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Effects of operational conditions on the supercritical solvent impregnation of acetazolamide in Balafilcon A commercial contact lenses

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ABSTRACT

In this work we employed a supercritical solvent impregnation (SSI) process using a $scCO_2$ + EtOH (5% molar) solvent mixture to impregnate acetazolamide (ACZ) into commercially available silicone-based soft contact lenses (Balafilcon A, Pure Vision™, Bausch & Lomb®). Contact lenses (SCLs) drug-loading was studied at 40 °C and 50 °C, and from 15 MPa up to 20 MPa, and using low depressurization rates in order to avoid any harm to SCLs. The effect of impregnation processing time on the loaded ACZ amounts was also studied (1, 2 and 3 h). In vitro drug release kinetics studies were performed and the released ACZ was quantified spectrophotometrically. Several analytical techniques were employed in order to characterize the processed and non-processed SCLs in terms of some of their important functional properties. Obtained results demonstrated that ACZ-loaded therapeutic Balafilcon A SCLs can be successfully prepared using the employed SSI process. Furthermore, it was possible to control ACZ loaded amounts and, consequently, to adjust the final ACZ release levels into the desired therapeutic limits, just by changing the employed operational conditions (*P*, *T*, processing time and depressurization rate) and without change some of their most important thermomechanical, surface/wettability and optical properties. Obtained soft contact lenses can be potentially employed as combined biomedical devices for simultaneous therapeutic and correction of refractive deficiencies purposes.

1. Introduction

Glaucoma is an ophthalmic condition which is currently the leading cause of irreversible blindness in the world. Glaucoma major risk factor is the increase in intraocular pressure (IOP) as the result of an imbalance between the production (inflow) and the drainage (outflow) of aqueous humour (Kaur et al., 2002). IOP can be lowered using drugs designed to limit aqueous humour production and/or to enhance the aqueous humour drainage. One of these drugs is acetazolamide (ACZ), a carbonic anhydrase inhibitor (CAI) (Fig. 1). ACZ recommended defined daily dose (DDD) for oral and for parenteral administration is 0.75 g (WHOCC, 2011) and it is usually administered by an enteral route (as tablets) or topically (as eye drops and sometimes in conjugation with other anti-glaucoma drugs). However, and mostly due to ACZ low bioavailability, these administration routes normally require high drug doses which may promote the occurrence of several undesired systemic side effects.

To overcome most of these bioavailability issues and because topical eye application of ACZ and of other CAIs is an ideal administration route to reduce IOP with fewer side effects (Friedman et al., 1985), great research efforts have been made during the last years in order to develop newer and advantageous systems to safely and efficiently deliver CAIs by the ocular route (Kaur et al., 2002). ACZ drug delivery systems (DDSs) based on drug-loaded soft contact lenses (SCLs) can be envisaged as potential good alternatives to efficiently treat glaucoma, since SCLs are already known to improve drug permeation and absorption through the cornea and to promote longer drug residence times in the post-lens tear film, as well as adequate sustained release drug levels. Drug-loaded SCLs may also present other advantages such as the reduction of the amount of drug that is lost by tear drainage and the reduction in the amount of drug needed to produce the desired therapeutic effect, all of which will minimize the potential occurrence of systemic side effects. This is mostly due to the above-referred formation of a post-lens tear film between the SCL and the cornea that

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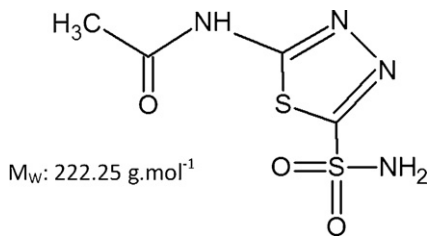

Fig. 1. Chemical structure of acetazolamide ($C_4H_6N_4O_3S_2$), N-5-(sulfamoyl-1,3,4-thiadiazol-2-yl) acetamide, CAS [59-66-5].

will act as a local and "closed" drug reservoir (Hiratani et al., 2005; Gulsen and Chauhan, 2004; Gulsen et al., 2005). When compared to other polymeric matrices, SCLs can be worn more frequently and for longer periods due to their excellent biocompatibility, comfort and patient compliance/acceptance. Moreover, their use as is widely spread and they are already approved as biomedical devices. Finally, therapeutic SCLs can combine their correction of refractive deficiency purposes with an intended therapeutic role, performing as a combination product according to FDA regulations (Novack, 2009; Lauritsen and Nguyen, 2009).

Despite they may present several disadvantages, commonly employed methods to obtain polymer film-based DDSs (such as therapeutic SCLs) usually encompass their preparation during synthesis/polymerization, during film processing procedures or by drug loading from liquid solutions/suspensions. Alternatively, a drug may also be loaded into already prepared polymeric films by a supercritical solvent impregnation/deposition (SSI) method, i.e., by dissolving this drug into compressed high volatile fluids such as carbon dioxide (CO_2), at subcritical or supercritical conditions, and then contact the resulting mixture with the polymeric matrixes to be loaded (Kazarian, 2004; Davies et al., 2008; Kikic and Vecchione, 2003; de Sousa et al., 2006a; Costa et al., 2010a). This technique can be extremely useful and present several additional advantages for the development of drug-loaded polymeric materials to be employed as DDSs for many biomedical applications including therapeutic SCLs (Kazarian, 2004; Davies et al., 2008; Kikic and Vecchione, 2003; de Sousa et al., 2006a; Costa et al., 2010a; Natu et al., 2008; Braga et al., 2008; Duarte et al., 2008; Costa et al., 2010b). The most commonly employed SCF is supercritical carbon dioxide ($scCO_2$) mainly because it is abundant and cheap, it is non-flammable and relatively inert and it is considered as a GRAS ("generally recognized as safe") solvent. It is quite soluble in aqueous media and it can swell, plasticize and decrease the glass transition temperature of most polymeric materials. Furthermore, it has a low critical temperature (31 °C) that allows the processing at relatively low temperatures which is a very helpful feature for thermally labile substances (Kazarian, 2004; McHugh and Krukonis, 1994). Therefore, $scCO_2$ impregnation/deposition allows the incorporation of $scCO_2$-soluble drugs (usually of the hydrophobic/low polarity type) into most polymeric matrixes and, when properly employed, it will not change and/or damage their most important physical, chemical and mechanical properties, as well as it will not degrade any involved thermally labile materials. In the end, the drug will be homogeneously impregnated/deposited in relatively short treatment times and without the presence of any harmful solvent residues. The manipulation of the employed operational conditions (such as pressure, temperature, processing time, depressurization rates and cosolvent addition) will control drug loading and drug depth penetration. SSI also permits to have previously prepared polymeric articles or biomedical devices (such as commercial SCLs) and impregnate them later with the desired drugs, taking in consideration the envisaged therapeutic application and without interfering with the polymeric article/device manufacture and/or processing method. This particular feature is an important advantage of the method that may lead to the development of several other biomedical and pharmaceutical applications (de Sousa et al., 2006a;Costa et al., 2010a,b; Natu et al., 2008; Braga et al., 2008; Duarte et al., 2008).

Continuous wear monthly Balafilcon A (Pure Vision™, Bausch & Lomb®) SCLs are prepared by the free radical copolymerization and cross-linking of tris(trimethylsiloxy)silylpropylvinyl carbamate (TPVC), *N*-vinyl pyrrolidone (NVP), *N*-carboxyvinyl ester (NCVE) and poly(dimethylsiloxy)di(silylbultanol)bis(vinyl carbamate) (PBVC) (Karlgard et al., 2004; Bausch & Lomb, 2008). They are coloured and cross-linked by Reactive Blue 246 and, in addition, they possess a Performa® surface modification treatment in order to increase hydrophilicity and thus to improve their wettability and wearing comfort (Karlgard et al., 2004; Bausch & Lomb, 2008). These lenses present low water content (around 36%, w/w), but their silicon-based oxygen permeable structure will help oxygen transport into the eye thus compensating their low water O_2 transport. On the contrary and for other commercial SCLs (without oxygen permeable polymers), higher water contents are usually necessary in order to carry oxygen into the eye. Other important commercial SCLs functional properties that should be considered are thermomechanical behaviour (such as glass transition temperature), optical properties (such as frontal power and light transmittance) and geometrical and dimensional consistency.

This work is part of a wider research project involving the development of polymer-based ophthalmic drug delivery systems using conventional and $scCO_2$ impregnation/dispersion methods, namely on the preparation of therapeutic contact and intraocular lenses, of hydrogels and particles for topical applications and of biodegradable copolymer blends for ophthalmic implants (de Sousa et al., 2006a; Costa et al., 2010a,b; Natu et al., 2008; Braga et al., 2008; Duarte et al., 2007, 2008; Coimbra et al., 2008; Yañez et al., 2011). In a recent work (Costa et al., 2010b), Balafilcon A SCLs were already impregnated with two anti-glaucoma drugs (ACZ – hydrophobic – and timolol maleate – hydrophilic) using a SSI methodology. Pressure and temperature, as well as the processing time and the depressurization rate, were kept constant while the nature and the effects of cosolvents (ethanol and water) concentration were studied. Based on some of those previous results, we now apply the same SSI process to impregnate ACZ on Balafilcon A SCLs, but this time by using a $scCO_2$ + EtOH (5% molar) drug-loading high pressure solvent mixture and by employing different pressure, temperature, impregnation time and depressurization rate operational conditions. The objective of this work is thus to study the effects of these experimental conditions on the final drug-loading yields, on the obtained drug-release profiles, as well as on some of the SCLs important functional properties. Drug-loaded contact lenses were characterized by optical measurements, FTIR, DMTA, TGA, DSC, contact angle/surface energy measurements, SEM and by in vitro kinetics of drug release experiments. Other analytical methods were also employed such as X-ray diffraction (WAXD) and elemental analysis.

2. Materials and methods

2.1. Chemicals

Commercial continuous-wear silicone-based SCLs were obtained from Bausch & Lomb® and from Mart-Optic (Coimbra, Portugal): Balafilcon A, Pure Vision™, 36% (w/w) water content, 8.6 mm (Base Curve), −4.00 D (PWR-power, spherical), Ø14 mm and center thickness from 0.05 up to 0.5 mm. Light transmittance is at least 95% (CIE value) (Bausch & Lomb, 2008). The employed ophthalmic drug was acetazolamide (≥99%, Sigma–Aldrich, Germany), Fig. 1, which will be abbreviated as ACZ. Employed solvents were carbon dioxide (99.995%, Praxair, Spain) and ethanol (99.5%,

Panreac Química, Barcelona, Spain). Saline water (NaCl isotonic solution, pH 6, 154 mEq for Na^+ and Cl^-, Osm ~285 mOsm/kg, Fresenius Kabi, Portugal) was used as the release media for the drug release experiments.

2.2. Supercritical impregnation experiments

The employed supercritical impregnation apparatus and the general impregnation procedures were already described by patent applications EP 1611877A1 and US 20060008506A1 (Unit I) (de Sousa et al., 2006a), as well as by other recent works (Costa et al., 2010a,b; Natu et al., 2008; Braga et al., 2008; Yañez et al., 2011). In general terms, the SSI apparatus (not represented) is comprised by a high pressure CO_2 liquid pump, a sealed high pressure stainless steel vessel with sapphire windows (having an internal volume of 10 cm^3), a temperature-controlled water bath, a magnetic stirring plate and pressure transducers. Wet SCLs were introduced into the high pressure cell (on specially designed stainless steel support structures) and in a vertical position, in order to reduce the probability of drug deposition on lenses surfaces during depressurization. Before any SSI experiment, SCLs were always kept in their hydrated state (36% of water content, according to manufacturer). Wet lenses were previously weighed and their mass was found to be 33.6 ± 1.9 mg. Their (wet) volume was found to be ~25 mm^3 per lens. CO_2 was then introduced into the impregnation vessel which was also previously loaded with ACZ and with the cosolvent (EtOH) and pressure and temperature were adjusted to the desired operational conditions. The added amounts of EtOH were previously calculated taking in consideration the available cell volume (lenses volume and the volume of the stainless steel support (5.5 cm^3) were discounted). The operational pressure and temperature conditions to be employed during impregnation experiments were also considered in order to achieve the desired cosolvent molar composition (5% molar) in $scCO_2$. The employed amounts of ACZ were calculated according to the corresponding drug solubilities in CO_2 + EtOH (5% molar) mixtures, at the employed pressure and temperature operational conditions (Duarte et al., 2005). Magnetic stirring was employed for the fast dissolution and homogenization of the drug in the compressed fluid mixture. Following the pre-established processing time, the system was then slowly depressurized (at approximately 0.06 MPa/min) so that the ophthalmic devices were not damaged during this procedure.

A first SSI group of assays was performed in order to study the effects of employed operational temperature (40 °C and 50 °C) and pressure (from 15 MPa up to 20 MPa) conditions. As referred, EtOH (5% molar) was used as the cosolvent. All these SSI experiments were carried out for 1 h and the employed depressurization rate was, in average, ~0.06 MPa/min. Two contact lenses were impregnated in each impregnation batch and all assays were duplicated.

Using the same compressed fluid mixture (ACZ + CO_2 + EtOH, 5% molar), a second SSI group of assays was carried out: this time changing the depressurization rate (from 0.06 MPa/min up to 0.15 MPa/min) and the processing/impregnation time (1, 2 and 3 h). These experiments were performed at constant temperature and pressure conditions (50 °C and 17 MPa). In this case, two contact lenses were impregnated in each impregnation batch and all SSI assays were triplicated.

2.3. Contact lenses storage

Processed/loaded SCLs were sterilized (under UV light, 360 nm, during 30–35 min at room temperature) and then immersed and maintained in a small-volume sterile saline water solution (1 ml), in the absence of light, until further analysis. After lenses removal from these storage solutions, the saline water was analyzed (spectrophotometrically, at 200–800 nm) in order to detect the presence of substances generated from polymer degradation, and/or from ACZ leaching and solubilization in the storage solution.

2.4. In vitro drug release kinetics

Drug release kinetics studies were performed for all prepared ACZ-loaded systems using a UV–vis spectrophotometric method (Jasco, model 530, Japan), at 265 nm. In order to simulate the envisaged daily-wear ophthalmic use, release experiments were carried out for 8 h, in 10 ml of saline water, at 37 °C and under orbital stirring. In order to simulate the typical hourly in vivo lachrymal fluid renovation rate (Baeyens and Gurny, 1997), an average 6% release fluid volume renovation rate was employed between each analysis.

Drug quantification in the release media was performed using a previously determined ACZ-saline water calibration curve. Two (or three) ACZ-loaded lenses from each impregnation batch were released in order to verify the homogeneity of the impregnation process and its reproducibility. After the above referred 8 h period of kinetics of drug release studies, SCLs were removed and placed into temperature-controlled (37 °C) stirred glass vials (containing 10 ml of saline water), for several days and in order to leach out the remaining drug still present in SCLs. Samples were then removed and analyzed spectrophotometrically in order to quantify the amount of ACZ which was released during these leaching experiments. Then, release solutions were replaced by 10 ml of fresh saline water and this procedure was repeated, twice a day, until no drug can be observed in the release solution. Finally, the total (accumulated) released drug was quantified taking in consideration the ACZ amounts released during the previously performed drug release studies and the ACZ leaching procedures. In addition, remaining (residual) ACZ amounts were also quantified in the small volume storage liquid solutions and in order to determine the ACZ amounts previously released during lenses storage. Results can be presented as released ACZ (during the 8 h release period) or as total released ACZ (sum of released ACZ in release experiments, of released ACZ in leaching experiments and of released ACZ during storage). Therefore, we can assume that the total released ACZ will correspond to the total impregnated/loaded ACZ by the SSI process.

For some selected ACZ-loaded SCLs (those obtained from SSI experiments performed at 18 MPa, 50 °C, processed for 1 h and depressurized at ~0.06 MPa/min), elemental analysis was employed to quantify any residual ACZ that was still present in SCLs (after the performed release and leaching experiments), and in order to confirm the above referred spectrophotometric quantification analysis. A Fisons Instruments Elemental Analyzer (model EA1108) coupled to a thermal conductivity detector (TCD) was employed for this purpose. Analyses were performed at 900 °C and using a helium flow rate of 120 mL/min. Processed SCLs (released and leached) were analyzed for their sulphur contents.

2.5. ACZ degradation

The evaluation of ACZ degradation in saline water was carried out for 19 days, which was also the maximum employed storage period of processed SCLs. This analysis was performed in the absence of light, at room temperature (at approximately 21 °C) and at 7 °C, for pure/non-processed ACZ and for high-pressure processed ACZ (at 20 MPa, 50 °C, 1 h of high-pressure processing and 0.06 MPa/min of depressurization rate). After the above-referred period of time, the resulting solutions were observed spectrophotometrically from 200 nm up to 400 nm (Jasco, model 530, Japan), and following other ACZ degradation literature data (Vargas et al., 1998).

2.6. Samples characterization (SCLs and ACZ)

SCLs were analyzed by Scanning Electron Microscopy/Energy Dispersive using X-Ray (SEM–EDX) (Hitachi, model S-2700, Japan), at 20 kV. Dehydration of the samples was conducted by a series of 15, 30, 50, 70, 96 and 100% ethanol in 0.9% NaCl (Vargas et al., 1998). Samples were further dried by using a Critical Point Dryer (CPD, Emitech). These samples were mounted on aluminium stubs and then coated with gold, approximately 120 Å by using a Sputter Coater (Emitech). To analyze the cross-section, samples were fractured with liquid nitrogen. SCLs were analyzed before and after the performed SSI experiments (carried out at 20 MPa, 50 °C, 1 h of high-pressure processing and 0.06 MPa/min of depressurization rate). A non-processed ACZ (commercial sample) and a $scCO_2$-processed ACZ sample (processed at the above referred conditions) were also analyzed by SEM–EDX.

Experimental wide angle X-ray diffraction (WAXD) patterns were obtained for a non-processed ACZ (commercial sample) and for a $scCO_2$-processed ACZ sample (at 20 MPa, 50 °C, 1 h of high-pressure processing and 0.06 MPa/min of depressurization rate). A X-ray diffractometer (Philips, model X-Pert), with a cobalt irradiation source and a Bragg-Brentano geometry, was employed. Data were collected in the continuous-scan mode using a step size of 0.025° and a step time of 0.1 s. Scanned 2θ range was from 5° up to 45°.

Non-processed and $scCO_2$-processed ACZ (processed at the above referred conditions), as well as drug-loaded and non-processed SCLs, were also analyzed by FTIR-ATR spectroscopy (Magma-IR Spectrometer 750, Nicolet Instrument Corp.; ATR – Golden Gate MK II, Specac, DTGS KBr detector, 32 scans and 4 cm^{-1} of resolution).

Processed (at different operational conditions and after being released) and non-processed wet Balafilcon A SCLs water contents and degradation profiles were obtained by thermogravimetric analysis (TGA) (TA Instruments, model SDT Q600). Experiments were performed in hermetic alumina pans. Temperature and heat-flow calibrations were performed using pure indium as the standard. Samples were equilibrated at 25 °C, with nitrogen (100 mL/min), and experiments were carried out using a heating rate of 5 °C/min (until 1200 °C). All assays were duplicated.

DSC analysis on processed (at different operational conditions and after being released) and on non-processed wet Balafilcon A SCLs was also performed using a modulated differential scanning calorimeter (MDSC – model Q100, Modulated FC, TA Instruments). Nitrogen was used at 50 mL/min and samples were equilibrated at 25 °C. A heating rate of 5 °C/min was employed (until 600 °C) and the process was modulated (sinusoidal modulation with an amplitude of 0.40 °C every 30 s). A hermetic alumina pan was used as the reference and SCLs were analyzed after rehydration and freeze-drying. All assays were duplicated.

Processed and non-processed dehydrated Balafilcon A SCLs were also analyzed by dynamic mechanical thermal analysis (DMTA) (Triton, model Tritec 2000). All samples were freeze-dried during 24 h before analyses. The constrain layer damping mode was used at multi-frequencies (1 and 10 Hz) and a displacement of 0.05 mm. Temperature profile was 2 K/min (until 300 °C) with a delay time of 1 s. The glass transition temperature (T_g) was determined as the peak in the damping factor curve ($\tan\delta = E''/E'$, where E'' and E' are the loss and the storage modulus, respectively).

Drug-loaded and non-processed SCLs contact angle measurements were obtained by the sessile drop method and using four different liquid substances: bi-distilled water, ethanol, formamide and 1-bromonaphthalene (Dataphysics Instruments, model OCA-20). Surface free energies were calculated using the OWRK method (Owens and Wendt, 1969). SCLs were cut into four parts and all of these pieces were measured. Average values were obtained and considered to verify the in-lens drug-loading homogeneity of the SSI process.

2.7. Optical measurements

The optical properties of non-processed, of SSI-processed and of SSI-processed/released SCLs were measured by an optical apparatus specifically implemented for measuring the frontal power of corneal contact lenses made of a somewhat hydrophilic material and having a predetermined index of refraction in a liquid having itself a predetermined index of refraction. This apparatus comprises a sighting optical system and a measuring optical system spaced from each other and having a common optical axis extending substantially vertically at least in the gap between said two optical systems. It further comprises a small cup disposed in said gap and filled with said liquid, the cup bottom comprising a fluid-tight window centered to said optical axis and means for supporting a contact lens in said liquid in a position centered to said optical axis for measuring the frontal power of contact lenses (frontofocometer). SCLs frontal power can be related to their produced image focus, as well as to the radiation transmittance through SCLs materials. Obtained data were frontal power (as dioptres, D ±0.125), focus position (cm ±0.02–0.04), light absorption and light transmittance (% ±0.5). These values were obtained as averaged values from ten different experimental measurements. Visual observations were obtained by a stereoscopic loupe at 64×, 160× and 400× magnifications, and in order to detect any possible damages caused on SCLs surfaces by the SSI process. All assays were duplicated.

2.8. Kinetics of drug release correlation

Experimental data on the kinetics of ACZ release were correlated using a linear regression analysis by a curve formed by three straight lines. Fitting was done by minimizing the least regression error (in the least squares sense) and using the fminsearch function of Matlab (R2007a). The first straight line is assigned to the constant release rate period (CRR), the second straight line to the falling release rate period (FRR) and the last to the diffusional release rate period (DRR). The corresponding kinetic parameters were correlated: mass transfer rate (M_{CRR}, M_{FRR} and M_{DRR}, μg/min) and the corresponding duration (t_{CRR}, t_{FRR} and t_{DRR}, min). Statistical analysis was performed by the Tukey HSD test and analysis of variance (ANOVA/MANOVA), using of Statistica 6.0.

3. Results and discussion

It is already recognized that the efficiency/yield of the SSI process is controlled and strongly affected by the employed operational conditions as well as by the physicochemical interactions that may be established between all the involved substances in the process and by their relative magnitudes (Kazarian, 2004; Kikic and Vecchione, 2003; de Sousa et al., 2006a; Costa et al., 2010a; Natu et al., 2008; Braga et al., 2008; Kikic, 2009). In the present case, these substances are the copolymers which are comprised in the employed SCLs, the drug (ACZ), $scCO_2$, the cosolvent (EtOH) and water. Thus, in the studied system, the most relevant physicochemical interactions to be considered are the $scCO_2$/ACZ/EtOH interactions (which will determine ACZ solubility in the $scCO_2$/EtOH mobile phase), the water-swollen SCLs/$scCO_2$/EtOH interactions (which will control SCLs additional swelling and plasticization) and the water-swollen SCLs/ACZ/EtOH interactions (which will control ACZ "solubility" in SCLs and its partition between SCLs and the $scCO_2$ mobile phase) (de Sousa et al., 2006a; Costa et al., 2010a,b; Natu et al., 2008; Braga et al., 2008).

$scCO_2$ is a non-polar solvent (despite the fact that, under certain conditions, it can form quadrupoles) which is known to have a high

affinity for low polarity small hydrophobic drugs. ACZ is a polar drug that can be considered of a hydrophobic nature because of its relatively low water solubility (~0.6 to 1.0 g/L) (Loftsson et al., 1996; Yalkowsky and He, 2003). Because of ACZ relatively high molecular weight, of its chemical functionalities and polarity, its solubility in pure $scCO_2$ is very low and thus it is necessary to add a small amount of a polar cosolvent to enhance ACZ solubility in $scCO_2$. By doing this, the ACZ solubility in a $scCO_2$ + EtOH (5% molar) mixture was found to be 0.023–0.038 g/L, at 40 °C, and 0.026–0.036 g/L, at 50 °C, in the 15–20 MPa pressure range for both isotherms (Duarte et al., 2005).

Taking in consideration the chemical structures of ACZ (Fig. 1) and of the co-monomers that constitute Balafilcon A SCLs (Karlgard et al., 2004; Bausch & Lomb, 2008), the potential favourable physical–chemical interactions between ACZ, $scCO_2$, copolymers, water and EtOH that may occur can be easily identified: polar and hydrogen-bond interactions (water, carboxylic acid, hydroxyl, sulphonyl, carbonyl groups), and methyl/carbonyl/sulphonyl/nitrogen-$scCO_2$ interactions (Kazarian, 2004; Costa et al., 2010a,b; Kazarian and Martirosyan, 2002; Xu and Chang, 2004; Kikic, 2009).

3.1. Drug impregnation/deposition experiments

As referred in Section 2, two different groups of SSI assays were performed: a first group of assays to study the effects of operational temperature (40 °C and 50 °C) and of pressure (from 15 MPa up to 20 MPa) conditions (carried out for 1 h and depressurized, in average, at ~0.06 MPa/min) and a second group of assays, performed at 40 °C and at 17 MPa to study the effects of the variation of depressurization rate (from 0.06 up to 0.15 MPa/min) and of the impregnation duration (1, 2 and 3 h). On both groups of assays, EtOH (5% molar) was always used as a cosolvent in order to increase the ACZ solubility in $scCO_2$. Two SCLs were impregnated in each performed SSI batch and experiments were duplicated (for first group of assays) and triplicated (for second group of assays). Therefore, it was possible to obtain two different standard deviations for the SSI process: one inside the each assay (intra-experimental deviations) and another between assays (inter-experimental deviations).

Elemental analysis for some selected ACZ-loaded SCLs (released for 8 h, leached for several days and previously processed at 18 MPa, 50 °C, processed for 1 h and depressurized at ~0.06 MPa/min) showed that the remaining amounts of sulphur (and thus of ACZ) in SCLs were undetectable (<100 ppm). Therefore these results confirmed the spectrophotometric analysis of leaching media and thus we can assume that the total released ACZ (sum of released ACZ in release experiments, of released ACZ in leaching experiments and of released ACZ during storage) corresponds to the total impregnated/loaded ACZ by the employed SSI process.

3.1.1. Influence of temperature and pressure

For the first group of assays, the total ACZ loaded amounts per mass of wet lens are presented in Table 1. The corresponding average inter-experiments ACZ loading results are presented at Fig. 2 for both isotherms: as a function of $scCO_2$ + EtOH (5% molar) density (Fig. 2A) And the ACZ partition coefficient (between the SCLs and the supercritical fluid phase) and the ACZ solubility are presented in Fig. 2B as a function of $scCO_2$ + EtOH (5% molar) density.

As can be seen in Fig. 2A, the total amount of loaded ACZ is not strongly dependent on the operational temperature and the observed differences can be considered as of the same magnitude as the obtained standard deviations. However, and for both experimental temperatures, the operational pressure seems to start playing a quite favourable role at its highest studied value (20 MPa)

Table 1
Total amounts of loaded ACZ in Balafilcon A SCLs (loaded by SSI for 1 h, depressurized at ~0.06 MPa/min). Intra-experimental and inter-experimental standard deviations are also presented.

Temperature (°C)	Pressure (MPa)	Loaded ACZ (μg/mg wet lens)[a]	
		Intra-experiments	Inter-experiments
40	15	6.54 ± 0.80 10.15 ± 1.53	8.35 ± 2.55
	17	7.01 ± 1.34 5.13 ± 2.16	6.07 ± 1.33
	18	6.28 ± 0.69 5.42 ± 0.80	5.85 ± 0.61
	20	16.91 ± 1.34 15.05 ± 0.78	15.98 ± 1.31
50	15	5.84 ± 0.40 9.68 ± 3.24	7.76 ± 2.72
	17	5.00 ± 1.16 5.30 ± 0.06	5.15 ± 0.21
	18	5.05 ± 1.48 9.01 ± 5.71	7.03 ± 2.80
	20	19.93 ± 2.31 16.74 ± 2.52	18.33 ± 2.26

[a] Value ± standard deviation.

in which the amount of loaded ACZ duplicates its value. Nevertheless, it should be noted that the impregnation duration for these experiments was just 1 h.

Despite other aspects, it is well-known that if higher amounts of a drug can be rapidly dissolved and loaded into a $scCO_2$ mobile

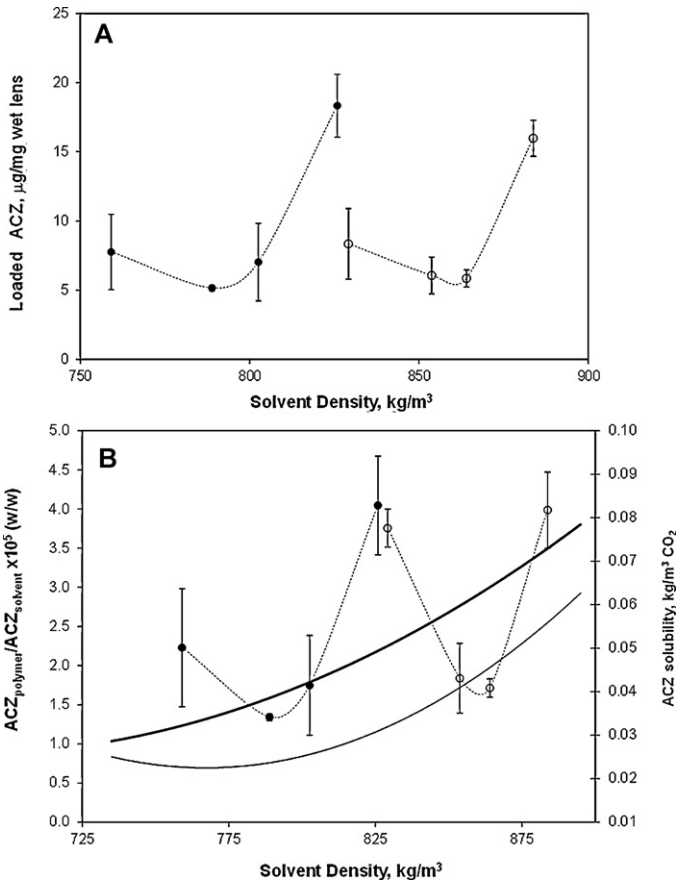

Fig. 2. Impregnation of ACZ in Balafilcon A SCLs (loaded by SSI for 1 h and depressurized at ~0.06 MPa/min). (A) Total amounts of loaded ACZ as a function of $scCO_2$ + EtOH (5% molar) density; and (B) ACZ partition coefficient and ACZ solubility in CO_2 as a function of $scCO_2$ + EtOH (5% molar) density. Experimental: 40 °C (◯) and 50 °C (•). Dashed lines are just guides for the eye. Correlated ACZ solubility (Duarte et al., 2005): 40 °C (——) and 50 °C (——).

phase, more efficient and successful the SSI process will be (de Sousa et al., 2006a; Costa et al., 2010a; Kikic, 2009). On the other hand, the solubility of organic drugs in high pressure and SCF mixtures is also directly related to the density of this mobile phase which also depends on the employed operational temperature and pressure conditions. The relative magnitude of ACZ/$scCO_2$ + EtOH interactions and of water-swollen SCLs/ACZ/EtOH interactions should control the ACZ "solubility" in the swollen SCLs as well as its partition coefficient between the SCLs and the $scCO_2$ phase. If the ACZ/$scCO_2$ + EtOH interactions are less favourable than the water-swollen SCLs/ACZ/EtOH interactions, then ACZ will have a higher partition coefficient into the SCLs, which is a very positive aspect for the SSI process. Otherwise, the drug may be easily removed from the SCLs into the $scCO_2$ mobile phase during depressurization. However and considering the relatively low solubility of ACZ in $scCO_2$ + EtOH mixtures and that this solubility is not strongly dependent on pressure and temperature (Duarte et al., 2005), we may thus consider that density, drug solubility and the specific ACZ/$scCO_2$ + EtOH interactions are only slightly relevant for the entire process.

This can be also verified taking in consideration the results presented in Fig. 2A. In this figure we plot the total amount of ACZ that was loaded (per mass of wet lens) as a function of the density of the $scCO_2$ + EtOH (5% molar) mobile phase. Density was calculated according to $scCO_2$–EtOH solubility data (Duarte et al., 2005; Durling et al., 2007). As can be seen, the loaded amounts of ACZ are quite similar for all experimental densities with the exception of those assays carried out at 20 MPa. Therefore, and for similar densities of the $scCO_2$ mobile phase (~830 kg/m^3), the loaded ACZ amounts were strongly increased at this higher pressure condition. This means that pressure is playing an additional role and not just affecting the specific ACZ/$scCO_2$ + EtOH interactions and, consequently, the ACZ solubility in the SCF mobile phase.

Finally, the ACZ partition coefficients (between the SCLs and the supercritical fluid phase) and the ACZ solubilities in $scCO_2$ + EtOH (5% molar) are presented in Fig. 2B. Obtained results confirmed that the ACZ partition coefficients (as a function of the mobile phase density) are not clearly related or follow the same tendency as the one observed for the ACZ solubility (and for both isotherms). In contrast to the loaded ACZ amounts (Fig. 2A), the ACZ partition coefficients became quite similar for $scCO_2$ mobile phase densities of around 830 kg/m^3, despite the operational pressure and temperature conditions were quite different. This confirms our previous assumption that pressure is influencing the ACZ loaded amounts in a different way and not just because of its effect on the $scCO_2$ + EtOH (5% molar) density and on the corresponding ACZ solubility.

As the specific water-swollen SCLs/ACZ/EtOH interactions are not supposed to be strongly affected by operational pressure and temperature, pressure should be then primarily influencing the water-swollen SCLs/$scCO_2$/EtOH interactions which will promote the SCLs additional swelling and plasticization. Despite the transport properties of the mobile phase (namely viscosity and diffusivity) are not favoured at higher pressures, the above referred interactions seem to become very favourable at 20 MPa and thus it can be considered that larger amounts of the ACZ/$scCO_2$/EtOH mobile phase can be loaded/absorbed into the water-swollen SCLs, leading to a higher swelling degree and plasticization effect which will promote to the impregnation of higher amounts of ACZ (Costa et al., 2010a).

To the best of our knowledge, no scientific or technical literature covering the $scCO_2$ sorption/diffusion into SCLs was published so far. However, we already employed a gravimetric method to measure the $scCO_2$ sorption/desorption behaviour of some cross-linked acrylate-based copolymers which are typical constituent materials of ophthalmic devices (Duarte et al., 2006) and of commercial Hilafilcon B and Balafilcon A SCLs (Braga et al., 2011). The obtained results showed that the different employed SCLs, as well as experimental temperature/pressure and sorption duration, had significant effects on the $scCO_2$ sorption degrees and they can be straightforward correlated to the corresponding drug impregnation yields in these ophthalmic devices. In particular, it was found that the pressure increase (at constant temperature) led to higher $scCO_2$ sorption degrees into Balafilcon A SCLs. These results will be published in a near future (Braga et al., 2011).

Table 2
Total amounts of loaded ACZ in Balafilcon A SCLs (loaded by SSI at 40 °C and at 17 MPa). Intra-experimental and inter-experimental standard deviations are also presented.

	Loaded ACZ (μg/mg wet lens)[a]	
	Intra-experiments	Inter-experiments
SSI processing time, h (depressurization at 0.06 MPa/min)		
1	6.85 ± 1.24	12.07 ± 7.38
	17.29 ± 1.04	
2	15.33 ± 2.56	15.23 ± 0.14
	15.13 ± 2.34	
3	18.14 ± 3.46	19.70 ± 2.21
	21.26 ± 2.99	
Depressurization rate (MPa/min) (2 h of processing time)		
0.06	15.33 ± 2.56	15.23 ± 0.14
	15.13 ± 2.34	
0.15	8.73 ± 4.26	10.48 ± 2.48
	12.23 ± 3.36	

[a] Value ± standard deviation.

Finally and in addition, if more CO_2 and EtOH can diffuse and dissolve into the water-swollen SCLs the inner pH as well as the polarity of water will decrease, thus favouring the incorporation of more ACZ which is more soluble at very low pH values and has a higher solubility in EtOH than in water. The pH of pure water in contact with $scCO_2$ was reported to vary between 2.80 and 2.85 (for pressures ranging between 15 and 20 MPa and temperatures between 40 °C and 50 °C) (Toews et al., 1995) or to be around 3.0–3.2 (for pressures ranging between 4 and 8 MPa and temperatures between 25 °C and 35 °C) (Qi et al., 2004). ACZ water solubility decreases very quickly from 1 g/L (at pH 7.3) down to 0.57 g/L (at pH 5.9) which is the typical pH value for water when in contact with air at atmospheric pressure. Then, it still decreases down to 0.52 g/L (at pH 4.5) and finally it starts to increase again up to 0.63 g/L (for pH < 3.6) (Loftsson et al., 1996). This means that ACZ water solubility is higher at the employed experimental pressure and temperature conditions than in water in contact with air at room temperature and at atmospheric pressure. Moreover and besides the consequent decrease in water polarity, the dissolution of EtOH also contributes to the water pH decrease (Qi et al., 2004).

3.1.2. *Influence of impregnation/deposition duration and of depressurization rate*

For the second group of assays, those covering the effects of the impregnation duration (1, 2 and 3 h) and the variation of depressurization rate (0.06 and 0.15 MPa/min) and keeping pressure and temperature constant (17 MPa and 40 °C), the total amounts of loaded ACZ (per mass of wet lens) are presented in Table 2.

As can be seen, at constant pressure and temperature, the impregnation duration can be a critical process variable and longer processing periods clearly improved ACZ loading yields. This happens because the initial stages of the diffusion and sorption process (of the $scCO_2$/ACZ/EtOH mobile phase into SCLs), as well as the associated swelling and plasticization phenomena, are time-dependent processes. Therefore, for short processing periods, the maximum/equilibrium swelling/plasticization degree is not yet completely achieved and, only when the sorption and swelling equilibria are finally reached (for longer exposure periods), higher

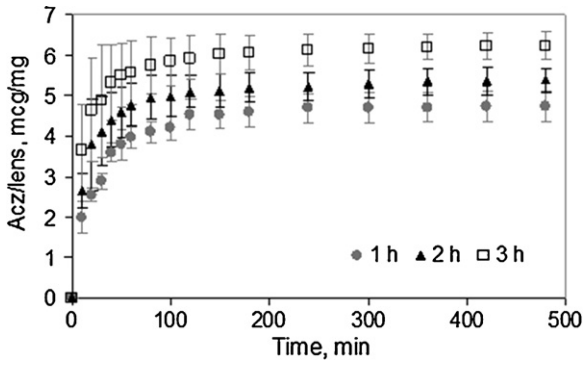

Fig. 3. In vitro kinetics of ACZ release from Balafilcon A SCLs as a function of employed processing time (1, 2 and 3 h). SCLs were SSI-loaded at 40 °C and 17 MPa.

loading yields can be observed (Costa et al., 2010a; Braga et al., 2011). Similar results were already found for a large number of $scCO_2$-polymer systems (Kikic, 2009; Duarte et al., 2006; Weinstein and Papatolis, 2006; Nalawade et al., 2006; Jiménez et al., 2007).

It can also be observed (Table 2) that system depressurization may as well play an important role on final loading efficiency: as the depressurization was rate increased (from 0.06 MPa/min to 0.15 MPa/min), slightly lower ACZ loading yields were obtained. As previously referred, the relative magnitude of the specific ACZ/$scCO_2$ + EtOH and of water-swollen SCLs/ACZ/EtOH interactions will control the ACZ "solubility" in the swollen SCLs as well as its partition coefficient between the SCLs and the mobile phase. However and based on the results for the first group of assays (at 17 MPa), density, ACZ solubility and the specific ACZ/$scCO_2$ + EtOH interactions did not seem to be very relevant for the ACZ loading efficiency. On the contrary, the same process conditions (pressure, temperature and EtOH and CO_2 incorporation into water-swollen SCLs) led to a local pH decrease inside SCLs that clearly favoured the water-swollen SCLs/ACZ/EtOH interactions. Thus, we should expect that, for systems presenting relevant drug–polymer interactions (when compared to the drug–$scCO_2$ interactions), more drug will be impregnated/deposited if depressurization rates are kept low. On the contrary and for systems with unfavourable drug-polymer interactions, faster depressurization rates are probably preferable, in order to promote a quick drug trapping and incorporation inside the polymeric material and to avoid its removal into the $scCO_2$ mobile phase.

Therefore, and in conclusion, the employed SSI method proved to be a feasible and "tunable" process for the impregnation/deposition of ACZ into commercial Balafilcon A SCLs, as the total ACZ loaded amounts can be controlled simply by changing the operational conditions (pressure, temperature, processing time and depressurization rate). Consequently and for therapeutic purposes, this helpful and advantageous feature will permit to adjust the final ACZ release levels into specific and desired therapeutic limits.

3.2. Drug release kinetics

The in vitro kinetics of ACZ release results are presented at Fig. 3, only for those systems involving the study of processing time. SCLs were impregnated at 40 °C and 17 MPa and the corresponding drug release experiments were carried out for 8 h, in saline water, at 37 °C and under orbital stirring. All kinetic drug release curves (for systems involving the study of processing time and depressurization rate) were fitted using the procedures referred in Section 2 and the corresponding correlated parameters and correlation statistics are presented at Table 3.

As expected, these kinetics release results reflect the already discussed effects of processing time in terms of the obtained ACZ loading yields (higher yields for longer processing times) and, as can be seen, all processed SCLs present similar ACZ release profiles: an initial burst release period (12–30 min), followed by a release period in which ACZ release was more sustained (until 120 min) and finally, a very slow release period (until 8 h/480 min of release). Nevertheless, and by the simultaneous wear of two SCLs by patients, the discrete (non-accumulated) released mass of ACZ (considering the total mass of two wet SCLs) is well above the required therapeutic limit for glaucoma treatment (even for those late slow release periods). For example and despite several ACZ therapeutic limits values can be found in literature for different ACZ formulations (Kaur et al., 2002; Friedman et al., 1985; Kaur et al., 2000; El-Gazayerly and Hikal, 1997), the ACZ release rates that led to an efficient IOP decrease were attained for 0.024–0.066 mg/h and for liposomal formulations (El-Gazayerly and Hikal, 1997). The ACZ release kinetic parameters (Table 3) were correlated using a linear regression analysis (by three different straight lines). All obtained drug release profiles fitted quite well to a drug delivery process that encompasses three well-defined regions, each one of them presenting different release rates. The most relevant release periods are the constant release rate period (CRR), which is mainly due to drug at/near the SCLs surface, and the final diffusional release rate period (DRR). The corresponding kinetic parameters are the mass transfer rate (M_{CRR} and the M_{DRR}, μg/min) and the duration of it (t_{CRR} and t_{DRR}, min). The falling rate period (also characterized by M_{FRR} and t_{FRR}), is located between the two above described release periods.

As referred, all ACZ-loaded contact lenses presented initial burst-release behaviour and the CRR period (highest release rate) was completed in short time intervals (with t_{CRR} always lower than 30 min). The mass transfer rates (in the CRR period) clearly increase as processing time increases, and decrease as employed depressurization rate increases. As a result, the duration of the CRR period follows an opposite trend. For the diffusional release rate period (DRR), the mass transfer rates are much lower than the corresponding values for the CRR period, despite the fact that they follow the same trend with the processing time. However, the effect of the depressurization rate in the DRR period is the opposite of the one observed for the CRR period. These kinetic results may be an indication that, and despite it leads to lower ACZ loading yields, faster depressurization rates may favour drug depth impregnation over surface/near-surface impregnation, thus decreasing initial burst release (at the CRR period) and promoting a more prolonged and sustained ACZ release (at the DRR period).

A practical application of these release kinetics results could be the estimation of the total ACZ that was released during 8 h release period (which is a period quite similar to the typical SCLs daily wear). For example and for each SCL (processed during 1 h), ACZ was released at a 1.1×10^{-1} μg/min rate, during approximately 28 min, followed by 1.3×10^{-2} μg/min rate during 117 min, and finally a 0.6×10^{-3} μg/min rate until the end of the 8 h of release. In the end, and considering the wearing of two SCLs and their wet weights, the total ACZ released (for 8 h) was approximately 0.33 mg/day. Same calculation procedures can be employed for those SCLs loaded at other experimental conditions.

Finally, it is expected that the observed in vitro initial ACZ burst release profiles will not be problematic and will be strongly diminished at in vivo studies (or in final therapeutic applications) due to the formation of the post-lens tear film between the SCLs and the cornea. The post-lens tear film is a closed and limited liquid environment that will accumulate the drug that was burst-released, protecting it by the tear flow and the tear drainage, and thus retaining it for prolonged residence time periods. This will force drug permeation and absorption through the cornea and, consequently, it will promote drug sustained release as well as it will increase

Table 3
Correlation results of the in vitro kinetics of ACZ release from Balafilcon A SCLs (loaded by SSI at 40 °C and 17 MPa).

Experimental processing conditions	Correlated kinetic parameters						
	1st line		2nd line		3rd line		[a]Error × 10^2
	$M_{CRR} \times 10$ (μg/min)	t_{CRR} (min)	$M_{FRR} \times 10^2$ (μg/min)	t_{FRR} (min)	$M_{DRR} \times 10^3$ (μg/min)	t_{DRR} (min)	
SSI processing time, h (depressurization at 0.06 MPa/min)							
1	1.11 ± 0.15	27.9 ± 8.9	1.3 ± 0.3	117.3 ± 13.0	0.6 ± 0.01	480	6.6 ± 1.1
2	2.40 ± 0.78	17.1 ± 6.3	1.7 ± 0.6	78.7 ± 6.3	1.0 ± 0.29	480	3.2 ± 3.0
3	3.63 ± 1.08	12.0 ± 0.7	2.9 ± 2.0	61.4 ± 3.1	1.3 ± 1.00	480	1.6 ± 1.0
Depressurization rate, MPa/min (2 h of processing time)							
0.06	2.40 ± 0.78	17.1 ± 6.3	1.7 ± 0.6	78.7 ± 6.3	1.0 ± 0.29	480	3.2 ± 3.0
0.15	1.44 ± 0.70	27.0 ± 7.6	1.9 ± 0.7	110.5 ± 0.2	1.3 ± 0.51	480	5.7 ± 0.5

[a] Legend: Fitting error $= \frac{1}{N}\sqrt{\sum\left(\frac{y^{calc}-y^{obs}}{y^{obs}}\right)^2}$.

drug bioavailability. These studies were not performed in this work but several other works dealing with the use of therapeutic SCLs confirmed these suppositions (Hiratani et al., 2005; Gulsen and Chauhan, 2004; Gulsen et al., 2005).

3.3. SCLs and ACZ characterization

3.3.1. ACZ degradation

ACZ degradation studies during SCLs storage in saline water were carried out for 19 days (the maximum employed storage period of all processed SCLs) and in the absence of light. Analyses were performed at room temperature (~21 °C) and at 7 °C, for pure/non-processed ACZ and for some selected ACZ-loaded SCLs (processed at 20 MPa, 50 °C, 1 h of processing and 0.06 MPa/min of depressurization rate). The UV spectra of these ACZ solutions were followed spectrophotometrically (from 200 nm up to 400 nm) and obtained results indicated that ACZ degradation did not occur, for both temperatures, despite the fact that other authors previously reported that ACZ was photo-labile under aerobic and anaerobic conditions (Vargas et al., 1998).

3.3.2. Scanning electron microscopy

SEM–EDX analyses were performed in order to identify the presence of ACZ on the surface/inside SCLs as well as to observe if any visible and relevant morphological changes that may have occurred in SCLs and in ACZ during processing. For these studies, SCLs and ACZ were processed in $scCO_2$ at 20 MPa and 40 °C, for 1 h and depressurized at 0.06 MPa/min. Some of these SEM micrographs are presented in Fig. 4. No visible surface changes were found on SCLs after processing (concave and convex surfaces) for all tested magnifications. In addition, the obtained SCLs surfaces seem very similar to those observed by other authors and by other morphological surface analysis methods such as atomic force microscopy (AFM) and cryo-SEM (Guryca et al., 2007). For non-processed SCLs, sodium chloride salt small particles (from the SCLs commercial storage liquids) were indentified and are visible on lenses surfaces. After the SSI processing, it is evident the presence of precipitated ACZ particles (of different sizes and shapes) on both convex and concave SCLs surfaces. This can help to explain the already discussed initial burst release profiles that were observed for all processed SCLs (even admitting that most of this surface deposited ACZ was released to the storage liquids prior to the drug release experiments). No apparent morphological differences were observed between non-processed (commercial) ACZ and processed ACZ. In addition, non-processed ACZ particles seem to be quite similar (in sizes and shapes) to those precipitated in SCLs surfaces after the SSI processing: ACZ particles are mostly of a plate-like type despite some needle-like particles can also be found (but in lower amounts). However, the presented micrographs (Fig. 4) are not fully representative of all precipitated/processed ACZ particles.

In Fig. 5 other SEM micrographs are presented (obtained at different magnifications). The existence of ACZ on processed SCLs convex and concave surfaces can be observed. Some ACZ particles can be found on SCLs cross-sections but their presence should be due to the employed lenses cutting procedures which probably drag these particles onto these cross-section surfaces. In fact, it is not plausible to observe large amounts of precipitated ACZ on SCLs cross sections since, and by the SSI processing, it was molecularly dispersed inside water-swollen SCLs (the SEM preliminary dehydration SCLs treatment removed water from lenses but ACZ was kept inside the polymeric structure without precipitating as particles).

Surface-precipitated ACZ particles present again different sizes and shapes. However, and in presented micrographs, the precipitated ACZ particles are mostly of a needle-type (despite some plate-like crystals can also be found). This may indicate that the employed SSI changed the morphology of surface-deposited ACZ particles. However, caution should be taken since SEM is not the appropriate method to positively conclude about this. In addition, sample preparation for the SEM–EDX analyses, like dehydration using ethanol solutions, may also affect ACZ particle morphology.

3.3.3. X-ray diffraction and FTIR-IR spectroscopy

X-ray (WAXD) diffraction data figure of non-processed and of $scCO_2$-processed ACZ (at 20 MPa and 40 °C, for 1 h and depressurized at 0.06 MPa/min) is presented as supplementary online material. These experiments were thus carried out in order to verify if ACZ crystallinity and polymorphism can be really affected by $scCO_2$ processing. ACZ is known to exist in two different crystalline forms (polymorphs), namely monoclinic (mod. I) and triclinic (mod. II), having needle- and plate-like crystal shapes, respectively, and presenting different IR spectra and WAXD patterns (Griesser et al., 1997; JCPDS-ICDD, 2001). The predominant type (of most commercial ACZ samples) is known to be the triclinic (plat-like shape) polymorph (Griesser et al., 1997). This was confirmed by our WAXD analyses and supplementary material also shows that no apparent crystallinity/polymorphism change was promoted by the scCO2 processing since non-processed and processed ACZ samples present quite similar patterns, and the observed peak intensity variations may be simply due to the preferential orientation of ACZ particles during sample preparation (artefacts or even to their spatial random arrangement) or to some small differences in the ACZ mass of the analyzed samples. These results were also confirmed by FTIR-ATR (not presented) in which the spectra of non-processed and of $scCO_2$-processed ACZ were identical and the ACZ crystalline form was found to be triclinic (mod. II) (Griesser et al., 1997).

The FTIR-ATR spectra (not presented) of non-processed SCLs (control) and of ACZ-loaded SCLs did not show any differences between them and thus it seems that the SSI process did

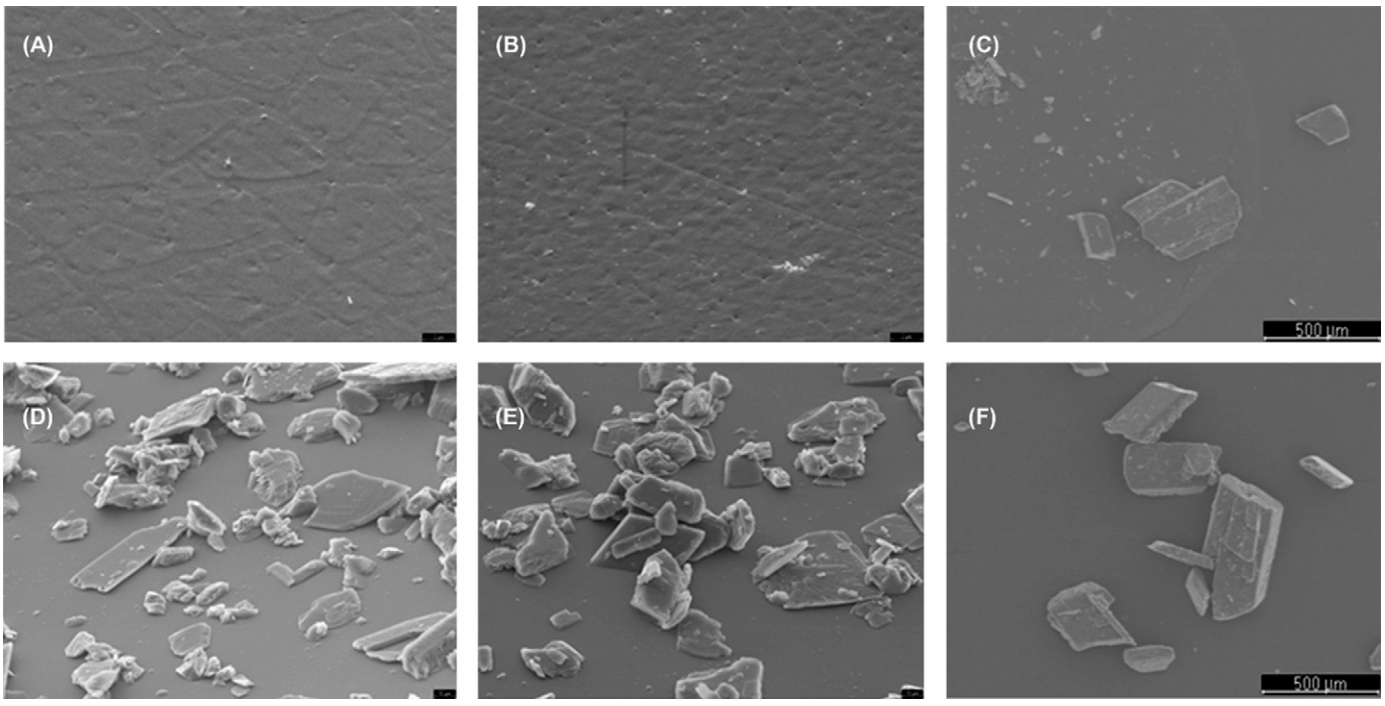

Fig. 4. SEM–EDX micrographs. (A) Non-processed Balafilcon A SCLs, concave surface, 4000× (scale 2 μm). (B) Non-processed Balafilcon A SCLs, convex surface, 4000× (scale 2 μm). (C) Non-processed ACZ. (D) Processed Balafilcon A SCLs, concave surface, 500× (scale 10 μm). (E) Processed Balafilcon A SCLs, convex surface, 500× (scale 10 μm). (F) Processed ACZ. SCLs and ACZ were processed at 20 MPa and 40 °C, for 1 h and depressurized at 0.06 MPa/min.

not promote relevant changes in any of the characteristic SCLs structural peaks (Roeges, 2004). In addition, the spectra of processed SCLs did not indicate the presence of ACZ, which is probably due to the relatively low amounts of loaded/deposited ACZ (when compared to wet SCLs mass) and to the ATR method limitations which just analyses the SCLs polymeric surface and regions nearby.

3.3.4. Thermal analyses – DMTA, DSC and TGA

Thermomechanical characteristics are very important commercial SCLs properties since they will control some of their functional features as ophthalmic devices, including comfort. TGA, DSC and DMTA results are presented in Table 4 for processed (at different operational conditions and after being released) and for non-processed (control) SCLs. Wet SCLs water contents were quantified

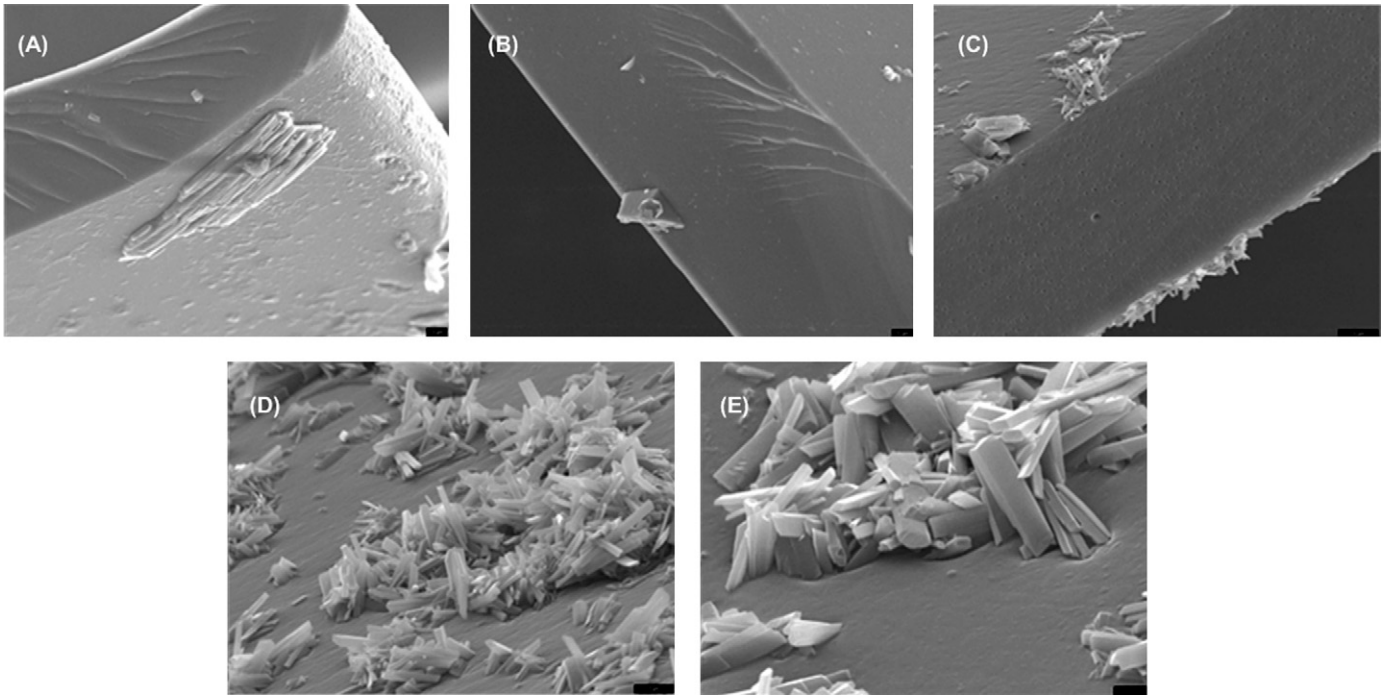

Fig. 5. SEM–EDX micrographs for processed Balafilcon A SCLs (at 20 MPa and 40 °C, for 1 h and depressurized at 0.06 MPa/min). (A, B) Lens surface and cross-section, 500× (scale 10 μm). (C) Lens surface and cross-section, 1000×. (D) Lens surface, 2000× (scale 5 μm). (E) Lens surface, 4000× (scale 2 μm).

Table 4
Thermomechanical analyses (TGA, DSC and DMTA) of processed (at different experimental conditions) and of non-processed (control) Balafilcon A SCLs.

Experimental processing conditions				TGA[a]	DSC[a]	DSC[b]	DMTA[b]
Impregnation time (h)	Depressurization rate (MPa/min)	T (°C)	P (MPa)	Water content (%)	T_g (°C)		
1	0.06	40	15.0	31.8 ± 1.9	32.30	–	39.4
			17.0	36.8 ± 4.0	33.53	–	44.1
			18.0	34.2 ± 3.5	30.45	–	51.7
			20.0	40.0 ± 1.4	30.25	–	44.2
1	0.06	50	15.0	30.1 ± 4.2	31.14 ± 0.16	–	51.1
			17.0	35.5	31.97	–	49.3
			18.0	33.1	30.79	–	38.7
			20.0	35.0 ± 0.5	30.92	–	45.3
2	0.06	40	17.0	–	30.00 ± 0.29	–	–
3	0.06			–	30.82 ± 0.59	–	–
1	0.15			–	31.31 ± 0.49	–	–
Control (non-processed lenses)				34.0 ± 1.4	31.20 ± 0.50	51 ± 3	57.9

Average values ± standard deviation.
[a] Wet SCLs.
[b] Dehydrated SCLs.

by TGA while their glass transition temperatures (T_g) were obtained by DSC. DMTA was also employed to obtain T_g values for dehydrated SCLs while DSC was employed to obtain the same property for dehydrated control (non-processed) SCLs.

TGA results showed that there are some small differences between processed (30.1–40.0%) and non-processed SCLs water contents (34%). There are no clear trends between final SCLs water contents and the employed SSI operational conditions. These values are also slightly different from the value indicated by manufacturer (36%). However, and considering the experimental standard deviation ranges and the handling difficulties of such fragile and complex materials (namely the observed quick evaporation of superficial water), all presented values can be considered quite similar. Therefore, we may assume that the employed SSI processing method and the subsequent ACZ release did not change the ability of SCLs to absorb and to retain water. Finally, and for all processed and non-processed SCLs, TGA thermograms (not presented) indicated that SCLs dehydration started around 70 °C while degradation events started to occur around 230 °C.

Like for SCLs water contents, the glass transition temperatures of processed wet (hydrated) SCLs were not altered after the impregnation process and after the release process (30.00–33.53 °C), at different employed experimental conditions and when compared to the T_g value obtained for control SCLs (31.20 °C). Again, no clear tendencies were found between obtained T_g values and the employed SSI operational conditions. Moreover, the obtained T_g values (by DSC) are in good agreement with other literature values for hydrated Balafilcon A SCLs. The same agreement with literature values was found for the T_g of dehydrated/freeze-dried SCLs (51.0 °C) obtained by DSC (Tranoudis and Efron, 2004; Fornasiero et al., 2005) despite the fact that, and as expected, T_g values are quite sensitive to the drying method and to the SCLs residual water contents (bonded water).

The glass transition temperatures of dehydrated (freeze-dried for 24 h) of processed (at different operational conditions) and of non-processed (control) SCLs were also obtained by DMTA. For the non-processed dehydrated SCLs the T_g values obtained by DSC and by DMTA are, as expected, not equal since the employed techniques are different (Menard, 2008). DMTA is considered to be more sensitive than DSC (in wide ranges of temperature) and it is usually recommended for the T_g determination of polymers namely for highly crosslinked polymeric materials such as SCLs (Menard, 2008; Duncan, 2008). Once again, a variation in the obtained T_g values can be observed (38.7–51.1 °C) and no evident tendency with the employed processing conditions can be noticed. The undetermined and unknown SCLs remaining water contents (after freeze-drying) and any later vapour water sorption (during storage) as well as the associated handling difficulties during analyses of these materials, can be accountable for these variations. The same reasons can be pointed out to explain why the T_g of non-processed SCLs (57.9 °C) is higher than those obtained for processed SCLs.

Two of the obtained DMTA thermograms are presented in Fig. 6, for non-processed and for processed SCLs (at 40 °C, 20 MPa, 1 h of

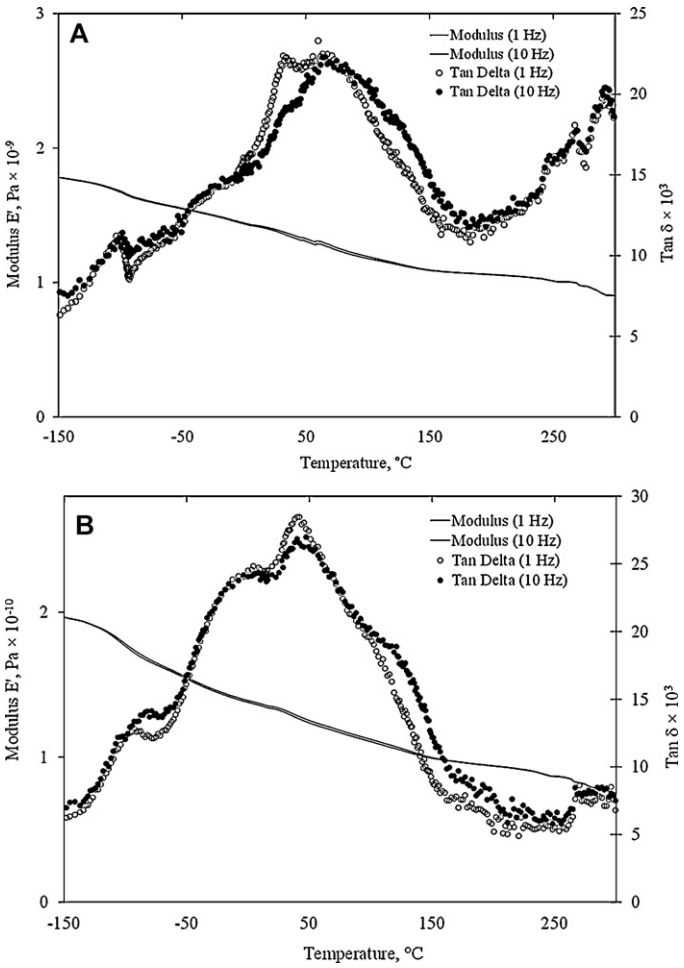

Fig. 6. DMTA analyses for dehydrated Balafilcon A SCLs. (A) Non-processed (control) SCLs; and (B) processed SCLs (at 20 MPa and 40 °C, for 1 h and depressurized at 0.06 MPa/min).

Table 5
Water contact angles and surface free energies (calculated by the Owens–Wendt–Rabel–Kaelble (OWRK) method) of processed (at different experimental conditions) and of non-processed (control) Balafilcon A SCLs.

Temperature (°C)	Pressure (MPa)	Contact angle (H_2O,°)	σ_S (mN/m)	σ_S^D (mN/m)	σ_S^P (mN/m)
Control lenses	–	95 ± 12	22.7 ± 2.16	22.1 ± 2.04	0.64 ± 0.72
40	15	103 ± 13.7	21.9 ± 0.870	21.3 ± 0.840	0.72 ± 0.23
	17	96 ± 10	20.9 ± 1.77	20.5 ± 1.72	0.46 ± 0.42
	18	102 ± 3.10	21.1 ± 0.870	20.5 ± 0.850	0.64 ± 0.17
	20	104 ± 6.60	20.9 ± 0.870	19.8 ± 0.840	1.08 ± 0.250
50	15	116 ± 10.5	23.5 ± 0.870	23.5 ± 0.870	0.01 ± 0.02
	17	103 ± 9.60	22.9 ± 1.47	22.8 ± 1.46	0.090 ± 0.19
	18	102 ± 8.40	22.8 ± 1.40	22.8 ± 1.39	0.050 ± 0.14
	20	93 ± 5.9	22.1 ± 1.79	21.4 ± 1.72	0.74 ± 0.50

Average values ± standard deviation; σ_S: surface free energy; σ_S^D: dispersive component; σ_S^P: polar component.

processing and 0.06 MPa/min of depressurization rate). The tan δ curves exhibit a first peak around −100 °C, for processed and for control SCLs, which usually corresponds to bonded water according to Lee et al., 1975 (cited by Tranoudis and Efron, 2004). Bonded water is considered to be "non-freezable" at temperatures around and below the atmospheric frozen point of water (0 °C) (it is considered to be "freezable" just at/or below −93 °C). Then, at temperatures around 0 °C, other thermal event appears which is more pronounced for processed SCLs and that corresponds to the freezing process of the still existing non-bonded water (Tranoudis and Efron, 2004; Fornasiero et al., 2005). This confirms what was previously referred concerning the incomplete drying of SCLs and explains why the glass transition temperature of non-processed SCLs (57.9 °C) was higher than for the processed SCLs (in this case, 44.2 °C). After the T_g peaks, other thermal events can be observed: those starting around and after 100 °C, which should correspond to the free-water evaporation and, at higher temperatures, other events that probably should correspond to the beginning of the thermal degradation processes.

3.3.5. Contact angles and surface free energy measurements

Table 5 presents the contact angle measurements and the free energy results (calculated using the OWRK method) for non-processed (control) and for ACZ-loaded SCLs (processed at various experimental conditions). Results indicated that the employed SSI processing method did not greatly affect the average water contact angles for Balafilcon A SCLs (93–116°) and the average surface free energies (20.9–23.5 mN m^{-1}) when compared to control SCLs (95°, 22.7 mN m^{-1}, respectively). Moreover, the obtained contact angle values were quite similar to those already reported in literature (as 110°) (Jones et al., 2006). The variability as well as the standard deviations observed in these results can be explained by drying (water loss) of the SCLs during the measurements, since the sessile drop method is highly sensitive to SCLs surface dehydration (Costa et al., 2010a; Read et al., 2009). It is clear that the most important contributions to the surface energies obtained derive from the dispersive surface energy component. Therefore, all processed SCLs kept their wettability properties and their surface characteristics after processing.

3.3.6. Optical measurements

In order to verify if drug loading and the employed SSI and release processes affected SCLs optical characteristics, several experimental optical measurements (frontal power, focus position and light transmittance) of non-processed (control), of SSI-processed and of SSI-processed/released SCLs were performed and are presented in Table 6. From these results it can be verified that focus position values were quite similar for all tested SCLs (17.1–17.4 cm) and were in good agreement with the corresponding value for control SCLs (17.3 cm). The same was observed for the obtained light transmittance values (92.5–96.0%), for SSI-processed SCLs despite some small differences can be noticed. These differences were probably due to the typical handling/measurement problems of such fragile ophthalmic devices. For processed/released samples, the obtained light transmittance values are a little lower but these differences are not statistical

Table 6
Optical properties of processed (at different experimental conditions), of processed and released and of non-processed (control) Balafilcon A SCLs.

Pressure (MPa)	Temperature (°C)	Frontal power (D)	Focus position (cm)	Light transmittance (%)
Control lenses	−4.00	17.3	95.0	
Processed samples				
15	40	−4.00	17.3	96.0
17		−4.00	17.1	95.0 ± 1.4
18		−4.00	17.2	93.0 ± 1.4
20		−4.00/−4.25	17.4	94.5 ± 0.7
15	50	−4.00	17.1	95.0 ± 0.0
17		−4.00	17.4	92.5 ± 2.1
18		−4.00	17.1	96.0 ± 0.0
20		−4.00/−4.25	17.4	94.0 ± 1.4
Processed and released samples				
15	40	−4.00	17.2	92.0
17		−4.00	17.2	93.0
18		−4.00	17.2	96.0
20		−4.00/−4.25	17.4	93.0
15	50	−4.00	17.2	93.0
17		−4.00	17.3	93.0
18		−4.00/−4.25	17.4	93.0
20		−4.00/−4.25	17.4	92.0

Average values ± standard deviation.

significant (at p-value < 0.05) for all samples (Analysis of Variance and Tukey test). The frontal power measurements were similar for processed and processed/released SCLs (−4.00 D/−4.25 D) and were in good agreement with control SCLs (−4.00 D). Despite the observed increase for the higher employed pressures at both temperatures (−4.25 D), these values were not statistical significant (at p-value < 0.05). Furthermore, those lenses presenting this frontal power variation (−0.25 D) are the same that showed the higher focus position values (17.4 cm) and these can be responsible for these frontal power variations. Therefore, and in conclusion, all processed and processed/released SCLs kept some of their most important optical properties which demonstrates that, and despite their potential therapeutic applications, they still can be employed for correction of refractive deficiencies purposes and perform as a combination product according to FDA regulations, for example (Novack, 2009; Lauritsen and Nguyen, 2009).

4. Conclusions

This work continued our recent research activities on the development of polymer-based ophthalmic drug delivery systems, namely on the preparation of therapeutic soft contact lenses. Previously, Balafilcon A SCLs were already impregnated with two anti-glaucoma drugs (ACZ and timolol maleate) using a SSI methodology and in which pressure and temperature, as well as the processing time and the depressurization rate, were kept constant while the nature and the effects of cosolvents (ethanol and water) concentration were studied. Now, the SSI process was employed to impregnate ACZ on same SCLs but this time always using a $scCO_2$ + EtOH (5% molar) and employing different pressure, temperature, impregnation time and depressurization rate operational conditions and in order to study the effects of these experimental conditions on the final drug-loading yields, on the obtained drug-release profiles, as well as on some of the SCLs important functional properties.

Results indicated that the employed SSI process is feasible and it proved to be a "tunable" process for the impregnation/deposition of ACZ into commercial Balafilcon A SCLs, as the total ACZ loaded amounts were easily controlled by changing the operational pressure, temperature, processing time and depressurization rate conditions. Consequently and for therapeutic purposes, this feature will permit to adjust the final ACZ release levels into specific and desired therapeutic limits.

Another great advantage of the SSI method is that it permits the impregnation/deposition of a specific and desired drug (considering the foreseen therapeutic application) into commercially available SCLs without interfering with the ophthalmic article manufacture and/or processing.

Finally, and like it was demonstrated in our recent works, ACZ-loaded SCLs prepared by the SSI method kept some of their most important thermomechanical, surface/wettability and optical properties, which shows that they can be simultaneously employed for medical applications and for correction of refractive deficiencies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.08.040.

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