) Impregnated at 20 MPa and 333 K (Cef_6) and d) impregnated in presence of ethanol (5%) a) at 8 MPa and 308 K (Cef_7)

A drug release study was performed on some impregnated IOLS in a solution simulating the aqueous humor. The release profiles are illustrated in **Figure 4** and the accumulated released mass are reported in Table 2.

Table 2 : Accumulated released mass (rapid depressurised batches)

Label	P (MPa)	T (K)	Cosolvent	Impregnated mass for 5 IOLs ± 0.1 (mg)*	Release duration (day)	Accumulated released mass from 5 IOLs ± 0.02 (mg)
Cef_1	8	308	No	0.1	36	0.19
Cef_2	8	308	No	0.1	21	0.16
Cef_3	8	333	No	0.4	21	0.3
Cef_7	8	308	Yes	5.7	36	0.38

For all the studied conditions, drug releases exhibit the same profile ; an initial rapid release period due the dissolution of drug located at/or near the surface of the lenses, followed by a second release period corresponding to the diffusion of the drug retained inside the polymeric matrix. A plateau was reached in almost 15 days of release for the different samples.
For IOLs impregnated without foaming phenomena (8 MPa without cosolvent), results confirm the low impregnation yields determined gravimetrically. However, for IOLs impregnated in presence of cosolvent (Cef_7), only 6% of the impregnated drug was released. This low release rate can be explained by a deposition of the drug in the porosity formed during the foaming process. The foamed cells are mostly closed [20], therefore, the impregnated drug can not be released when immersed in the solution simulating the aqueous humor.

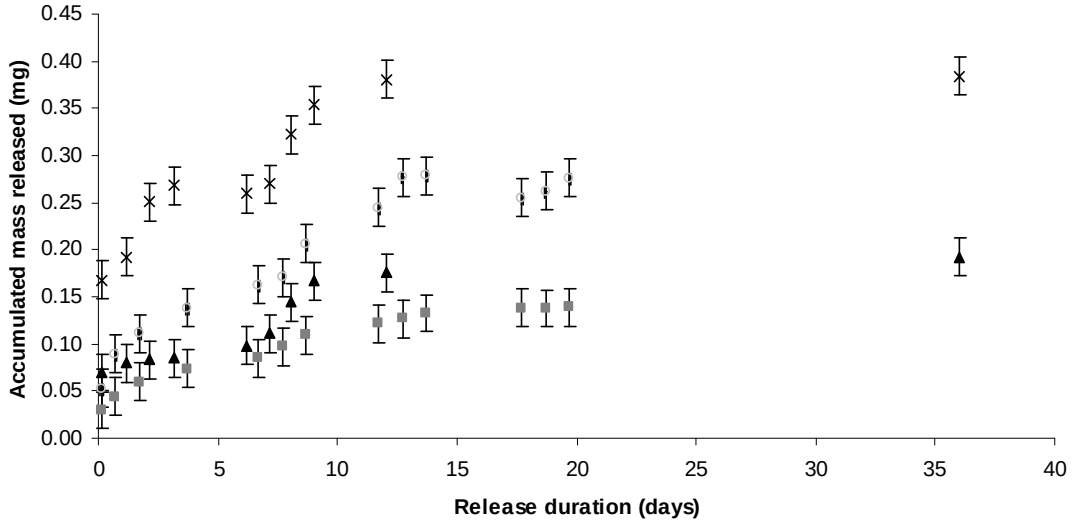


Figure 4 : Accumulated released drug from impregnated IOLs for 2 hours without cosolvent at (▲) 8 MPa and 308 K (Cef_1); (■) 8 MPa and 308 K (Cef_2); (●) 8 MPa and 333 K (Cef_3) and with cosolvent (x) at 8 MPa and 308 K (Cef_7)

Slow depressurisations

In order to avoid the foaming phenomena, the depressurisation steps were carried out slowly while maintaining a fixed rate of 0.2 ± 0.05 MPa/min. The experimental conditions are sumed up in Table 3. All the resulting impregnated IOLs (with or without cosolvent) were free of bubbles. However, the impregnation yields for all the experiments were too much low to be quantified gravimetrically. This result was confirmed by drug release tests. Indeed, even after 15 days of release, spectrophotometric analyses show very low absorbance (in the order of magnitude of the measure precision).

Table 3: Gravimetric impregnation yields of cefuroxime sodium and aspects of IOLS from slow depressurised batches

Label	P (MPa)	T (K)	Cosolvent	Foaming
Cef_10	14	333	No	No
Cef_11	20	333	No	No
Cef_12	8	308	Yes	No

The partition of the cefuroxime between the supercritical fluid (CO_2 or CO_2/ethanol mixture) and the polymeric matrix seems to be favourable towards the high pressure phase. Indeed, when depressurised slowly, a high quantity of drug quits the polymer solubilised in the dense fluid. At higher depressurisation rates, the density of the high pressure fluid and therefore its solvating power decrease rapidly, leading to an instantaneous supersaturation and therefore deposition of the active ingredient inside the polymer. This result also supports the hypothesis of the deposition of the drug inside the porosity formed during the foaming process.
On the base of these results, the mechanism governing the impregnation in our experimental conditions seems to be the APi deposition rather than the molecular dispersion. This phenomenon is enhanced in the conditions of rapid depressurisation, because of a porosity creation within the polymer matrix.

Dexamethazone impregnation

In order to avoid the damaging of the optical properties of the impregnated IOLs, experiments with dexamethasone were carried out while maintaining a fixed depressurisation rate of 0.2 ± 0.05 MPa/min. The influence of the variation of the pressure as well as that of the use of a ethanol as a cosolvent were studied for two dioptres; +21.0 D and +30.0 D. The experimental conditions are sumed up in Table 4. The reproducibility of the results was verified for the dipotre +21.0 D in the most favorable impregnation conditions (8 MPa in the presence of a cosolvent, Dexa_3, Dexa_4 and Dexa_5). The experiment was reproducible with a mean deviation of 0.003 which is close to the precision error of 0.002.

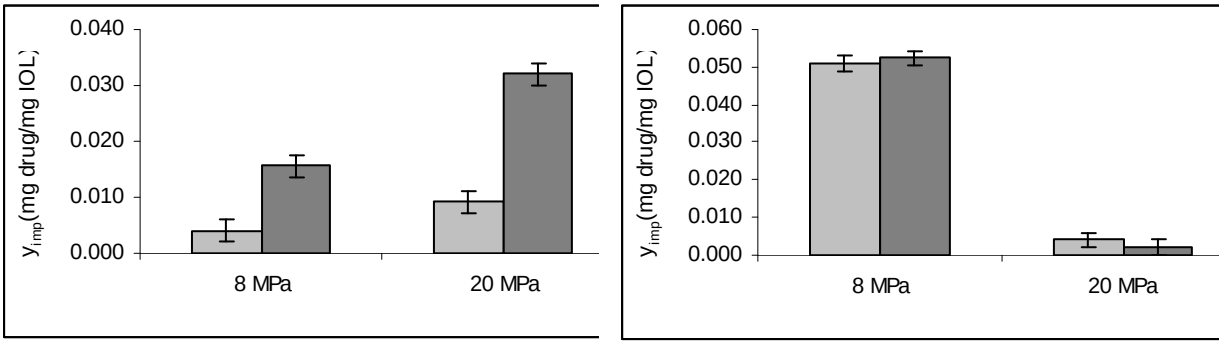
Table 4 : Gravimetric Impregnation yields of Dexamethasone on IOLs

Label	Dipotre	P (MPa)	T (K)	Cosolvent (5% mol.)	$Y_{imp} \pm 0.002$ (g_{drug}/g_{IOL})
Dexa_1	+21.0 D	8	308	NO	0.004
Dexa_2		20	308	NO	0.009
Dexa_3		8	308	Yes	0.049
Dexa_4		8	308	Yes	0.055
Dexa_5		8	308	Yes	0.051
Dexa_6		20	308	Yes	0.004
Dexa_7	+30.0 D	8	308	NO	0.016
Dexa_8		20	308	NO	0.032
Dexa_9		8	308	Yes	0.052
Dexa_10		20	308	Yes	0.002

In all the experiments, the impregnated IOLs were free of bubbles, which confirms that the foaming phenomena is avoided by low depressurisation rates. In comparison with the results obtained with cefuroxime sodium when slow depressurisations were carried out, higher impregnation yields were obtained for the same conditions of slow depressurisation. Indeed, yields varying between 0.002 and 0.055 were obtained and seem to be dependant on the operating conditions.

First, the variation of the dioptre is illustrated in Figure 5. It can be observed from the histograms that in the absence of a cosolvent, increasing the dioptre leads to an increase in the impregnation yield. When a cosolvent is used, the results are almost similar for both dioptres.

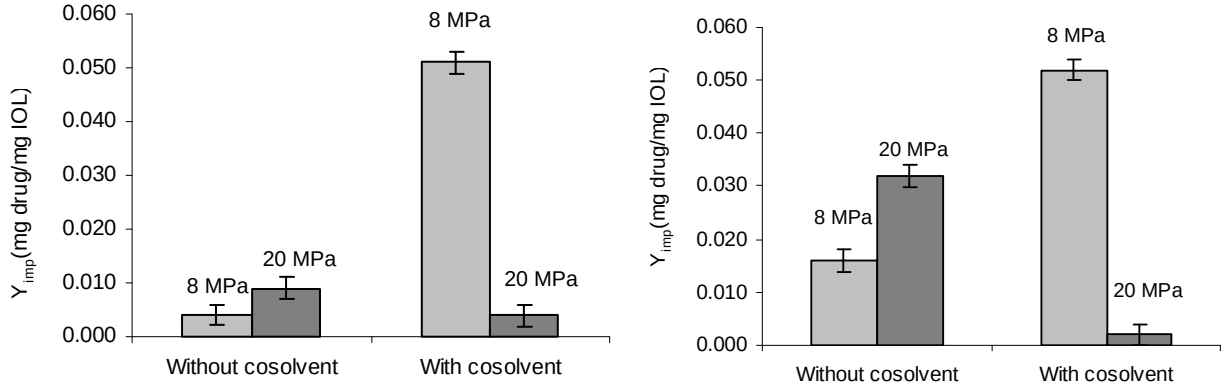
Increasing the dioptre of the IOLs modifies among other parameters their mass (0.0194 g/IOL for the dioptre +21.0 D and 0.0278 g/IOL for the dioptre +30.0 D), their curve radius and their thickness.
One possible explanation of the increase of the impregnation yield with the dioptre, is that the lower the dioptre the higher ratio of the surface by the volume. Therefore, during the depressurisation, higher quantities of drug are dragged with CO2 out of the IOL.



Without cosolvent With cosolvent

Figure 5 : Influence of the dipotre on the impregnation yield (y_{imp}) respectively without cosolvent and with cosolvent (dioptre +21.0 D and +30.0 D)

The influence of the pressure as well as that of adding ethanol as a cosolvent on the impregnation yield is illustrated in Figure 6 and Figure 7. It can be observed from the histograms, that the tendancy is similar for the two dioptres. Indeed, in the absence of a cosolvent, increasing the pressure is favorable to impregnation. However, when a cosolvent is used the pressure increase is rather an unfavorable factor. The influence of the pressure is more pronounced for the dioptre +30.0D.



Dioptre : +21.0 D Dioptre : +30.0 D

Figure 6 : Influence of the pressure on the impregnation yields (Y_{imp}) respectively for the dioptre +21.0 D and +30.0 D (8 MPa and 20 MPa)

Increasing the pressure can favor the impregnation process through two routes. First, higher pressure leads to an increase in the CO2 density and therefore in its solvating power. Furthermore, the swelling/plasticizing effect of the compressed fluid on the polymer is promoted though pressure raise. As a consequence of these two phenomena, higher quantity of drug is carried within the polymeric matrix. During the depressurisation phase, the APi can

be deposited within the polymer and/or molecularly dispersed if the partition is favorable toward the polymer.

When using a cosolvent, the opposite effect of the pressure is obtained. Adding ethanol during the impregnation process has a great influence on the impregnation at 8 MPa. Indeed, the impregnation yield increases from 0.004 to almost 0.051 for the dioptre +21.0 D and from 0.016 to 0.052 for the dioptre +30.0 D. However, at higher pressure (20 MPa), the opposite influence is obtained and the addition of the cosolvent seems to be unfavorable to the impregnation especially for the dioptre +30.0 D. The impregnation yield is reduced from 0.009 to 0.004 for the dipotre +21.0 D and from 0.032 to 0.002 for the dipotre +30.0 D

The addition of a cosolvent enhances the polarity of the compressed fluid and therefore its solvating power. Furthermore, the swelling/plasticizing effect of the high pressure phase is also promoted. Once again, adding a cosolvent should increase the quantity of drug carried by the high pressure phase within the polymeric matrix. Such an effect seems to be favorable for impregnation at moderate pressures but unfavorable at higher ones. Taken into account these observations, it can be considered that by combining the effect of the high pressure and the use of a cosolvent, drug molecules interact more favorably and have a relatively higher affinity with the compressed fluid than with the polymer. The partition is then more favorable towards the high pressure phase and the drug leaves the polymeric matrix during the depressurisation phase. In the absence of a cosolvent and/or at lower pressure (in the presence of ethanol), the drug seems therefore to have some affinity with the polymeric matrix which could explain the impregnation yields obtained.

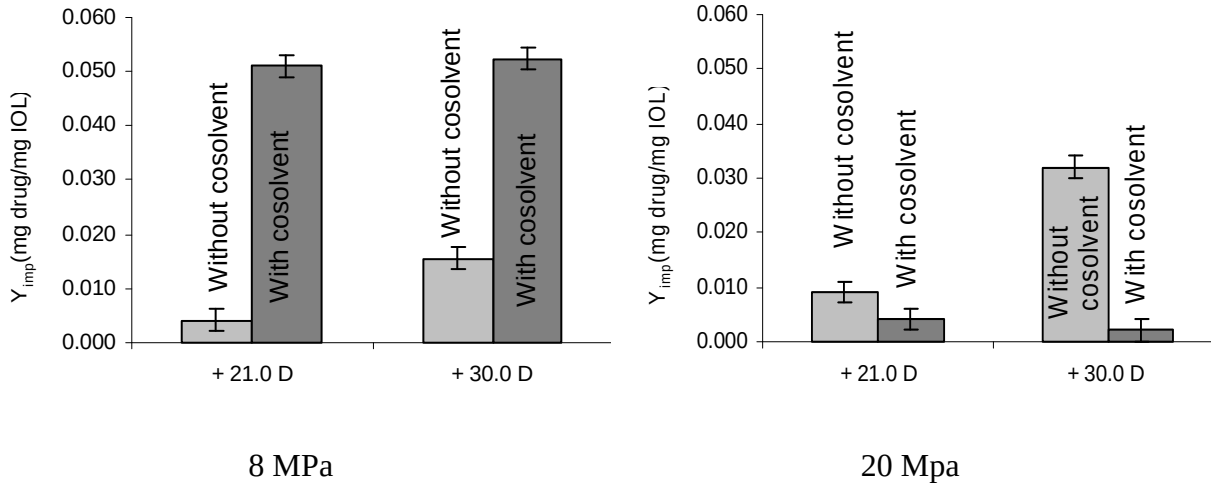


Figure 7 : Influence of the use of a cosolvent on the impregnation Yields (y_{imp}) respectively for the pressure of 8 MPa and 20 MPa (without cosolvent and with cosolvent)

CONCLUSION

This work aims to impregnate intraocular lenses with drug components in order to combine cataract surgery and postoperative treatment in a single procedure. Two drugs usually used to prevent cataract postoperative infectious complications were tested, cefuroxime sodium, an antibiotic and Dexamethasone 21-phosphate disodium, an anti-inflammatory drug.

Supercritical impregnations were carried out through a batch process, composed of two main steps; a high pressure impregnation step, the duration of which was fixed to 2 hours, followed by a depressurisation step.
First studies on cefuroxime sodium were carried out with rapid depressurisations (few minutes). At 8 MPa and in the absence of a cosolvent, transparent IOLs presenting low impregnation yields were obtained (≤ 0.004 g_{drug}/g_{IOL}).
Increasing the pressure or adding a cosolvent enhance significantly the impregnation yield to lead respectively to yields up to 0.029 g_{drug}/g_{IOL} and 0.063 g_{drug}/g_{IOL}). However, the optical properties of the IOLs were damaged by the apparition of a foaming phenomenon resulting from the combined effect of the swelling and plasticising effect of the supercritical fluid and rapid depressurisation rates.

In vitro drug release studies carried on some IOLs confirms the very low impregnated quantity at low pressures. In the presence of cosolvent, the cumulative mass released reaches a plateau in almost 15 days, and only 6.6% of the impregnated drug was released. Such a few released rate can be explained by a deposition of the drug within the formed pores during rapid depressurisation (mostly closed).
By carrying out slow depressurisations (0.2 MPa/min), the foaming phenomena was avoided. However, the impregnation yields were very low to be quantified (either gravimetrically or through release analysis).

On the base of these results, it was estimated that for cefuroxime sodium and in our experimental conditions, the partition of the drug between the supercritical fluid (CO_2 or CO_2/ethanol mixture) and the polymeric matrix is favourable towards the high pressure phase. Therefore, impregnation yields obtained at rapid depressurisation result essentially from the deposition phenomena.

For experiments carried out with dexamethasone, the depressurisation rate was fixed to 0.2 MPa/min. Transparent IOLs were obtained. In the absence of a cosolvent, increasing the pressure promotes the impregnation yield. When a cosolvent is used, impregnation was further promoted at 8MPa. However, by coupling high pressure and the use of a cosolvent, very low impregnation yields were obtained. In the absence of a cosolvent or at low pressure with the cosolvent, the partition seems to be favourable towards the polymeric matrix. At high pressure and in the presence of cosolvent, the affinity of the drug with the compressed fluid is promoted and the partition is more favourable towards the high pressure phase.

Results obtained with dexamethasone drug are encouraging when compared to those of Cefuroxime since transparent IOLs presenting an effective impregnation are obtained. Nevertheless, drug release studies should be carried out on the impregnated IOLs to confirm the hypothesis of molecular dispersion inside the polymer.

Experiments of impregnation are currently carried out with the two drugs on foldable IOLs since they are the most used nowadays for cataract surgery.

REFERENCES :

[1] ANDERSON, E. M., NOBLE, M. L., GARTY, S., MA, H., BRYERS, J. D., SHEN, T. T., RATNER, B. D., Biomaterials, Vol. 30, **2009**, p. 5675

[2] BARRY, P., SEAL, D.V., GETTINBY, G., LEES, F., PETERSON, M. REVIE, C.W., Journal of Cataract & Refractive Surgery, Vol. 32, **2006**, p. 407.

[3] ÜZER, S., AKMAN, U., HORTAÇSU, Ö., The Journal of Supercritical Fluids, Vol. 38, **2006**, p. 119.

[4] KAZARIAN, S.G., VINCENT, M.F., WEST, B.L., ECKERT, C.A., J. Supercritical Fluids, Vol. 13, **1998**, p. 107.
[5] KAZARIAN, S.G., BRANTLEY, N.H., WEST, B.L., VINCENT, M.F., ECKERT, C.A., Applied Spectroscopy, Vol. 51, **1997**, p. 491.
[6] KIKIC, I., VECCHIONE, F., Current Opinion in Solid State and Materials Science, Vol. 7, **2003,** p. 399.
[7] COSTA, V.P., BRAGA, M.E.M., GUERRA, J.P., DUARTE, A.R.C., DUARTE, C.M.M., LEITE, E.O.B., GIL, M.H., DE SOUSA, H., J. Supercritical Fluids, Vol. 52, **2010**, p. 306.
[8] KIKIC, I., VECCHIONE, F., Current Opinion in Solid State and Materials Science, Vol. 7, **2003,** p. 399.
[9] GUNEY, O., AKGERMAN, A., AIChE Journal, Vol. 48, **2002,** p. 856.
[10] DUARTE, A. R. C., MARTINS, C., COIMBRA, P., GIL, M. H.M., DE SOUSA, H. C., DUARTE; C.M.M., J. Supercritical Fluids, Vol. 38, **2006**, p. 392.
[11] DUARTE, A. R. C., SIMPLICIO, A. L., VEGA-GONZALEZ, A., SUBRA-PATERNAULT, P. COIMBRA, P., GIL, M.H., DE SOUSA, H. C., DUARTE, C.M.M., J. Supercritical Fluids, Vol. 42, **2007,** p. 373.
[12] NATU, M. V., GIL, M.H., DE SOUSA, H. C., J. Supercritical Fluids, Vol. 47, **2008**, p. 93.
[13] BRAGA, M.E.M., VAZ PATO, M.T., COSTA SILVA, H.S.R., FERREIRA, E.I., GIL, M.H., DUARTE, C.M.M., DE SOUSA, H.C., J. Supercritical Fluids, Vol. 44, **2008,** p. 245.
[14] COSTA, V.P., BRAGA, M.E.M., DUARTE, C.M.M., ALVAREZ-LORENZO, C., CONCHEIRO, A., GIL, M.H., DE SOUSA, H. C., J. Supercritical Fluids, Vol. 53, **2010,** p. 165.
[15] YAÑEZ, F., MARTIKAINEN, L., BRAGA, M.E.M., ALVAREZ-LORENZO, C., CONCHEIRO, A., DUARTE, C.M.M., GIL, M.H., DE SOUSA, H. C., Acta Biomateriala, Vol. 7, **2011,** p. 1019.
[16] MASMOUDI, Y., BEN AZZOUK, L., FORZANO, O., ANDRE, J-M., BADENS, E., Proceedings of the 9th Conference on Supercritical Fluids and their Applications, Sorrento, Italy, September 5-9, 2010.
[17] MASMOUDI, Y., BEN AZZOUK, L., FORZANO, O., ANDRE, J-M., BADENS, E., The Journal of Supercritical Fluids, accepted.
[18] GOEL, S.K., BECKMAN, E.J., Polymer Engineering and Science, Vol. 34, **1994**, p.1137.
[19] GOEL, S.K., BECKMAN, E.J., Polymer Engineering and Science, Vol. 34, **1994**, p. 1148.
[20] REVERCHON, E., CARDEA, S., The Journal of Supercritical Fluids, Vol. 40 (2007) 144–152.