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Review

Supercritical solvent impregnation of ophthalmic drugs on chitosan derivatives

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Abstract

In this work, three chitosan derivatives (*N*-carboxymethyl chitosan (CMC), *N*-carboxybutyl chitosan (CBC) and *N*-succinyl chitosan (SCC)) were impregnated with flurbiprofen (an anti-inflammatory drug) and timolol maleate (an anti-glaucoma drug), using a supercritical solvent impregnation (SSI) technique (and employing high pressure CO_2 and CO_2 + EtOH mixtures) in order to develop hydrogel-type ophthalmic drug delivery applications. Impregnation experiments were carried out from 9.0 up to 14.0 MPa, and at 303.0, 313.0 and 323.0 K. The resulting polymeric drug delivery systems, as well as other polymeric samples processed in CO_2, were characterized by FTIR spectroscopy and scanning electron microscopy (SEM). Drug release kinetics studies were performed for all prepared systems. The effects of impregnation pressure and temperature on the release kinetics results were studied and compared to the traditional soaking impregnation method. For the same operational conditions, results confirmed that the three different (chemically and physically) polymeric structures conditioned the impregnation and the drug release processes. Despite the final released drug mass is always the result of the employed operational impregnation conditions and of the very complex relative specific interactions that may occur between all species present in the system (drugs, polymers, CO_2 and ethanol), results showed that, for *N*-carboxymethyl chitosan, the predominant effects in the impregnation process seemed to be the solubility of drugs in CO_2 and in CO_2 + EtOH mixtures, as well as the swelling and plasticizing effect of CO_2 and ethanol on the polymer. Finally, the SSI method proved to be a more efficient and "tunable" impregnation process than the traditional impregnation of drugs by a soaking method. Therefore, and using this "tunable" SSI method, these *N*-chitosan derivatives-based ophthalmic drug delivery systems can be easily and efficiently prepared taking in consideration the desired drug levels according to patients needs.

Keywords: *N*-chitosan derivatives; Ophthalmic drug delivery systems; Supercritical solvent impregnation (SSI); Flurbiprofen; Timolol maleate

Contents

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1. Introduction

Some ocular diseases are very common in the ageing population namely cataracts, glaucoma, diabetic retinopathy and age-related macular degeneration (AMD) [1]. Commonly, medicines are applied on the eye surface in order to treat eye's outer surface diseases like infections or to provide intraocular treatment through the cornea as glaucoma or AMD. However, typical topical current therapeutic procedures and formulations present several limitations, like poor drug bioavailability and the possibility of occurrence of systemic toxicity [2].

Poor ocular drug bioavailability is owed essentially to the relative impermeability of the corneal membrane, to blinking and tear dynamics, and to nasolacrimal drainage. Generally, the delivery of ocular drugs to the eye posterior segment is reduced by the same factors that are also responsible for poor ocular bioavailability and also by the blood-retinal barrier which limits the intravenous route in posterior drug delivery [2–4]. Poor drug absorption across mucosal membranes is due essentially to the drug/membrane relative hydrophobic/hydrophilic natures. Although, some ophthalmic drugs may present an important therapeutic value for a specific pathology, these relative and different natures may reduce or even impede drug membrane permeability and, consequently, their therapeutic value is lost [5,6]. Moreover, tear dynamics and nasolacrimal duct drainage is also the major way of entry into the circulatory system of potent ocular drugs applied by topical administration. This may cause undesired and toxic systemic side-effects [7–9].

Pulse-drug delivery is a recurrent but undesired pharmacokinetic characteristic associated with topical eye drops drug formulations. Normally, in these systems, only less than 1–4% of the instilled dose reaches the aqueous humor. As a consequence, and despite the fact of causing a potential risk of systemic toxicity, clinicians are forced to recommend frequent drug dosing, at high concentrations, in order to avoid the poor drug bioavailability at the posterior segment of the eye [10,11]. Nevertheless, and despite their inherent disadvantages and associated risks, these conventional dosage forms account for almost 90% of the commercially available therapeutic ophthalmic products mostly because of their favorable costs, simplicity of formulation, self-application and good acceptance by patients.

To overcome these problems, and in recent years, a considerable effort has been made in order to develop capable ophthalmic controlled drug release systems (CDR's), focusing essentially on two main objectives: (i) to find or make newer, more effective and safer drug molecules for the various ocular conditions and diseases and (ii) to improve the already existing ocular dosage forms and to exploit newer and more efficient delivery systems in order to improve ocular bioavailability of already existing drug molecules. Actual trends in ocular therapeutics and ophthalmic drug delivery indicates that the existing dosage forms may and probably will be substituted by new CDR's, which can offer improved biopharmaceutical properties, with a better capability to deliver therapeutic agents to the targeted receptors in the eye, and in a more predictable and reproducible way [12].

Presently, there are several proposed ophthalmic CDR's designed to treat most of the common diseases of the eye, some of them already are commercially available, like: enhanced topical administration of water-soluble eye drops; water-insoluble drugs in ointments; polymeric hydrogels; in situ activated gelling systems; mucoadhesive and bioadhesive hydrogels; colloidal systems, penetration enhancers; collagen shields; prodrugs and ocular inserts [2–4,7].

Hydrogels are highly swollen hydrophilic polymer networks which increase their volumes by absorbing large amounts of water or aqueous solutions [13]. Hydrogel formulations usually prolong the residence time of drugs at the absorption site, maintaining drug release and improving drug bioavailability for convenient periods [14]. Different ophthalmic hydrogel formulations like, for example ophthacoil (a drug-loaded adherent hydrogel coating on a thin metallic wire), were developed and are commercially available as an alternative to topical eye drop administration for sustained drug delivery [15,16]. Other ophthalmic hydrogel-based devices like therapeutic contact lenses (TCL), intraocular lenses, glaucoma filtration implants, keratoprostheses, intracorneal implants, scleral buckles and viscoelastic replacement agents are also commonly used in ophthalmology [2,7,12,13,17–19]. These hydrogel devices may be composed by synthetic (or natural) biocompatible and/or biodegradable polymers, copolymers, polymeric blends or also by polymeric composites.

Chitosan-based and chitosan derivatives-based hydrogels already showed a great potential to be used as CDR's for several pharmaceutical and biomedical applications [2,13,14,20,21] including ophthalmic CDR's. Chitosan is an abundant and natural polysaccharide which presents some interesting and useful properties such as biocompatibility, biodegradability, non-toxicity, adsorption and adhesion properties and the ability to interact with different substances (like hydrophilic and hydrophobic drugs) [13,22,23]. These characteristics are extremely important for medical and pharmaceutical applications, namely for the development controlled drug release devices [13,24,25]. For ocular applications, it is also recognized that chitosan-based formulations present a prolonged precorneal residence time, an ability to increase solution viscosity and excellent mucoadhesive properties [26,27].

Most of the chitosan functional biological properties are related to molecular weight, charge density and distribution, degree of deacetylation and to the pH value of the media in which they can be placed into. Consequently, these factors can restrain some chitosan applications as drug delivery systems [13,14]. For example, chitosan have limited solubility at pH above 6 (the physiological pH of the buccal cavity) and some incompatibility with anionic drugs [28].

As a result, usually there is a practical necessity to develop several chitosan derivatives with improved solubility/non-solubility, at different pH values, or, for example, to obtain a better permeability to anionic drugs and to avoid the undesired formation of drug-polymer complexes [28,29]. This can be done by introducing some specific chemical modifications into the chitosan chain molecule. These "tailored" modifications may change the polymeric solubility/non-solubility and biodegradability/biocompatibility nature, the polymeric ability to interact with drugs or with other biomolecules as enzymes, for example, and the kinetics of drug release for any new or particular envisaged controlled drug delivery application. For example, Sandri et al. [28], studied different chitosan derivatives as acyclovir penetration enhancers adding the drug to several modified chitosans: 5-methyl-pyrrolidinone chitosan (MPC), low molecular weight chitosans, a partially reacetylated chitosan and chitosan hydrochloride. In this study, MPC presented the best mucoadhesive and penetration/permeation enhancement properties. Thanou et al. [29] studied, the use of high viscosity chitosan (HCS) and low viscosity (LCS) chitosan as well as mono-*N*-carboxymethyl chitosan (MCC) with low and high grade viscosities (LMCC and HMCC). On other work, the permeation and adsorption of low molecular weight heparin (LMWH) was increased using *N*-carboxymethylated chitosan via intestinal epithelia [30]. Other chitosan derivative, trimethyl chitosan chloride (TMC), showed an increased peptide intestinal absorption and bioavailability for buserelin and octreotide analogs across intestinal epithelia [30]. In addition to *N*-carboxymethyl chitosan (CMC), other *N*-carboxyalkyl chitosans like *N*-carboxybutyl chitosan (CBC) and succinyl chitosan (SCC) (Fig. 1) had been studied as polymeric drug carriers [31–33].

Polymeric dispersed drug CDR's are usually prepared by two conventional methods. A first method consists in mixing the drug, or drugs, in the polymer synthesis reactive mixture, containing monomers, co-monomers, cross-linkers, initiators, etc. Drugs can be soluble or insoluble in this mixture. Then, the resulting mixture is polymerized, for example, by a thermal or photochemical route. A second method consists in immersing and soaking the polymeric particles/articles, previously synthesized, into a solution, or dispersion, containing the drugs to impregnate/infuse. Usually an aqueous solution, or dispersion, is used but organic solutions or dispersions can also be employed, depending essentially on drug and polymer solubility or on other considerations like, for example, solvent toxicity and solvent facility to be removed/evaporated [34–39]. However, these methods present several disadvantages, like the use of sometimes toxic organic solvents, which have to be removed by heating, undesired drug reactions, drug and polymer photochemical and thermal degradation, low incorporation yields and heterogeneous drug dispersion.

Drugs may also be impregnated by dissolving them in compressed high volatile fluids like supercritical carbon dioxide, at temperatures and pressures near or above their critical temperatures and pressures, and contacting the resulting mixture with the polymeric matrixes to be infused. In these conditions, the compressed fluid can act also as a swelling and plasticizer agent for polymers, helping drugs' diffusion into them. This happens because, and despite the usually low polymer solubility in CO_2, the sorption and solubility of CO_2 in some polymers is considerably high, promoting polymer swelling and changing polymeric mechanical properties by a plasticization effect [40–42].

This recent technique, presents several advantages for the development of drug impregnated polymeric materials which can be used as drug delivery systems for many biomedical applications. It is called supercritical solvent impregnation (SSI) [40–43] and permits the drug impregnation of a great part of polymeric matrixes. Furthermore, some interesting hydrophobic drugs, which can not be impregnated by aqueous solution/suspension soaking, can be incorporated by this method. Drug loading and drug depth penetration can be considered as a "tunable" process since it can be performed by controlling the depressurization step (which can be performed at several rates), the time of impregnation or by changing the solvent density (and consequently the drug solubility in it) by pressure and temperature control, in opposition of conventional impregnation processes [40–42]. Furthermore, and in most cases, it can be carried properly in order to not alter and/or damage polymeric materials physical, chemical, and mechanical properties and without degrading drugs, additives and polymers. It also may permit to have previously prepared polymeric matrixes and, later, impregnate them with the desired drugs,

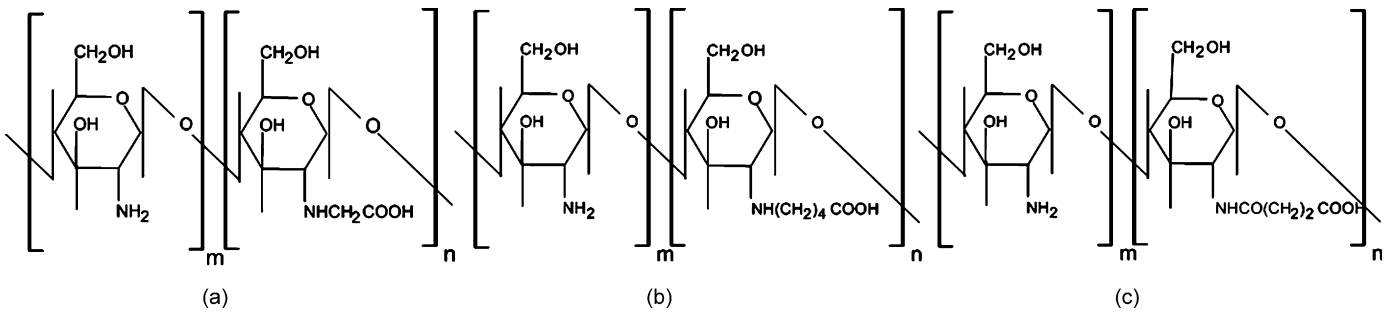
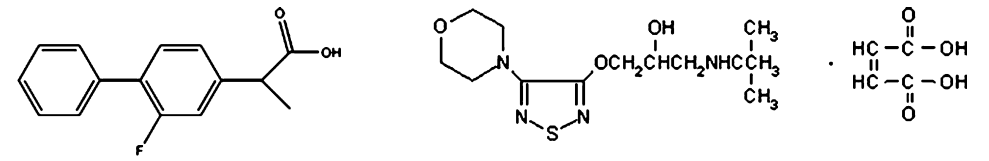


Fig. 1. Structural formula of employed chitosan derivatives: (a) *N*-carboxymethyl chitosan; (b) *N*-carboxybutyl chitosan and (c) *N*-succinyl chitosan.



Flurbiprofen

Molecular Formula: $C_{15}H_{13}FO_2$

Molecular Weight: 244.261 gm ol^{-1}

Timolol Maleate

Molecular Formula: $C_{17}H_{28}N_4O_7S$

Molecular Weight: 432.493 gm ol^{-1}

Fig. 2. Structural formula of impregnated drugs.

according to desired applications and patients' needs and leaving no harmful solvent residues.

Several ophthalmic drugs were already incorporated into polymeric matrixes with the intention of preparing ophthalmic CDR's systems and are reported in the literature: for example, cortisone and retinol [44], ibuprofen [45], pilocarpine [46] and indomethacin [47]. Some systems were already prepared using the SSI technique and using CO_2 as the high pressure solvent [48,45,49]. Some ophthalmic drugs as timolol maleate and flurbiprofen, a beta adrenergic blocker, and, a non-steroidal analgesic/anti-inflammatory, respectively (Fig. 2) had also been used as active principles in several polymeric-based (acrylates and chitosan/carbopol mixtures, respectively) ocular CDR's prepared by reverse phase evaporation [50] and by quasi-emulsion solvent diffusion technique [51].

The solubility of these two solid drugs was already measured in carbon dioxide. The maximum solubility for flurbiprofen was found to be 0.90 g/L, at 323 K and 234 bar [52]. For timolol maleate the maximum CO_2 solubility was determined as 0.56 g/L [53]. The minimum amount of flurbiprofen to be kept in the eye must be, at least, 0.03–0.15 μg, the therapeutic limit in order to prevent and treat inflammatory processes. Its chronic toxicity is 5 mg/(kg day) for 26 weeks [54], based on data obtained at clinical essays in baboons. For timolol maleate, the maximum recommended therapeutic dose (MRTD) is 1 mg/(kg-bw day) [55].

The main objectives of this work were to study the impregnation of two ophthalmic drugs (flurbiprofen and timolol maleate) into three chitosan derivatives: *N*-carboxymethyl chitosan (CMC), *N*-carboxybutyl chitosan (CBC) and *N*-succinyl chitosan (SCC), using the supercritical solvent impregnation (SSI) methodology employing high pressure CO_2 and CO_2 + EtOH mixtures, in order to prepare hydrogel-type drug delivery systems for ophthalmic applications. Other goals were to study the pressure and temperature effects on the resulting CDR's systems and to compare the employed SSI method with the conventional polymer soaking method.

2. Materials and methods

2.1. Chemicals

The three chitosan derivatives used in this work: *N*-carboxymethyl chitosan (CMC), *N*-carboxybutyl chitosan (CBC) and *N*-succinylchitosan (SCC) were prepared according to Silva [56], Fig. 1. Employed ophthalmic drugs were: flurbiprofen (97%, Sigma–Aldrich, CAS [5104-49-4], Germany) and timolol maleate (≥98%, Sigma–Aldrich, CAS [26921-17-5], Germany), Fig. 2. Employed solvents were: carbon dioxide (99.998%, Praxair, Spain) and ethanol (99.8%, Riedel-de-Haën). Physiological serum (sodium chloride isotonic solution, pH 6, 154 mEq for Na+ and Cl−, Osm ∼285 mOsm/kg, Fresenius Kabi, Portugal) was used as drug release media.

2.2. Impregnation methods

The employed supercritical impregnation apparatus is described by Patent EP 1 611 877 A1 (Unit I), and it is presented at Fig. 3 [57]. This unit is comprised by a compressed air-operated CO_2 liquid pump, a visual stainless steel impregnation cell (with approximately 10 cm^3 of internal volume), equipped with sapphire windows, a thermostatic controlled water bath and a magnetic stirring plate as an auxiliary tool to homogenize the high pressure mixture (drug + CO_2 or drug + CO_2 + cosolvent). Ethanol (EtOH), 5%, mol, was used as the cosolvent and with the intention of increase timolol maleate's solubility in supercritical carbon dioxide [53]. Several supercritical solvent impregnation (SSI) experiments were performed in order to study the effects of operational temperature at ∼303, 313 and 323 K and pressure from ∼9 up to 14 MPa) for the systems CMC + flurbiprofen and CMC + timolol maleate.

Average depressurization average rates were 0.29 ± 0.01 MPa/s, for polymer + flurbiprofen systems, and 0.29 ± 0.02 MPa/s for polymer + timolol maleate systems. Higher

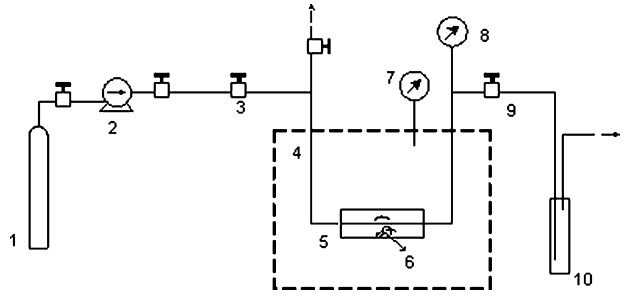


Fig. 3. Schematic diagram of the experimental supercritical impregnation apparatus: (1) CO_2 reservoir; (2) high pressure CO_2 pump; (3, 9) valves; (4) water bath; (5) high pressure stainless steel impregnation cell; (6) magnetic stirrer; (7, 8) thermometer and pressure transducer and (10) glass trap.

depressurization rates may cause the "explosion" of polymeric samples and, consequently, may destroy their structures.

The SSI method consists in introduce the CO_2 into the sealed high pressure impregnation cell, previously charged with the polymeric sample (around 0.004–0.015 g of polymer), the drug (and the cosolvent, when used), at the pre-established process temperature and pressure. Magnetic stirring was always employed in order to solubilize and homogenize the drug in the compressed fluid (CO_2 or mixture of CO_2 + EtOH). The amount of drug was established taking in consideration its solubility (saturated environment) at the operational conditions. The amount of added cosolvent was also calculated taking into consideration the desired cosolvent concentration and the operational temperature and pressure conditions. SSI experiments were carried out during 1 h. After this period, the compressed fluid, or the mixture of compressed fluid and cosolvent, was removed by slow expansion in order not to alter or damage the polymeric samples. Impregnated samples were then recovered in a dry final state.

Conventional drug soaking experiments were performed in a glass vial, containing the polymeric sample to be impregnated, during 1 h, in saturated drug + physiological serum solutions and at ambient conditions (0.1 MPa, 294 K).

2.3. Impregnated drug amounts

The amounts of impregnated drugs were quantified gravimetrically, before and after impregnation experiments, and compared to the results obtained by the drug release kinetics spectrophotometric method (to be described further on). The polymeric flurbiprofen and timolol maleate residual contents (after drug release experiments) were quantified by fluorine and sulphur, respectively, analysis using Ion Chromatography (Waters, 431 model, USA) with conductivity detector (Millipore, USA), IC-Pak A anions column and borate/gluconate as eluent, at 2.93 MPa, conductivity of 319 μS and flow rate of 1 mL/min.

2.4. In vitro drug release kinetics

Drug release kinetics studies were performed for all prepared systems using a spectrophotometer (Jasco, models 530 and 550, Japan), at 247 and 298 nm, for the release of flurbiprofen and timolol maleate, respectively. Release experiments were carried out during 8 h, in order to simulate the envisaged ophthalmic use.

Impregnated polymers were immersed in physiological serum, at 310 K, and an aliquot (1.6 mL) of the released drug solution was removed and analyzed, with or without renovation of the release medium with fresh serum. The release medium renovation was done, for each experimental release point, by removing an aliquot (1.6 mL) for UV analysis and by adding the same volume of fresh serum to the release medium. Drug concentration was calculated using previously determined drug calibration curves [57–59].

The operational central point of experiments was considered to be 11 MPa and 313 K (obtained for CMC + timolol maleate experiments) and, at these conditions, triplicate assays were performed in order to obtain the experiments standard deviation (±0.012 mg of drug/mg of polymer).

2.5. Degradation experiments

Non-impregnated CMC samples were also tested in terms of their ability to degrade over time in the physiological serum solution. CMC samples were pressurized, for 1 h, with supercritical CO_2 at 12 MPa and 313 K. These samples were then immersed and maintained in physiological serum for 168 h (one week). After this period, the physiological serum was analyzed spectrophotometrically (at 200–800 nm) in order to detect the presence of substances generated from polymer degradation and/or solubilization.

2.6. Estimation of diffusion kinetics coefficients

Drug release kinetics coefficients were estimated from the flurbiprofen/polymers drug release kinetics studies. Curves were fitted using Eq. (1), where M_t and M_∞ represent the amounts of flurbiprofen released at time t and after infinite time, respectively, n is the diffusion exponent and k the diffusion kinetic constant [60,61].

$$\frac{M_t}{M_\infty} = k.t^n \tag{1}$$

2.7. Sample characterization

Obtained controlled drug delivery systems were chemically and optically characterized by FTIR-ATR spectroscopy (Magma-IR Spectrometer 750, Nicolet Instrument Corp., Wisconsin, USA; ATR, Golden Gate MK II, Specac, USA) DTGS KBr detector, at 32 scans and 4 cm^{-1} of resolution) and by Scanning Electron Microscopy (SEM) (Jeol, JSM-5310 model, Japan), at 25 kV. Samples were coated with gold, approximately 300 Å, in an argon atmosphere, and analyzed before and after the polymer impregnation experiments.

3. Results and discussions

The drug release triplicate assays, which were performed at an operational central point (11 MPa and 313 K) for the timolol maleate + CMC system, presented *in vitro* drug release average values with a curve amplitude of 3% (in mass) until the second experimental point. After this point, curve amplitude was almost constant, 1–2% (in mass). These amplitude values were considered to be valid for all studied systems (Table 1).

Fig. 4 shows the accumulated flurbiprofen mass release profiles for the three employed chitosan derivatives (CMC, CBC and SCC), impregnated for 1 h at 12 MPa and 313 K by SSI. As shown, and after 8 h of release studies, the accumulated released amount of flurbiprofen was found to be higher for CMC, followed by SCC and CBC (0.125, 0.081 and 0.053 mg flurbiprofen/mg polymer, respectively). These *in vitro* release studies were performed without the renovation of the releasing

Table 1
Accumulated drug release results in physiological serum, obtained after 8 h of drug release from SSI and soaking flurbiprofen and timolol maleate impregnated chitosan derivatives (CMC, CBC and SCC). For Flurbiprofen, drug release was performed without physiological serum renovation. For timolol maleate, drug release was performed with physiological serum renovation

Impregnation process	Timolol maleate (5%) (mg/mg polymer)						
	∼0.1 (MPa)	9 (MPa)	10 (MPa)	11 (MPa)	12 (MPa)	13 (MPa)	14 (MPa)
CMC polymer							
SSI (30 °C)	–	0.443	0.541	0.587	0.528	0.478	0.449
SSI (40 °C)	–	0.394	0.462	0.531 ± 0.012	0.550	0.800	0.849
SSI (50 °C)	–	0.086	0.203	0.461	0.590	0.527	0.445
Soaking	0.674	–	–	–	–	–	–

Impregnation process	Flurbiprofen (mg/mg polymer)								
	CMC polymers							CBC polymers 12 (MPa)	SCC polymers 12 (MPa)
	∼0.1(MPa)	9 (MPa)	10 (MPa)	11 (MPa)	12 (MPa)	13 (MPa)	14 (MPa)		
SSI (30 °C)	–	0.080	–	–	–	–	–	–	–
SSI (40 °C	–	0.035	0.035	0.097	0.125	0.147	–	0.053	0.081
SSI (50 °C)	–	0.017	0.043	0.112	0.034	0.079	–		
Soaking	0.074	–	–	–	–	–	–	–	–

media (without the addition of a "fresh" volume of physiological serum). All impregnated samples presented almost the same drug release profile, a typical biphasic release pattern: (i) a diffusional period with an initial increase probably as a result of the rapid release caused by drugs deposited on and near the polymeric surface and which is related to the total surface of polymers and/or to its high porosity; (ii) a swelling phase, a period that occurs before the disintegration/dissolution of the polymers (which was not observed, because the degradation/solubilization tests indicated that the physiological serum solution did not degrade or solubilize the employed polymers after 168 h of contact, at ambient conditions). However, while flurbiprofen release from CBC reached the equilibrium at approximately 30 min; for CMC and SCC around 2.5 h were required to reach equilibrium.

The observed initial burst release profiles can be an indication that the impregnated drug was mainly located at the polymer surface or near it. And the different initial profiles obtained would then be the result of the different impregnation efficiencies due to the different interactions between CO_2, drugs and the three employed chitosan derivatives. Short impregnation periods can also be responsible for low depth impregnation but the impregnation duration was kept constant for these experiments. However,

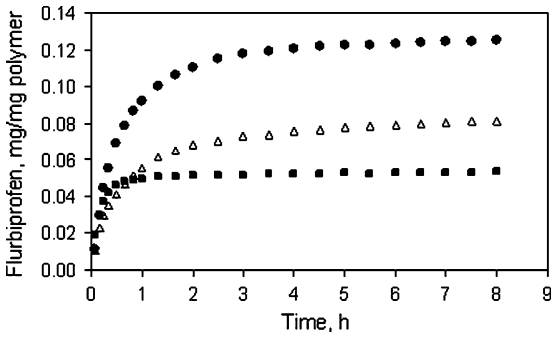


Fig. 4. Accumulated flurbiprofen mass released from chitosan derivatives, impregnated at 12 MPa and 40 °C by SSI: ● CMC; △ SCC and ■ CBC.

different release profiles can also be due to other factors related with the release experiments and not just to the impregnation process like, for example, polymer/drug/release medium physical and chemical properties and interactions and to polymer swelling. Therefore, this is an evidence that, at the same impregnation and release conditions, the three different (chemically and physically) polymeric structures somehow conditioned the impregnation and the drug release processes.

The conventional soaking method, employed for the same system (CMC + flurbiprofen) and soaking the polymeric sample for 1 h at ambient conditions (∼0.1 MPa and 294 K), showed an accumulated released drug amount of 0.074 mg flurbiprofen/mg of CMC (Table 1), which is almost half of the observed release for the sample impregnated by SSI at 12 MPa and 313 K. However, this observed more efficient SSI impregnation does not occur for all the employed operational temperature and pressure conditions and this subject will be discussed in detail further on. Table 1 also presents other results which show that, for the CMC derivative, SSI is a "tunable" impregnation process, i.e., varying the operational pressure and temperature conditions will result in the impregnation of different drug amounts which will lead to different drug released amounts (from 0.017 to 0.147 mg of flurbiprofen/mg of CMC). This is an evident advantage of the SSI technique. Furthermore, in some cases and for strongly hydrophobic drugs, it is not possible to use drug aqueous soaking solutions and the use of organic solvents is not recommendable for the development of CDR's applications.

Fig. 5 shows the absolute flurbiprofen mass released, as a function of release time, for the three used chitosan derivatives. It can be seen that the diffusional period may be extended over time until the released drug mass become almost constant: around 1 h (∼1–2 μg), 3 h (∼3–5 μg) and 4 h (∼3–6 μg), for CBC, SCC and CMC, respectively, and above the flurbiprofen therapeutic limit. The knowledge of these values is very important to develop efficient CDR devices, capable to keep drug concentrations between therapeutic and toxic limits. The mass variation showed in the

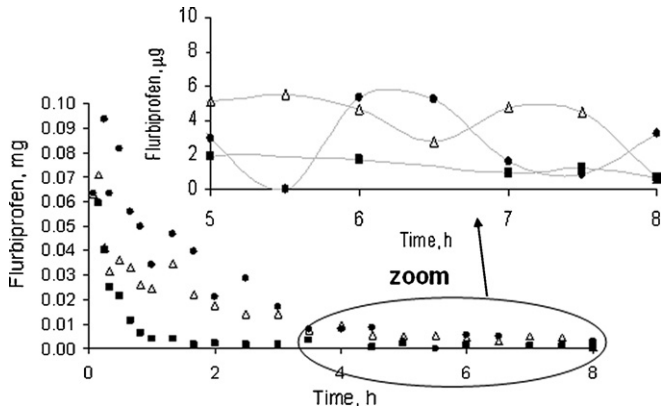


Fig. 5. Flurbiprofen mass released from chitosan derivatives, impregnated at 12 MPa and 40 °C by SSI: (●) CMC; (△) SCC and (■) CBC.

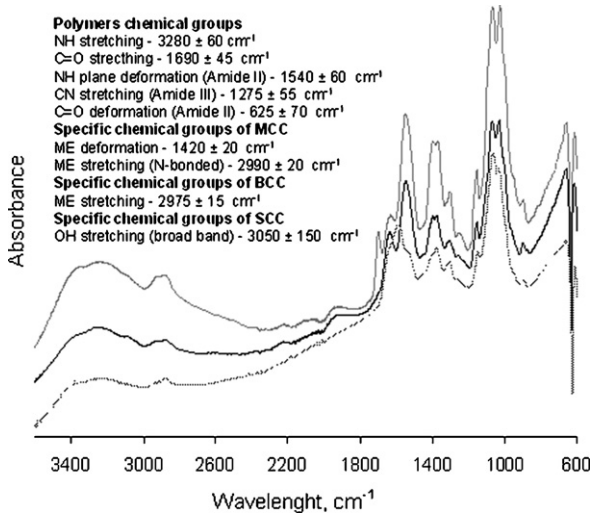


Fig. 6. FTIR analysis of chitosan derivatives before impregnation: (—) CBC; (—) SCC and (···) CMC.

zoomed part of Fig. 5 is still due to small fluctuations in drug release and not to any experimental uncertainties related to the method detection limit.

FTIR analysis (Fig. 6) shows the different spectrum profiles for the three chitosan derivatives. A characteristic peak for these polymers appears at the same wavelength (1541 cm^{-1}) and is probably indicative of the NH_2 group substitution [62]. FTIR analysis is not conclusive but it is an indication of the chemical differences between the three synthesized chitosan derivatives. Besides, the different attained substitution degrees in these derivatives must be also taken in consideration: for example, CMC may have more probability to be di-substituted (which will increase the number of polymer carbonyl groups) [63] leading to higher interactions with CO_2 and, consequently, to higher CO_2 sorption and polymer swelling, contrarily to CBC and SCC. Because impregnation yields are also strongly dependent on these factors (sorption and swelling) this can be a possible explanation for the higher amounts of impregnated flurbiprofen observed for CMC when compared to CBC and SCC at the same operational impregnation conditions (see Table 1 and Fig. 4).

Table 2 shows the calculated values for diffusional kinetic constants (k) and diffusional coefficients (n) for the kinetics of flurbiprofen release from the three chitosan derivatives, as a function of operational impregnation pressure. The n value is representative of the transportation mechanism and, for the studied systems, at 12 MPa, is higher for CMC followed by SCC and CBC (being 1.20, 0.86 and 0.57, respectively).

These values are in good agreement with the release curves presented at Fig. 4. All systems present a Non-Fickian behavior. Comparing the n values, as a function of pressure for the CMC + flurbiprofen system, it is possible to observe an increase at 11 and 12 MPa, showing that, in some way, the impregnation operational pressure has influence on the system. The diffusional kinetic constant (k), which usually defines the characteristics of a polymeric network system, is different for all polymers (at 12 MPa) and follows the order CMC > CBC > SCC (around 1.8, 1.5 and 1.2, respectively). Higher diffusional kinetic constant values can be an indication of the existence of more favorable interactions between the polymer and the drug (flurbiprofen) in the physiological serum medium [31].

Fig. 7 shows drug release assays, at 313 K and at 9, 12 and 13 MPa, performed with and without drug release media (serum) renovation. The experiments with renovation of the drug release media with fresh serum (1.6 ml) were done in order to simulate the *in vivo* eye conditions in terms of tear renovation, evaporation and drainage through the nasolacrimal system [8]. For 9 and 12 MPa, the difference (in terms of released mass) between the release experiments with serum renovation and without serum renovation was around 7%, with a slightly increase for the begin-

Table 2
Flurbiprofen drug release kinetic parameters for impregnated samples at 40 °C: CMC, CBC and SCC; (n) diffusion exponent and (k) diffusion kinetic constant

Impregnation process	Polymers								
	CMC			CBC			SCC		
SSI (MPa)	n	k	r^2	n	k	r^2	n	k	r^2
9.0	0.87	1.88	0.98	–	–	–	–	–	–
10.0	0.64	1.49	0.98	–	–	–	–	–	–
11.0	1.09	2.19	0.98	–	–	–	–	–	–
12.0	1.20	1.79	0.99	0.57	1.52	0.99	0.86	1.19	0.98
13.0	0.75	1.84	0.96	–	–	–	–	–	–
Soaking	0.64	1.19	0.99	–	–	–	–	–	–

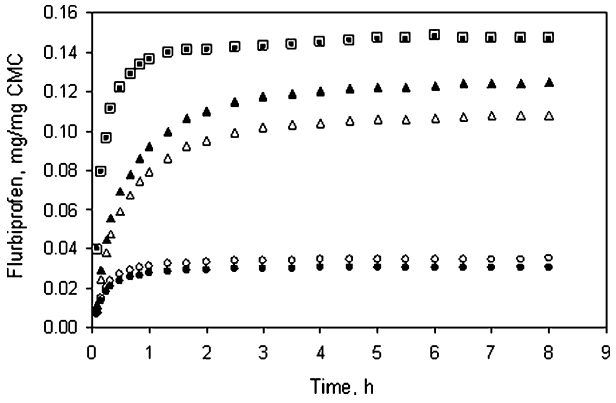


Fig. 7. Accumulated flurbiprofen mass released from CMC, impregnated at 40 °C by SSI, with serum renovation (SR) and without serum renovation: (●) 90 bar, (◯) 90 bar, SR; (▲) 120 bar, (△) 120 bar, SR and (■)130 bar, (□1)30 bar, SR.

ning of the 12 MPa release kinetics. At 13 MPa, the difference between the two drug release methodologies was around 0.8%, for the first release point, and 0.1% for all the rest.

Fig. 8 shows the three chitosan derivatives surface observed by SEM before impregnation (I), after pressurization/treatment with CO_2 (II) and impregnated with flurbiprofen by SSI (III), at 12 MPa and 313 K. CMC shows a pattern of quasi-parallel smooth sheets, in several layers, which seem to be just slightly changed after the CO_2 treatment and also after the flurbiprofen impregnation. Before impregnation and CO_2 treatment, CBC presents a kind of twisted flexible sheets pattern (in layers) and, after pressurization and impregnation, these sheets seem to become more twisted, like interlaced wires. Finally, SCC shows an interlaced wires pattern, which seems to become more interlaced after CO_2 treatment and impregnation. These observations are a clear confirmation of the well known high pressure/supercritical CO_2 plasticizing effect on the final morphological characteristics of these polymeric samples which, consequently, will influence their physical, mechanical and thermal properties. These effects (as well as others like induced crystallization) are already referred in the literature [41,42,64,65]. Finally, at Fig. 8, it is also possible to observe flurbiprofen's particles deposited on the surface of the chitosan derivatives. Despite the fact that only the deposited surface particles are visible, because the obtained drug release profiles and the notorious effect on samples' morphology (a strong indication that the CO_2 + drug high pressure mixture swelled and plasticized these samples), we may presume that, with some confidence, flurbiprofen was also precipitated and impregnated into the three chitosan derivatives.

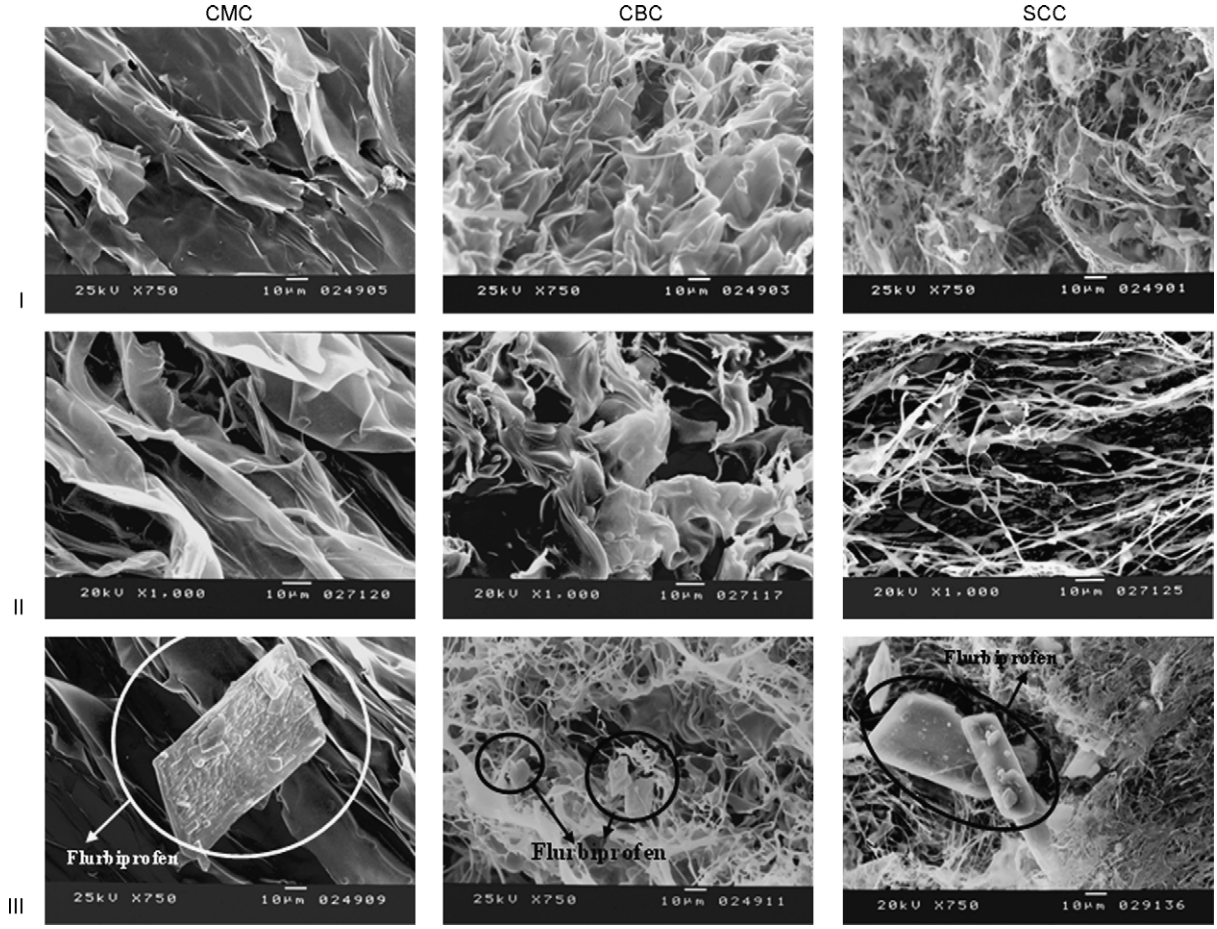


Fig. 8. Chitosan derivatives samples (CMC, CBC and SCC) observed by SEM before the impregnation (I); pressurized with CO_2 (II) and impregnated with flurbiprofen by SSI (III), at 12 MPa and 40 °C.

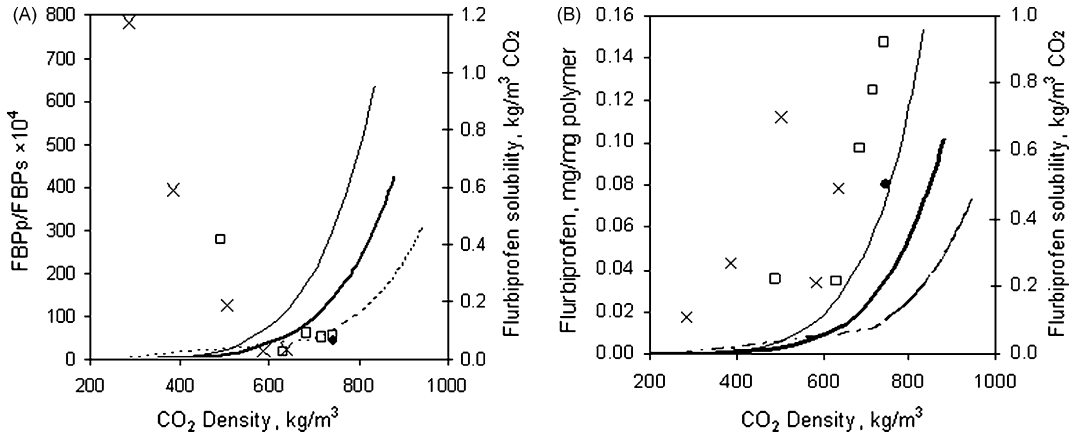


Fig. 9. (A) Flurbiprofen partition coefficient for CMC as a function of CO_2 density. (B) Accumulated flurbiprofen mass released from CMC impregnated by CO_2 SSI: (●) 30 °C; (□) 40 °C and (×) 50 °C. Flurbiprofen solubility in CO_2: (—) 30 °C; (—) 40 °C and (···) 50 °C.

Fig. 9(A) shows the flurbiprofen/CMC partition coefficient as a function of CO_2 density while Fig. 9(B) shows the accumulated flurbiprofen mass released (in absolute terms), also in function of CO_2 density, at 303, 313 and 323 K. In both figures it is also represented the flurbiprofen's solubility in CO_2 and, as expected, it is clear that the correlated flurbiprofen solubility in CO_2 increases with the CO_2 density for the three experimental solubility temperatures. Flurbiprofen is known to have a relatively high solubility in supercritical CO_2 and the experimental flurbiprofen's solubility in supercritical CO_2 was obtained and correlated by authors [52,53,64,66]. Fig. 9(A) shows that the flurbiprofen's partition coefficient (FBPp/FBPs ratio) decreases with the increase of the CO_2 density. However, Fig. 9(B) shows that, in general terms and as expected, flurbiprofen absolute impregnated mass tends to increase with the CO_2 density. This is due to above referred drug solubility increment and, probably, to the higher swelling and plasticizing effects promoted by high density supercritical (or liquid) supercritical carbon dioxide. We do not possess experimental data on these phenomena but they are recognized to be very common and important on these processes and are abundantly reported in literature [65,67,69].

For example, at 303 K and 9 MPa, the drug + supercritical CO_2 mixture is at a high pressure liquid state, which corresponds to a high density liquid state (thus promoting drug solubility in the high pressure phase). At the same pressure and at 313 K, the polymer is in the presence of a high pressure supercritical phase in which CO_2 densities are lower than at 303 K but higher than at 323 K. As a consequence, flurbiprofen was impregnated in a smaller extent than at 303 K and in a higher extent than at 323 K. However, this does not happen for all experimental pressures (for example at 11 and 12 MPa) which may indicate that other phenomena, and not just drug solubility, are involved in the process. In fact, the global impregnation process will always be the result of the relative specific interactions that may occur in the system: CO_2-drug interactions (which controls drug solubility in CO_2), polymer-CO_2 interactions (which controls CO_2 solubility in the polymer and, consequently, swelling and plasticization) and drug-polymer interactions (which controls solubility/compatibility of the drug in the polymer). Additionally, induced crystallization of the polymeric substrate can also influence the overall impregnation process. [65,67-69]. However, a reasonable approach is to consider that the operational (P and T) conditions will not affect the drug-polymer interactions as much as it will affect the two other possible interactions (the drug-polymer system – flurbiprofen/CMC – was the same for all these represented systems and pressure and

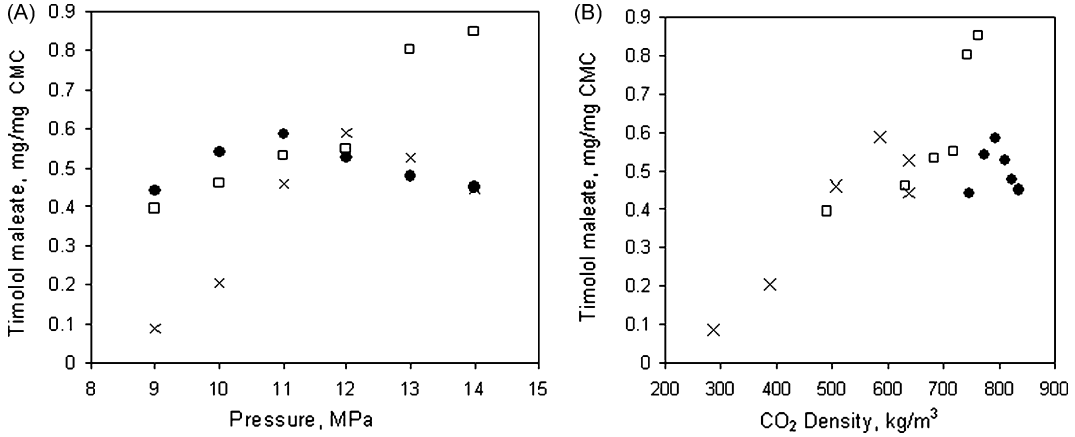


Fig. 10. Accumulated timolol maleate mass released from CMC impregnated by CO_2 + EtOH (5% mol) SSI, as a function of pressure and CO_2 density: (●)30 °C; (□) 40 °C and (×) 50 °C.

temperature are not expected to strongly influence the specific interactions between these two solids). Thus, we may consider that, in fact, the predominant effects in the impregnation process may be the solubility of the drug in CO_2 as well as the CO_2 swelling and plasticizing effect on the polymers. On this type of polymers, induced crystallization by supercritical CO_2 is not supposed to occur. Another important operational variable could be the system depressurization rate but it was kept constant for all studied systems.

So far, all the presented impregnated flurbiprofen amounts were determined by the drug release kinetics results (corresponding to the maximum of the released flurbiprofen accumulated mass). To verify these results, the amounts of impregnated flurbiprofen were also attempted to be quantified gravimetrically (weighing samples before and after impregnation experiments). However, experimentally, it was very difficult to measure gravimetrically these amounts because of the relatively low drug amounts involved and to the possibility of occurrence of some mass loss. This mass loss could be explained by the CO_2 extraction of any chemicals (or of CO_2 soluble low molecular weight polymeric chains) still present in the polymeric matrixes. Other explanation could be the removal of small solid polymer particles, pulled out of the system during depressurization. Flurbiprofen quantification by ion chromatography analysis also did not lead to satisfactory results because the very low flurbiprofen residual contents may induce high errors in results.

Fig. 10 shows the accumulated timolol mass released from CMC, impregnated by SSI using CO_2 + EtOH, as a function of operational pressure and of CO_2 density. These results are also presented in Table 1. All release experiments were carried out with renovation of the drug release media. The highest impregnated amount of timolol maleate was attained at 313 and 14 MPa (0.849 mg drug/mg polymer). For this system, and for all impregnation operational temperatures and pressures, after the two initial hours of drug release experiments (with serum renovation), the released mass of timolol maleate was found to be almost constant over time (5–10 μg) for the next 6 h of release. As can be seen, just for the 313 K isotherm there is a general tendency for the maximum impregnated timolol maleate mass increase with the operational pressure and with the CO_2 density. For 303 and 323 K isotherms, the impregnated mass increases with pressure and CO_2 density, passes through a maximum (around 0.5–0.6 mg of drug/mg of polymer) and then decreases. A possible explanation is that, probably, at higher densities, the interactions between the solute and the mobile phase increased and are detrimental to the bonding forces between the solute and the polymeric matrix, thus leading to lower impregnation yields. But this did not occur for the 313 K isotherm. As already referred for the flurbiprofen system, the final partitioning of the solute between the mobile phase and the solid matrix will be the result of the relative strength of all binary interactions involved in the system. And, for timolol maleate, the high pressure mobile phase has an extra compound, the cosolvent (ethanol), thus increasing even more the system complexity. It is well known from literature that drug solubility in CO_2 will increase when using a cosolvent with the same polar characteristics of the drug. It is also known that the same can happen with the polymer solubility in CO_2 and the CO_2 solubility in polymers. However, we do not have any information regarding these cosolvent effects on all the possible involved interactions that may be controlling the impregnation efficiency.

Fig. 11 shows the surface SEM micrographs of CMC impregnated with timolol maleate by SSI-CO_2 + EtOH, at 303 K (A), 313 K (B) and 323 K (C), and at 12 MPa. As can be seen, there is a large amount of drug deposited at polymer surfaces. It also looks like the drug is "plasticized", forming films on the polymer surface. This can be due to a cosolvent (ethanol) effect on the deposition of drug particles during depressurization. Unfortunately, timolol maleate is almost insoluble in high pressure CO_2 and we were unable to perform timolol impregnation experiments without the ethanol addition. Moreover, the presence of ethanol seems to have a strong influence on the amount of impregnated drug.

When we compare the relative amounts of released drugs (mg drug/mg polymer) between flurbiprofen and timolol maleate, Fig. 12(A) and (B), we can see that timolol maleate was impregnated in higher extents than flurbiprofen, despite the fact that it is much less soluble in CO_2 than flurbiprofen [52,53]. For both drugs, it is also clear the already referred pressure-driven "tunable" character of the SSI method which constitutes an advantage over the soaking method.

Like in the CMC + flurbiprofen case, in this system the drug impregnated final mass will also be the result of the operational impregnation conditions and of the very complex relative specific interactions that may occur between all species present in the system: drugs, polymers, CO_2 and, now a cosolvent

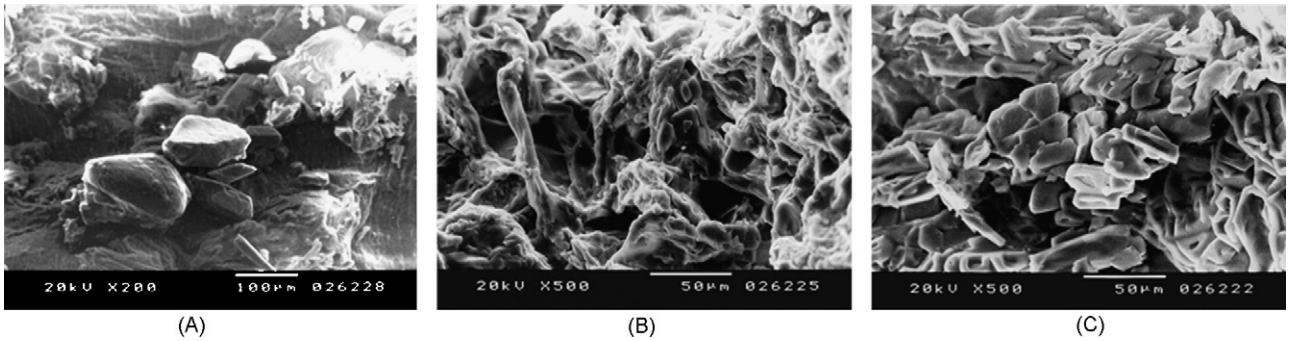


Fig. 11. Temperature effects of SSI-CO_2 + EtOH timolol maleate impregnation on CMC at 12 MPa. Surface samples observed by SEM: (A) 30 °C; (B) 40 °C and (C) 50 °C.

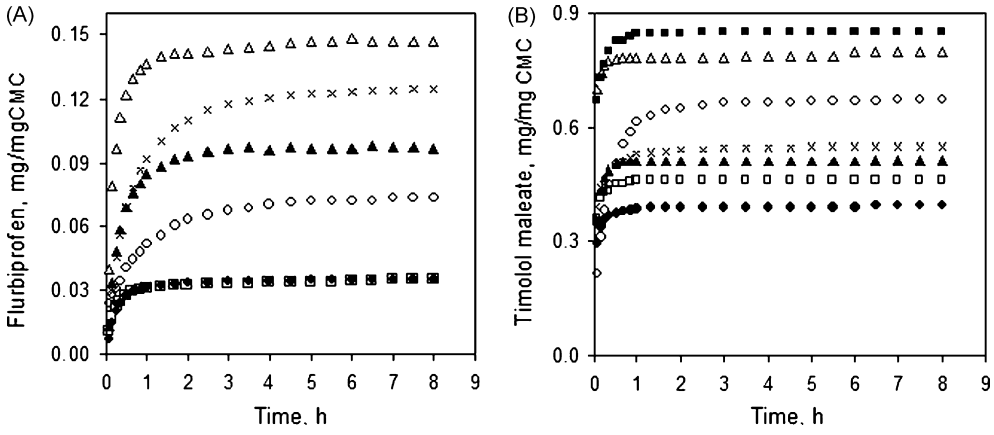


Fig. 12. Accumulated flurbiprofen (A) and timolol maleate (B) mass released from impregnated CMC SSI at 40 °C: (●) 9 MPa; (□) 10 MPa; (▲) 11 MPa;(×) 12 MPa; (△) 13 MPa and (■) 14 MPa; and (◯) ∼0.1 MPa soaking at 25 °C.

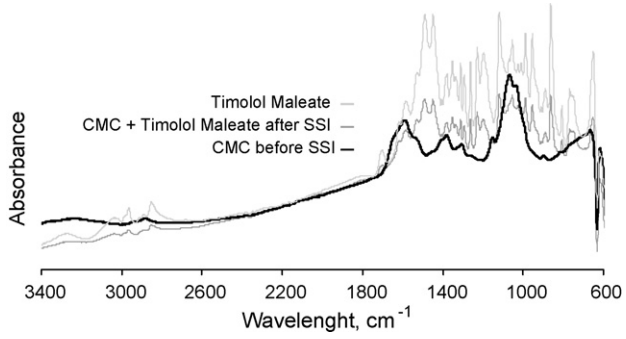


Fig. 13. FTIR analysis of timolol maleate CO_2 + EtOH (5% mol) SSI impregnated CMC: (—) CMC before SSI; (—) CMC + timolol maleate after SSI (at 313 K and 12 MPa) and (···) timolol maleate.

(ethanol). Thus, ethanol could be not just improving timolol maleate solubility in CO_2 but also increasing the solubility of the high pressure phase (containing also CO_2 and timolol maleate) in the CMC polymeric matrix, as well as improving the compatibility, swelling and plasticizing power of this phase.

Like for flurbiprofen impregnation, only the surface deposited particles/films are visible on micrographs. However, and due to the obtained drug release profiles and to the notorious swelling and plasticizing effects observed on polymeric samples' morphology, in this case we may consider that timolol maleate was also precipitated and impregnated inside the three chitosan derivatives. Samples loaded with timolol maleate were also analyzed by FTIR and, in Fig. 13, it is possible to observe the two spectra profiles, corresponding to the polymer (CMC) with and without impregnated drug. The individual FTIR spectrum for timolol maleate confirmed this result. Furthermore, sulphur quantification (performed by ion chromatography analysis) permitted to quantify the amount of timolol maleate still present in the polymeric matrix after the drug release experiments in physiological serum ($1.54 \pm 0.32\%$ of total impregnated mass, with $98.46 \pm 0.32\%$ of total released drug mass).

4. Conclusions

In this work three chitosan derivatives (*N*-carboxymethyl chitosan, *N*-carboxybutyl chitosan and *N*-succinyl chitosan) were impregnated with flurbiprofen and timolol maleate, using a SSI technique (and employing high pressure CO_2 and CO_2 + EtOH mixtures) in order to try to develop hydrogel-type ophthalmic drug release systems. Impregnation experiments were carried out from 9.0 up to 14.0 MPa, and at 303.0, 313.0 and 323.0 K. The resulting polymeric drug delivery systems, as well as other polymeric samples processed in CO_2, were characterized by FTIR spectroscopy and Scanning Electron Microscopy (SEM). Drug release kinetics studies were performed for all prepared systems. The effects of impregnation pressure and temperature on the release kinetics results were studied and compared to the traditional soaking impregnation method.

For the same operational conditions, results confirmed that, as expected, the three different (chemically and physically) polymeric structures conditioned the impregnation and the drug release processes. Furthermore, and even though the final impregnated drug mass is always the result of the employed operational impregnation conditions and of the very complex relative specific interactions that may occur between all species present in the system (drugs, polymers, CO_2 and ethanol), results showed that, for *N*-carboxymethyl chitosan, the predominant effects in the impregnation process seemed to be the solubility of drugs in CO_2 and in CO_2 + EtOH mixtures, as well as the swelling and plasticizing effect of CO_2 and ethanol on the polymer. Finally, the SSI method proved to be a more efficient and "tunable" impregnation process than the traditional impregnation of drugs by a soaking method.

Therefore, and using this "tunable" SSI method, these *N*-chitosan derivatives-based ophthalmic drug delivery systems can be easily and efficiently prepared taking in consideration the desired drug levels according to patients needs.

Acknowledgments

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