Carbohydrate Polymers 98 (2013) 380–390

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Characterization, dissolution and in vivo evaluation of solid acetazolamide complexes

María J. Mora, Luis I. Tártara, Renée Onnainty, Santiago D. Palma, Marcela R. Longhi, Gladys E. Granero*

Departamento de Farmacia, UNITEFA, CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, X5000HUA, Argentina

ARTICLE INFO

Article history:
Received 25 March 2013
Received in revised form 29 May 2013
Accepted 15 June 2013
Available online 20 June 2013

Keywords:
Hydroxypropyl-β-cyclodextrin
Polymorphism
Acetazolamide
Triethanolamine
Freeze-drying

ABSTRACT

The effects of binary and ternary systems of acetazolamide (ACZ) with hydroxypropyl-β-cyclodextrin (HP-β-CD) alone or with triethanolamine (TEA) on the crystalline properties, dissolution and intraocular pressure (IOP)-lowering effect were investigated.

It was found that the crystal structure of ACZ powder could be modified by the processing conditions. Freeze-drying ACZ powder affected not only the particle morphology but also its polymorphic form and the starting ACZ was converted to pure form A upon freeze-drying treatment. Results provided by DSC/TGA, XRPD, SEM and FT-IR suggested the formation of inclusion complexes between ACZ with HP-β-CD alone or with TEA, obtained by the freeze-drying method and the conversion of the drug into the amorphous state. Binary and ternary systems of ACZ obtained by freeze-drying exhibited significantly enhanced ACZ dissolution rates. The IOP-lowering effects of ACZ and its complexes with HP-β-CD alone or with TEA were studied in normotensive rabbits. Whereas the maximum IOP-lowering effect (~4 mmHg, ~33%), obtained with these binary and ternary lyophilized ACZ systems occurred at around 90 min, the ternary system exhibited a longer maximum IOP-lowering effect peak compared with that of the binary system. These results are in line with those obtained from the dissolution studies, where the ternary system exhibited longer dissolution times compared to the lyophilized binary one.

Results obtained from the dissolution studies, also showed that freeze-drying the native crystalline form of ACZ significantly increased the dissolution rate of ACZ, thus improving the IOP-lowering effect of this drug.

1. Introduction

Acetazolamide (ACZ) [5-acetamido-1,3,4-thiadiazole-2-sulfonamide], a carbonic anhydrase inhibitor (CAI), is used orally for the reduction of intraocular pressure (IOP) in patients suffering from glaucoma. It is utilized in the pre-operative management of closed angle glaucoma or as an adjunct therapy in the treatment of open angle glaucoma (Kaur, Smitha, Aggarwal, & Kapil, 2002). In addition it has been evaluated as an antiepileptic and diuretic agent, for treating acute high altitude sickness, and most recently as a remedy against respiratory diseases and in preventing the adverse effects of drugs in the treatment of influenza (Martindale, 2005).

Lindenberg, Kopp, and Dressman (2004) tentatively classified ACZ as Class IV of the biopharmaceutics classification system (BCS) (Amidon, Lennernas, Shah, & Crison, 1995; Taub, Kristensen, & Frokjaer, 2002), indicative of its low solubility and low permeability. Later, Wu & Benet (2005) classified ACZ as Class IV in their biopharmaceutics drug disposition classification system (BDDCS). By virtue of the fact that ACZ has low solubility (0.72 mg/mL in water at 25 °C), the development of a new formulation of ACZ for oral administration is relevant in order to improve its physical and/or chemical properties.

Various methods have been described to enhance the solubility of poorly soluble drugs (e.g. the use of pro-drugs, the addition of surfactants, salt selection, solid dispersions and particle size reduction) (Leuner & Dressman, 2000). In addition, cyclodextrins (CDs), which are macrocyclic oligosaccharides, represent an important group of excipients used for this purpose. CDs have a unique structure, with a hydrophobic cavity and a hydrophilic exterior that can act as a host and form inclusion complexes with a variety of guest molecules. Upon complexation with a sparingly soluble guest, CDs can increase their solubility. Moreover, CDs often afford additional beneficial properties (e.g. stabilization of unstable active pharmaceutical ingredients and taste masking) (Brewster & Loftsson, 2007; Loftsson & Duchene, 2007).

We have previously observed that the simultaneous complexation and salt formation with triethanolamine (TEA) significantly increased the HP-β-CD solubilizing power for the sparingly

* Corresponding author. Tel.: +54 351 4334163; fax: +54 351 4334127.
E-mail addresses: graneroglady s@gmail.com, glagra@fcq.un.edu.ar (G.E. Granero).

0144-8617/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.carbpol.2013.06.012

water-soluble ACZ by forming a drug:HP-β-CD:TEA multicomponent system (Granero, Longhi, et al., 2008; Granero, Maitre, et al., 2008). In addition, after the preparation of inclusion complexes in solution, the drying step can be carried out by different methods such as sublimation (freeze-drying) or evaporation (spray-drying) (Miller, Carrier, & Ahmed, 2007). However, this drying can generate particulate materials that show different characteristics. From a practical point of view, amorphous CDs such as HP-β-CD are useful for the control of solid properties of poorly water-soluble drugs, because they can convert crystalline drugs to amorphous complexes which then usually have better solubility (Hirayama, Usami, Kimura, & Uekama, 1997; Hirayama, Wang, & Uekama, 1994).

It is important to control the crystallization and polymorphic transition of solid drugs, since crystal modifications affect various pharmaceutical properties, such as stability, solubility, dissolution rate and bioavailability (Junginger, 1976; Borka, 1991). Therefore, we considered it of interest to investigate the effect of HP-β-CD, with or without TEA, on the particle morphology and structure of ACZ using a variety of techniques, including scanning electronic microscopy (SEM), X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), thermogravimetric analysis (TG) and Fourier transform infrared (FT-IR). Also, the correlation between the in vitro dissolution profiles of the ACZ formulations and their performance was evaluated in vivo by assessing the capacity to lower the intraocular pressure (IOP) in normotensive rabbit eyes.

2. Materials and methods

2.1. Materials

ACZ (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) was obtained from Sigma® (99%, USA). HP-β-CD (MW = 1325–1400; degree of molar substitution, 7.0) was a gift from Ferromet S.A. (agent of Roquette in Argentina) and TEA was obtained from Aldrich® (98%, USA). All experiments were performed with analytical grade chemicals and solvents. A Millipore Milli-QWater Purification System generated the water used in these studies.

2.2. Animals

New Zealand white normotensive rabbits weighting 2–2.5 kg were used. Water and commercial food pellets were freely available except during the 12 h before starting the study in a temperature-controlled room (21 ± 5 °C) with water being provided ad libitum. Rabbits were exposed to 12 h light:12 h dark cycles in a temperature-controlled room (21 ± 5 °C). Animal management procedures conformed to the ARVO (Association for Research in Vision and Ophthalmology) resolution on the use of animals in research, the European Communities Council Directive (86/609/EEC). The Institutional Care and Use Committee of the Chemistry Faculty of Córdoba University, Córdoba, Argentina, reviewed and approved the protocols. After a week of adaptation in the facilities, animals were admitted to the experimental sessions.

2.3. Preparation of solid systems

Stoichiometric ratios of 1:1 or 1:1:1 were used to prepare solid complexes of ACZ with HP-β-CD alone or with TEA, respectively and their physical mixtures (PMs).

These PMs were prepared for comparative purposes by the addition of ACZ to an agate mortar containing powdered HP-β-CD or with HP-β-CD and TEA under manual agitation. Freeze-drying products were obtained by dissolving ACZ or ACZ, HP-β-CD alone or with TEA accurately weighed in distilled water. The whole solution was stirred on a magnetic stirrer for 24 h. After filtration, the solution was frozen overnight and then lyophilized over a period of 30 h using a freeze-drier (Labconco freeze dry system).

2.4. Powder X-ray diffractometry (XRPD)

The X-ray diffractograms of the different samples were carried out using a Philips PW 1710 Based (Holand) X-ray diffractometer with a Cu Kα anode and a curved graphite monochromator, operated at a voltage of 45 kV and a current of 30 mA, with a diffraction angle range of 2–60° and a step size of 0.035. The analysis was carried out at room temperature under ambient conditions.

2.5. Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra were recorded on a Nicolet 5 SXC FT-IR Spectrophotometer (Madison, WI, USA) and the potassium bromide disks were prepared by compressing the powder.

2.6. Scanning electron microscopy (SEM)

Microscopic morphological structures of the raw materials and the binary and ternary systems obtained by the different treatments were investigated and photographed using a scanning electron microscope (LEO Model EVO 40XVP). The samples were fixed on a brass stub using a double-sided aluminum tape. To improve the conductivity, samples were gold-coated under vacuum employing a sputter coater PELCO Model 3 with the magnification selected being sufficient to appreciate in detail the general morphology of the samples under study.

2.7. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

The DSC curves of the different samples were recorded on a DSC TA 2920 and the TGA curves on a TG TA 2920, both by applying a heating rate of 10 °C min^{-1}. The thermal behavior was studied over a temperature range of 25–300 °C/350 °C, by heating 1–3 mg of samples in aluminum-crimped pans under nitrogen gas flow. Data were obtained and processed using the TA Instruments Universal Analysis 2000 software.

2.8. In vitro dissolution studies

The dissolution rate studies were performed in a dissolution apparatus (Hanson SRII 6 Flak Dissolution Test Station, Hanson Research Corporation, Chatsworth, USA) using the paddle method according to USP XXX, at 37 ± 0.5 °C, and stirring at 75 rpm/min. Samples containing 50 mg of ACZ or a corresponding amount of the respective test formulation were sprinkled over the surface of 500 ml of diluted hydrochloric acid (0.1 M), and aliquots of the dissolution medium (2 ml) were taken at suitable time intervals. The withdrawn samples were then replaced by equal volumes of fresh dissolution medium maintained at the same temperature. Each solution was diluted and determined spectrophotometrically (Agilent Carry 60 spectrophotometer) at 263 nm. The results were expressed as mean % of drug released (±SD) at the given sampling time.

The dissolution profiles were evaluated using the dissolution efficiency at intervals of 15 and 20 min (DE_{15} and DE_{20}) (Khan & Rhodes, 1972).

Data from the release of ACZ were analyzed using the similarity factor (f_2) which is a logarithmic reciprocal square root

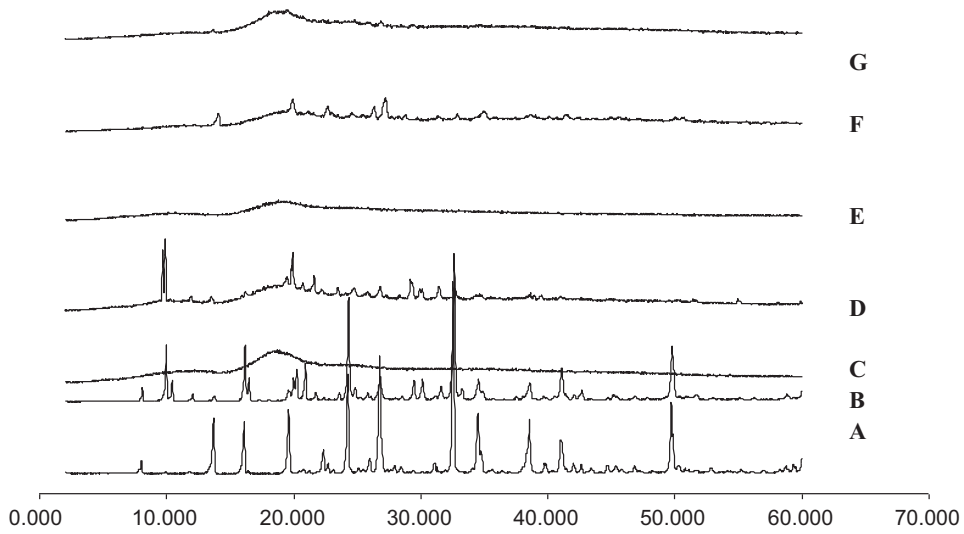

Fig. 1. Powder X-ray diffractograms (Cu-ja) for A: commercial ACZ, B: freeze-drying ACZ (ACZ_L), C: commercial HP-β-CD, D: physical mixture 1:1 ACZ:HP-β-CD (1:1 ACZ:HP-β-CD_{PM}), E: freeze-drying 1:1 ACZ:HP-β-CD (1:1 ACZ:HP-β-CD_L), F: physical mixture 1:1:1 ACZ:HP-β-CD:TEA (1:1:1 ACZ:HP-β-CD:TEA_{PM}) and G: freeze-drying 1:1:1 ACZ:HP-β-CD:TEA (1:1 ACZ:HP-β-CD:TEA_L).

transformation of the sum of the squared error and expresses the dissolution between the two curves as:

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t-1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where n is the number of sampling points, and R_t and T_t are the percentages dissolved of the reference and the test product, respectively, at each time point t.

For curves to be considered similar the f_2 values should be close to 100. Generally, f_2 values are greater than 50 (50–100), which implies an average difference of no more than 10% at the sample time points. This ensures equivalence of the two curves and thus of the performance of the test and the reference products.

2.9. IOP measurements

Intraocular pressure (IOP) was measured in mmHg using a Perkins MK2 tonometer (HS Clement Clarke, England) calibrated according to the manufacturer's instructions. Before tonometry, infant blepharostat was used to maintain the eyelids open during the measurements. Then, a mixture of topical anesthetic (0.5% solution of proparacaine HCl) and fluorescein salt (0.25% Solucion de Grant^{MR}, Alcon^{MR} Montevideo-Uruguay) were applied (50 μl)

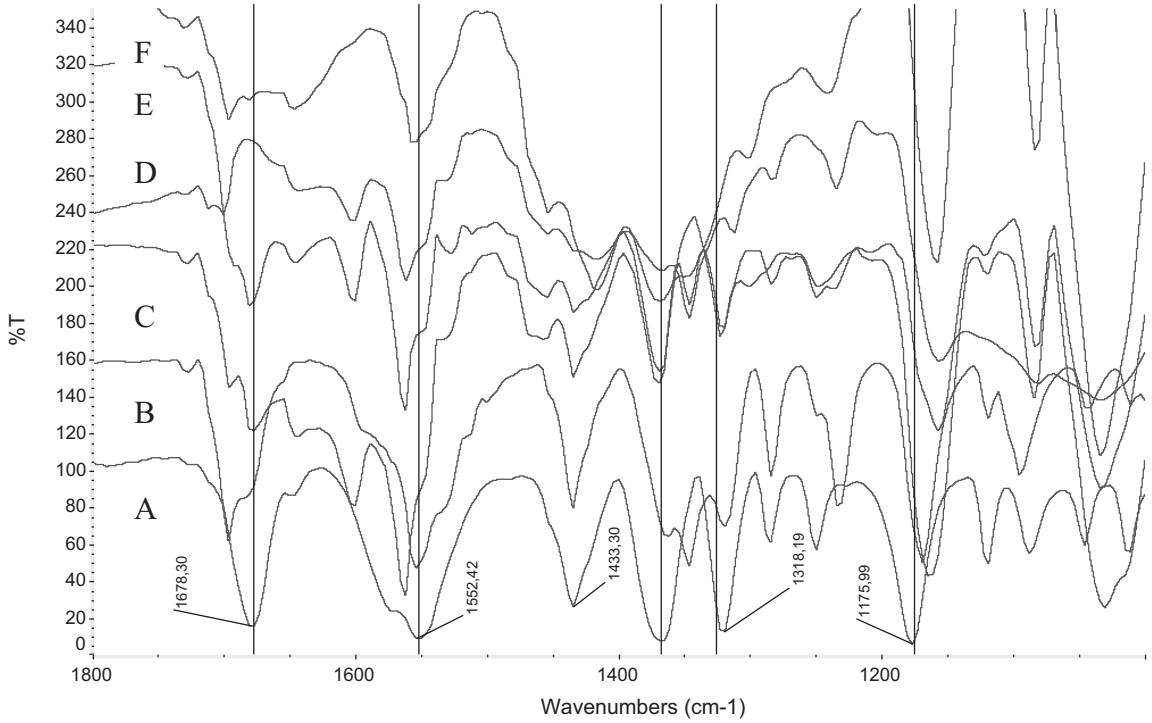

Fig. 2. FT-IR spectra for A: commercial ACZ, B: freeze-drying ACZ (ACZ_L), C: physical mixture 1:1 ACZ:HP-β-CD (1:1 ACZ:HP-β-CD_{PM}), D: freeze-drying 1:1 ACZ:HP-β-CD (1:1 ACZ:HP-β-CD_L), E: physical mixture 1:1:1 ACZ:HP-β-CD:TEA (1:1:1 ACZ:HP-β-CD:TEA_{PM}) and F: freeze-drying 1:1:1 ACZ:HP-β-CD:TEA (1:1 ACZ:HP-β-CD:TEA_L).

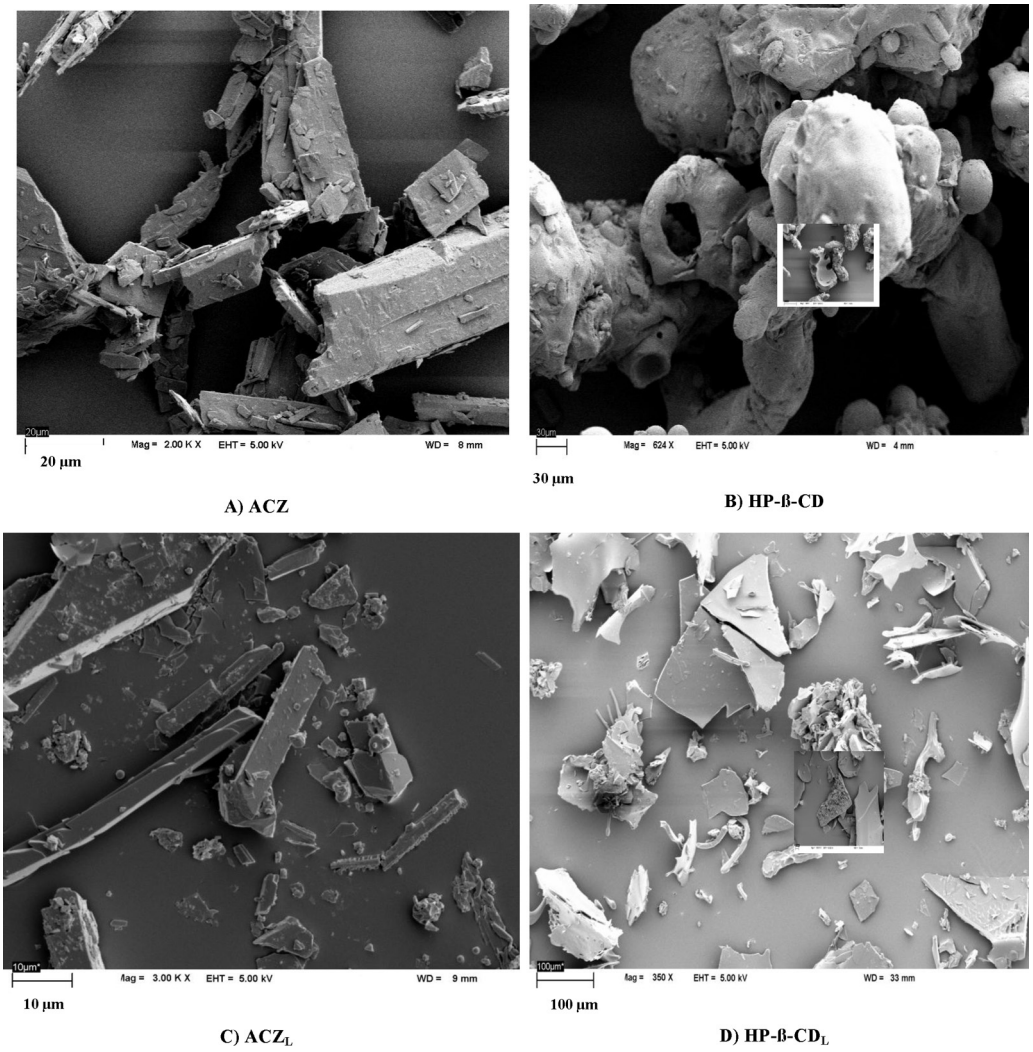

Fig. 3. SEM micrographs of A: commercial ACZ, B: commercial HP-β-CD, C: freeze-drying ACZ (ACZ$_L$), D: freeze-drying HP-β-CD (HP-β-CD$_L$).

to the cornea before each measurement of intraocular pressure was performed, in order to improve animal welfare during the test (proparacaine HCl) and to achieve the necessary contrast in the IOP measurement (fluorescein salt). The animals used were accustomed to the experimental procedure and IOP measurements were taken at baseline and at intervals of 30, 60, 90, 120, 150, 180 and 240 min with the experiments always being carried out at the same time of the day. Each formulation was administered as a suspension by oral route via a stomach tube with a manual restraint, after anesthesia had been applied by an intramuscular injection of ketamine hydrochloride (300 mg/kg, Ketafine® Brouwer, Buenos Aires, Argentina) with a wash-out time of three weeks. The dose of ACZ was always 9 mg/rabbit and the four different oral formulations of ACZ used were: (1) marketed ACZ power, (2) lyophilized ACZ power, (3) 1:1 ACZ:HP-β-CD lyophilized complex power, and (4) 1:1:1 ACZ:HP-β-CD:TEA lyophilized complex power. The percentage change of IOP from the baseline was determined for each animal for each IOP measurement. Group means and standard error of the mean (SEM) were calculated for each time point in six animals and the Student's *t*-test and ANOVA were used to analyze the data. Significance levels used were: $p < 0.05$; $\alpha = 0.05$.

3. Results and discussion

3.1. X-ray powder diffractometry (XRPD) analysis

Powder X-ray diffractograms were obtained from isolated samples of ACZ, physical mixtures (PM) and processed materials using the freeze-drying method (L), in order to investigate the characteristics of the obtained systems. The diffractograms obtained are shown in Fig. 1.

ACZ is known to exist in two crystal forms (A and B) (Griesser, Burger, & Mereiter, 1997). Two diffraction peaks characteristic of these forms were detected at ~9.9° and ~13.7° (2θ), respectively. The crystal form of the marketed ACZ powder was found to correspond to the polymorphic form B (Fig. 1A) (Baraldi, Gamberini, Tinti, Palazzoli, & Ferioli, 2009), and a polymorphic change representing form A was produced by lyophilizing the powder (Fig. 1B).

The XRD pattern of the physical mixture (PM) of the binary system 1:1 ACZ:HP-β-CD (1:1 AC:HP-β-CD$_{PM}$) mostly showed the diffraction peak characteristics of the individual components, excluding the occurrence of interactions within the CD cavity and/or amorphization phenomena during the blending of the components. It is important to note that ACZ underwent a polymorphic

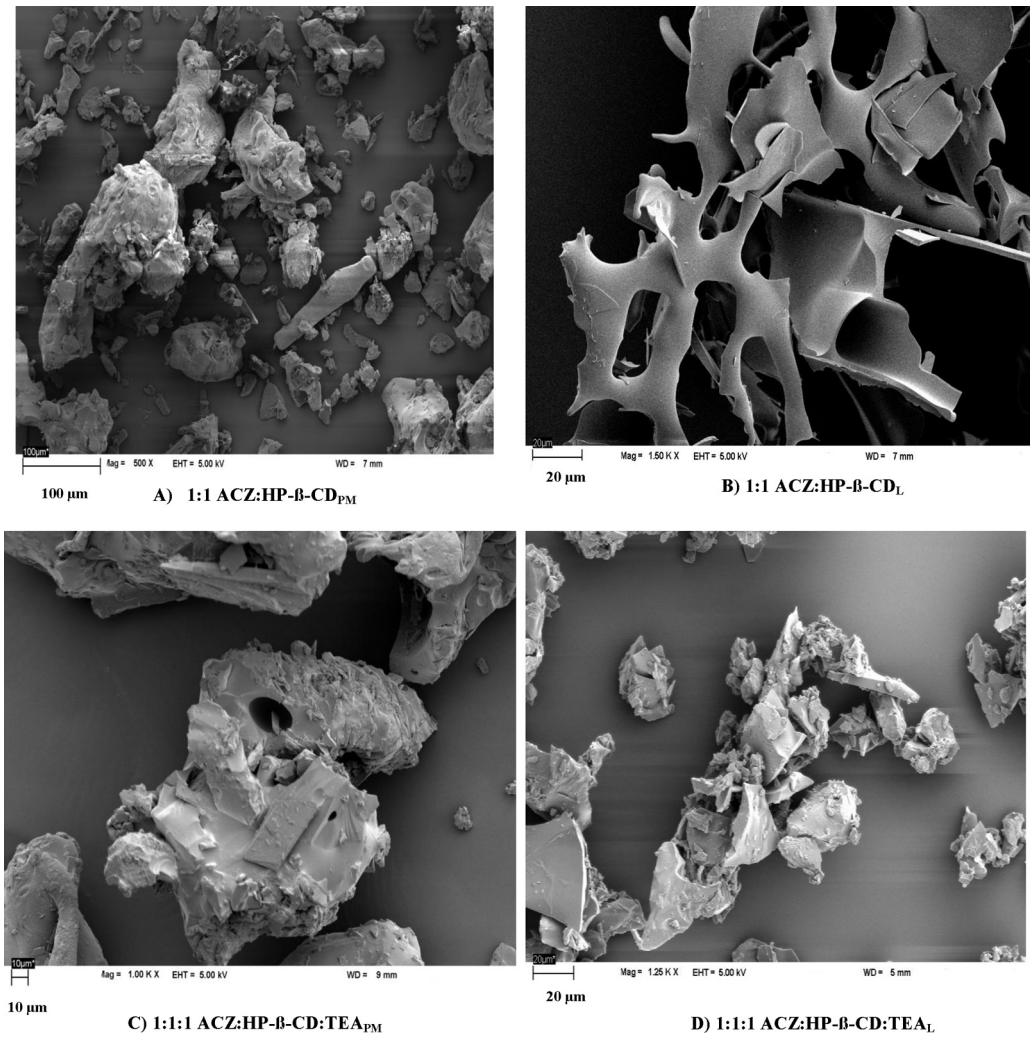

Fig. 4. SEM micrographs of A: physical mixture 1:1 ACZ:HP-β-CD (1:1 ACZ:HP-β-CD$_{PM}$), B: freeze-drying 1:1 ACZ:HP-β-CD (1:1 ACZ:HP-β-CD$_{L}$), C: physical mixture 1:1:1 ACZ:HP-β-CD:TEA (1:1:1 ACZ:HP-β-CD:TEA$_{PM}$) and D: freeze-drying 1:1:1 ACZ:HP-β-CD:TEA (1:1 ACZ:HP-β-CD:TEA$_{L}$).

transformation from form B to A (Fig. 1C) in this system. This phenomenon could be attributed to the fact that the solid-state properties of ACZ are mainly directed by strong intermolecular hydrogen bond forces that play an important role in stabilizing the molecular structure of this drug. ACZ crystallizes in the two polymorphic forms A and B, which differ in the spatial molecular arrangement and hydrogen bonding pattern (Seliger, Zagar, & Katisubsja, 2010). Thus, the physicochemical interactions that can be established between ACZ and the external surface of the HP-β-CD could affect the spatial arrangement and packing of the ACZ molecules causing different interaction forces between the ACZ molecules thereby producing the polymorphic change (Chatyrvedu, Gupta, Tandon, Sharma, Baraldi, & Gamberine, 2012; Iohara et al., 2012). In contrast, the freeze-dried product of the binary system (1:1 ACZ:HP-β-CD$_{L}$) showed a diffractogram typical of amorphous powders with traces of the residual crystalline drug being practically undetectable, thus suggesting that HP-β-CD inhibited the crystallization of ACZ through the formation of inclusion complexes (Fig. 1D). In the case of the ternary physical mixture of ACZ with HP-β-CD and TEA (1:1:1 ACZ:HP-β-CD:TEA$_{PM}$), its XRD pattern revealed the presence of several diffraction peaks that were indicative of the polymorphic form B of ACZ on a diffuse background, due to the amorphous carrier, indicating that ACZ had not suffered a polymorphic transformation in this system (Fig. 1E). In contrast, the XR diffraction pattern of the ternary system 1:1:1 ACZ:HP-β-CD:TEA obtained by the freeze-drying process (1:1:1 ACZ:HP-β-CD:TEA$_{L}$) was characterized by an amorphous halo between 10° and 30° (2θ), thus suggesting that the crystallization of ACZ was inhibited by the formation of inclusion complexes (Fig. 1F).

3.2. Fourier transform infrared spectroscopy (FT-IR)

IR spectroscopy is a valuable supplementary tool for the identification of new solid forms. Fig. 2 shows the FT-IR spectra of the ACZ powders and solids of the binary and ternary systems, where several characteristic FT-IR absorption bands and their assignments to ACZ can be observed as follows: 1678 cm^{-1} [ν(C=O)]; 1552 cm^{-1} [ν(N—H)]; 1552 cm^{-1} [ν(C=N)]; 1246 cm^{-1} [ν(C—N)]; 1318 and 1176 cm^{-1} [ν(SO$_2$)] (Chufán, Pedregosa, Ferrer, & Borrás, 1999).

The FT-IR spectrum of the lyophilized ACZ powder (ACZ$_{L}$) (Fig. 2B) was different from that one of the commercial ACZ (ACZ) (Fig. 2A). A significant shift of the C=O stretching vibration band to higher wave numbers (from 1678 cm^{-1} to 1697 cm^{-1}) of the ACZ$_{L}$ powder took place, which was not seen for the commercial ACZ, suggesting weaker intermolecular H-bonds. The presence of a different N—H···OH-bond was also responsible for the shift in the symmetrical and asymmetrical SO$_2$ stretching modes bands (1318 cm^{-1} and 1176 cm^{-1}) (Baraldi et al., 2009).

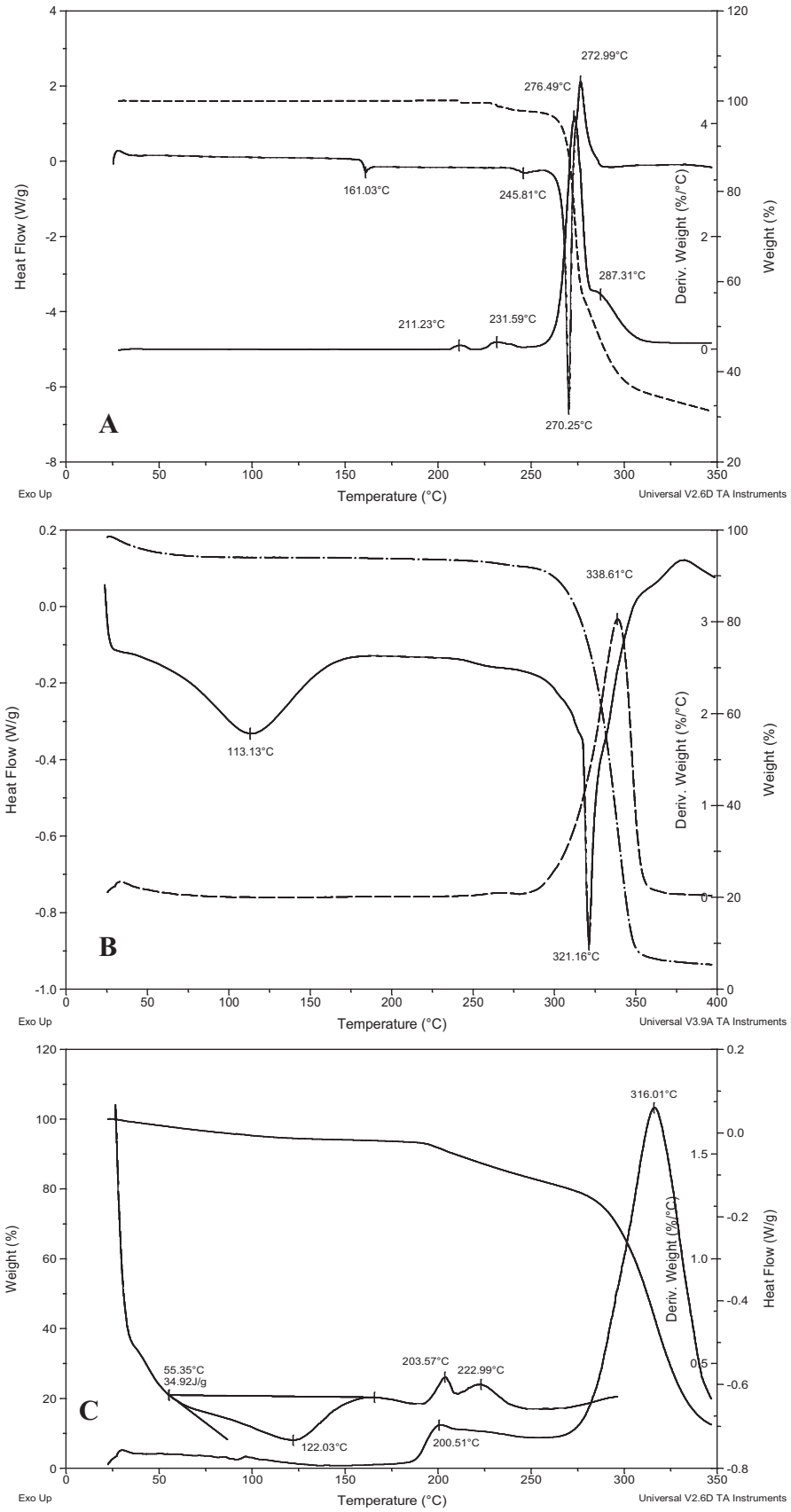

Fig. 5. DSC and TGA/DTG curves for A: commercial ACZ, B: freeze-drying ACZ (ACZ_L), C: physical mixture 1:1 ACZ:HP-β-CD (1:1 ACZ:HP-β-CD_{PM}).

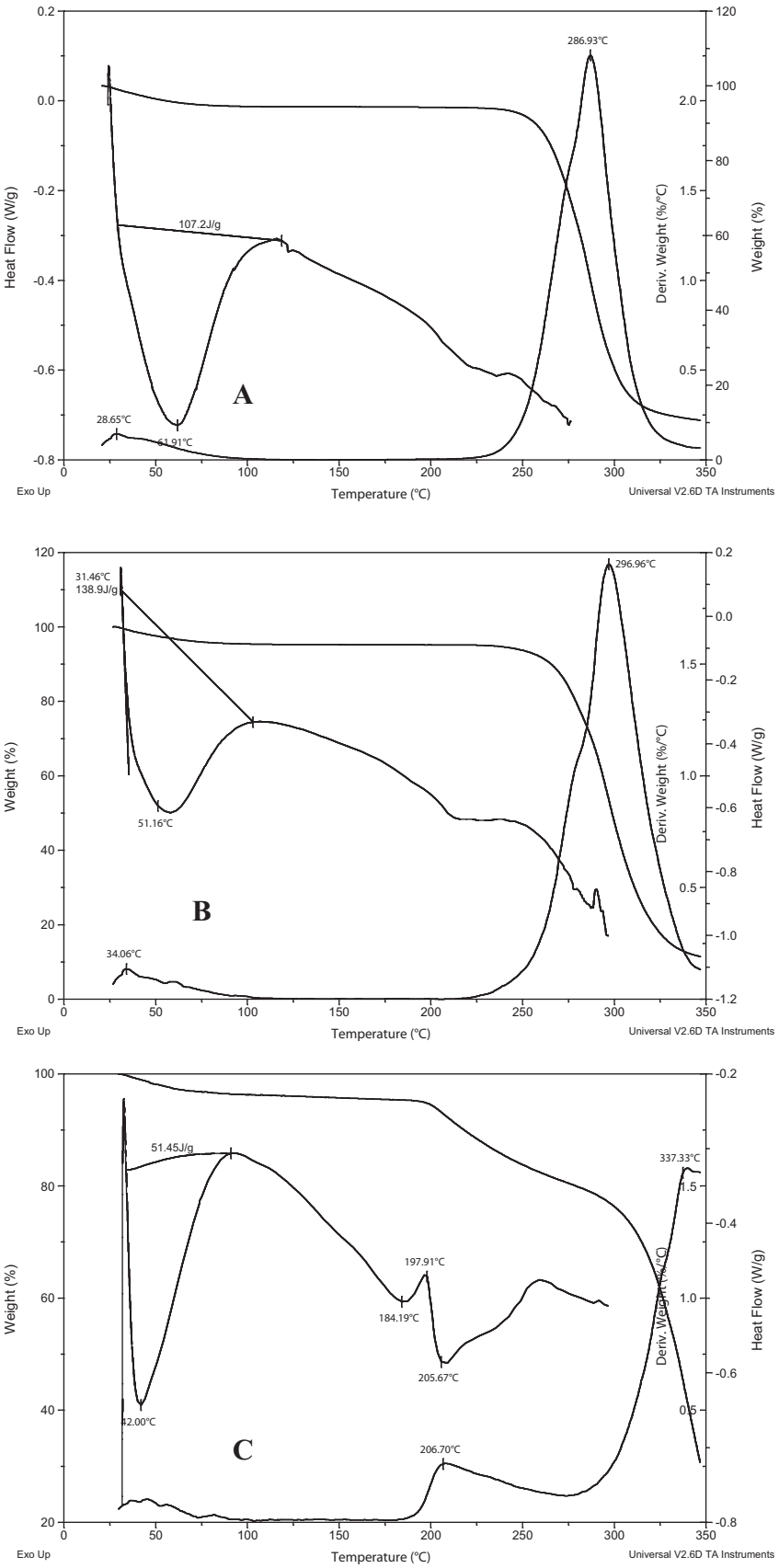

Fig. 6. DSC and TGA/DTG curves for A: freeze-drying 1:1 ACZ:HP-β-CD (1:1 ACZ:HP-β-CD_L), B: physical mixture 1:1:1 ACZ:HP-β-CD:TEA (1:1:1 ACZ:HP-β-CD:TEA_{PM}) and C: freeze-drying 1:1:1 ACZ:HP-β-CD:TEA (1:1 ACZ:HP-β-CD:TEA_L).

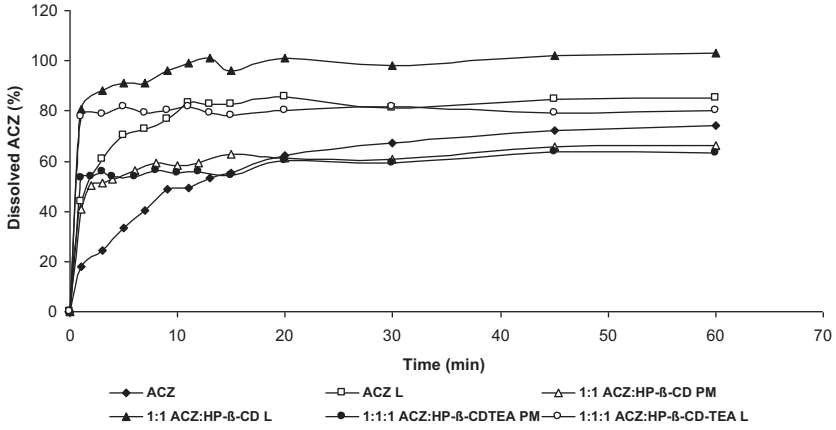

Fig. 7. In vitro release profiles of ACZ formulations.

The FT-IR spectra of both solid systems (corresponding to the 1:1 binary ACZ:HP-β-CD obtained by the freeze-drying process and the corresponding physical mixture in the region from 1800 cm^{-1} to 1000 cm^{-1}) are shown in Fig. 2C and D, respectively. Some bands of the host and the guest were affected by the formation of an inclusion complex, resulting in changes in the position and relative intensities. In the FT-IR spectrum of the freeze dried solid of the 1:1 ACZ:HP-β-CD binary system (1:1 ACZ:HP-β-CD_L) (Fig. 2D), the peak assigned to the C=O group of the ester of the drug was broad and shifted toward a lower frequency (i.e., 1681 cm^{-1})

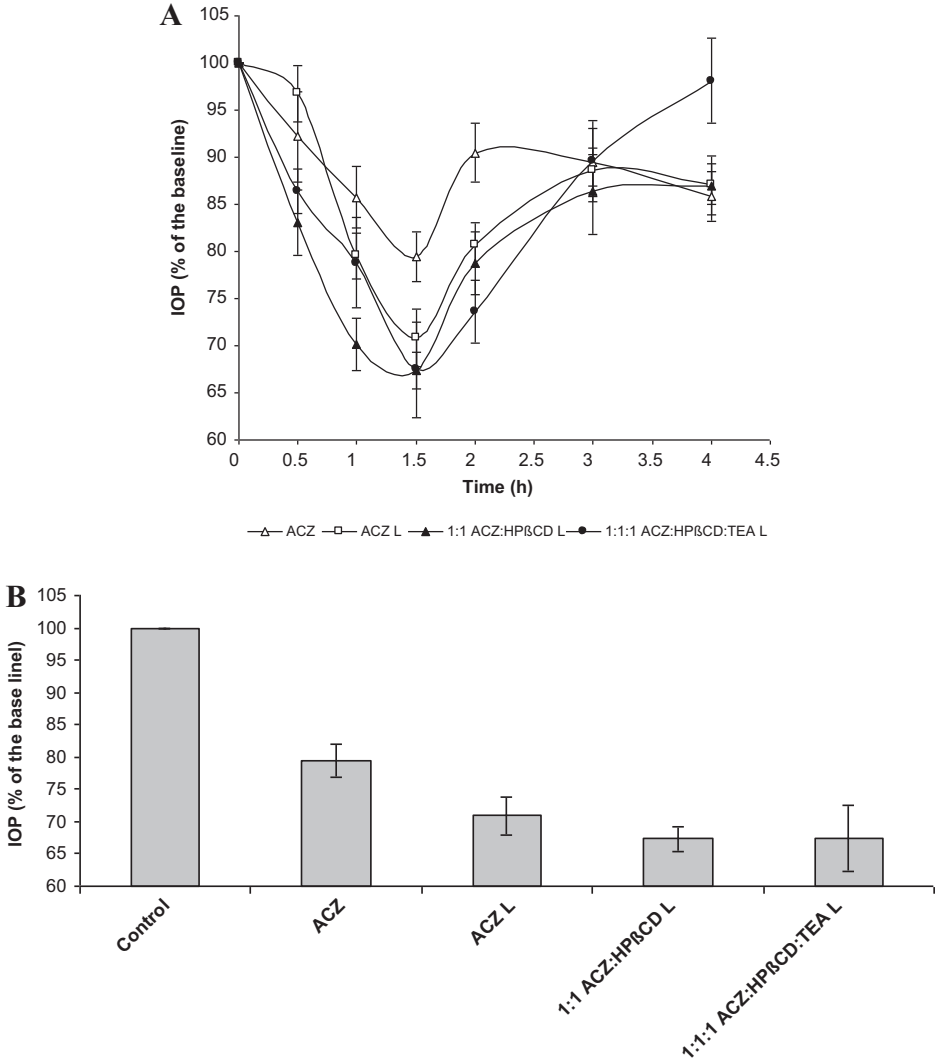

Fig. 8. A: Time-course of the different ACZ formulations-induced changes in intraocular pressure after a single instillation. B: Intraocular pressure measurement of treated eyes with various ACZ formulations in normotensive rabbits.

with respect to the one in the spectrum of the freeze-dried solid from the commercial ACZ powder (ACZ_L) (Fig. 2B), suggesting the formation of hydrogen bonds between the carbonyl group of ACZ and the hydroxyl groups of the host cavity during the inclusion complexation. These findings are in agreement with other authors (El-Nahhas, 1996; Fernandes, Vieira, & Veiga, 2002), who previously reported that when the carbonyl group is joined to a hydroxylic compound by hydrogen bonds, the stretching band is then displaced to a lower frequency due to a weakening of the carbonyl radical double bond.

In order to understand the effect of TEA on the association equilibrium between ACZ and HP-β-CD, the physical mixture and the lyophilized products of the ternary 1:1:1 ACZ:HP-β-CD:TEA system were obtained with their FT-IR spectra (1850–1000 cm^{-1}) being depicted in Fig. 2E and F, respectively. In the FT-IR spectrum of the freeze-dried ternary solid (1:1:1 ACZ:HP/β/CD:TEA_L) (Fig. 2F) it was observed that the band attributed to the carbonyl group of ACZ was up-shifted to higher wave numbers with respect to the corresponding frequency in the freeze-dried binary system (1:1 ACZ:HP-β-CD_L) (Fig. 2D). This effect may have been a result of the breakdown of the intermolecular hydrogen bonds formed between ACZ and HP-β-CD as a consequence of the weakening of the interaction between ACZ with HP-β-CD due to the presence of TEA. Related to this, a similar behavior had been previously found in the liquid state (Palma et al., 2009). In the FT-IR spectra of the physical mixtures of the binary and ternary systems (1:1 ACZ:HP-β-CD_{PM} and 1:1:1 ACZ:HP-β:CD_{PM}) there were no changes, since these were formed by the superposition of the spectra of the single components (Fig. 2C and E).

3.3. Scanning electron microscopy (SEM)

The SEM images for the raw materials (ACZ and HP-β-CD), 1:1 ACZ:HP-β-CD and ternary system with TEA, obtained by freeze-drying and their corresponding physical mixtures are shown in Figs. 3 and 4.

The image of the commercial ACZ power (Fig. 3A) revealed large acicular crystals together with small irregular particles, corresponding to the polymorphic form B of the drug (Griesser et al., 1997). A radical change in the original morphology of ACZ was observed as a consequence of the freeze-drying process of the pure drug. The lyophilization technique gave rise to amorphous pieces of irregular sizes with a lamellate aspect (Fig. 3C) with HP-β-CD appearing as amorphous particles without a definite shape (Fig. 3B). A drastic change in the shape and aspect of HP-β-CD particles was observed when the oligosaccharide was subject to the freeze-dried method, with the particles (Fig. 3D) being flat and angular, irregularly-shaped and without any relation to any known geometry.

In the physical mixtures of the binary 1:1 ACZ:HP-β-CD and ternary 1:1:1 ACZ:HP-β-CD:TEA systems the presence of unmodified particles of HP-β-CD was evident. However, as they were covered by drug crystals (Fig. 4A and C, respectively), no apparent interaction was revealed between both species in the solid state.

Freeze-dried particles of the 1:1 ACZ:HP-β-CD binary system (Fig. 4B) displayed a lamellate aspect, in which the original morphology of both components (ACZ and HP-β-CD) had disappeared.

The SEM microphotograph of the freeze-dried 1:1:1 ACZ:HP-β-CD:TEA product showed the formation of large masses of undifferentiated particles, which were different from those of raw materials. In this picture, it is possible to distinguish amorphous pieces with a lamellate aspect agglomerated with spherical particles, indicating the presence of two solid phases, probably as a consequence of a partial complexation of ACZ with HP-β-CD. The SEM analysis was found to be consistent with the FT-IR results.

3.4. Thermal analysis

The DSC and TGA/DTG curves of the raw materials (ACZ and HP-β-CD), 1:1 ACZ:HP-β-CD and the ternary system of ACZ with HP-β-CD and TEA, obtained by freeze-drying and their corresponding physical mixtures, are shown in Fig. 5. Unprocessed ACZ (Fig. 5A) was characterized by a single, sharp melting endotherm at 270.25 °C (ΔH = 176.1 J/g) during DSC analysis, thus confirming the crystalline nature and anhydrous state of the starting material, which was followed by an exothermic effect due to decomposition phenomena at higher temperatures (276.49 °C). The thermal profile of the ACZ was almost unaffected by the freeze-drying treatment (DSC curve is not shown). In the case of HP-β-CD (Fig. 5B) owing to its amorphous nature, a broad endothermic peak was observed at about 113.13 °C, corresponding to the dehydration process. In addition, the base shift around 338.61 °C may have resulted from a degradation process of HP-β-CD. It is known that when guest molecules are embedded in the cavity of the CD or in the crystal lattice, their melting, sublimating and/or boiling points generally shift to a different temperature or disappear, as in the case of CD decomposition (Marques, Hadgraft, & Kllaway, 1990).

The DSC curves of the binary and ternary lyophilized products (Fig. 6B and C) showed a broad endothermic band corresponding to cyclodextrin dehydration and the disappearance of the endothermic peak at 270.25 °C due to the melting of ACZ, thereby confirming its amorphous state and/or a drug–carrier interaction. This thermal behavior may indirectly point to the formation of an inclusion complex between the components, which reduced the intensity of the thermally induced interaction between these components. However, in the DSC curves of the solid products prepared by a physical mixture of ACZ with HP-β-CD alone or with TEA (Figs. 5C and 6A), the disappearance of the melting and decomposition peaks of ACZ may have being due to the overlapping of these peaks with the one attributable to the thermal decomposition of the HP-β-CD, although this latter peak shifted to a lower temperature, thus indicating a small degree of interaction between ACZ and HP-β-CD.

Fig. 5 also shows the TGA/DTG curve of the 1:1 ACZ:HPβCD binary solid product obtained by freeze-drying. From this TGA/DTG curve, the step from room temperature up to about 90 °C was due to the removal of water molecules located in the HP-β-CD cavities and in the interstices between the macrocycles. The solid binary product prepared by freeze-drying showed a mass loss of 4.61% (as mass fraction) of water, which plain HP-β-CD hydrate revealing a similar well-defined step from room temperature up to about 80 °C with a mass loss of 5.54%. As the partial occupation of the HP-β-CD cavities by the guests is usually accompanied by a reduction of hydration waters, this supports the assumption (from powder XRD and FT-IR data) that a new supramolecular structure was formed between HP-β-CD and the guest ACZ, involving a partial inclusion of the molecule. After the dehydration step, no further mass loss was observed until decomposition started. It is quite interesting to note that the weight loss curves for the 1:1 ACZ:HP-β-CD binary product had a lower initial decomposition temperature than pure HP-β-CD or free ACZ. These results indicate that the ACZ's usual thermal properties were altered after inclusion complexation, and provide further evidence for the existence of a significant host–guest interaction in this solid product.

The liberation of crystal water from the cavity of HP-β-CD in the ternary 1:1:1 ACZ:HP-β-CD:TEA system obtained by freeze-drying and physical mixture was observed as broad endothermic peaks in the range of 25–150 °C. The elimination of CH_2OH groups during the degradation of the TEA occurred in the second stage with a corresponding exothermic event in the temperature range of 190–270 °C (Yilmaz, Topcu, & Karadag, 2002) related to the decomposition of the ternary complex. It should be noted that the exothermic peak assigned to the decomposition of the TEA was visible on the DSC

Table 1
Dissolution efficiency after 15 min and 20 min and similarity factors among ACZ formulations.

Formulations	DE_{15}[a]	DE_{30}[a]	Reference formulation	Test formulation	f_2
ACZ	55.2	67.4	ACZ	ACZ_L	28.07
ACZ_L	82.9	84.4	1:1 ACZ:HP-β-CD_{PM}	1:1 ACZ:HP-β-CD_L	20.92
1:1 ACZ:HP-β-CD_{PM}	62.9	65.5	1:1:1 ACZ:HP-β-CD:TEA_{PM}	1:1:1 ACZ:HP-β-CD:TEA_L	32.36
1:1 ACZ:HP-β-CD_L	96.0	98.0	ACZ	1:1 ACZ:HP-β-CD_L	15.32
1:1:1 ACZ:HP-β-CD:TEA_{PM}	54.4	59.4	ACZ	1:1:1 ACZ:HP-β-CD:TEA_L	23.14
1:1:1 ACZ:HP-β-CD:TEA_L	78.1	81.6			

[a] Dissolution efficiency.

curve for the ternary lyophilized system, which seemed indicate of that TEA was not inside the HP-β-CD cavity but instead interacting with the external side of it. These results are in agreement with a previous report on these systems in the liquid state (Palma et al., 2009).

Derivative curves of the weight loss with temperature (d*m*/d*t*) of the solid ternary 1:1:1 ACZ:HP-β-CD:TEA systems obtained by physical mixture and freeze-drying methods were characterized (at least) by the presence of two mass loss events: one associated with decomposition of the TEA in the range 180–250 °C; and a second at a higher temperature in the range 250–350 °C, associated with the decomposition of the corresponding ternary system.

3.4.1. *In vitro dissolution study*

Poorly water-soluble drugs exhibit a number of negative clinical effects, such as rate-limiting dissolution, slow absorption and low bioavailability. These drugs tend to be eliminated from the gastrointestinal tract before they have the opportunity to fully dissolve and be absorbed into the systemic blood circulation. In this context, the dosage of a poorly soluble drug should be increased to reach a suitable therapeutic drug concentration in blood, potentially resulting in local toxicity and discomfort in patients. Thus, enhancement of the dissolution rate in biological fluids is the first objective in the delivery of poorly soluble drugs.

The dissolution profiles of the systems evaluated are presented in Fig. 7. The calculated 15 and 30 min DE values and similar factors among the different products are given in Table 1.

A distinctive difference in the ACZ release between the freeze-dried ACZ (ACZ_L) and the commercial ACZ powder (ACZ) was observed, with the faster release of ACZ_L in comparison with ACZ possibly being attributed to an increase in the surface area of the drug as a result of particle size reduction and the polymorphic change in the drug to Form A, which probably had a better solubility.

By comparing the dissolution profiles of the binary 1:1 ACZ:HP-β-CD systems obtained by freeze-drying and physical mixture (1:1 ACZ:HP-β-CD_L and1:1 ACZ:HP-β-CD_{PM}), it was observed that the drug dissolution rate of 1:1 ACZ:HP-β-CD_L was evidently higher than that one of its corresponding physical mixture. The fast dissolution of the lyophilized binary product might have been attributed to the inclusion of the drug into the HP-β-CD cavity and to the capability of HP-β-CD of improving the drug wettability. In addition, the formation of the amorphous state of drug, as indicated by the DSC and XRD studies, may also have contributed to the enhanced dissolution of this product.

Upon analyzing the dissolution curves of the ternary freeze-dried 1:1:1 ACZ:HP-β-CD:TEA system (1:1:1 ACZ:HP-β-CD-TEA_L), it was found that the addition of TEA into the binary 1:1 ACZ:HP-β-CD system clearly led to a delay in the ACZ dissolution rate (Fig. 7), as particle agglomeration could be observed in the SEM images of this ternary system. Concerning the dissolution performance, this is an indication that the agglomerates formed upon drying the ternary system were apparently so strong that their disintegration compromised the overall dissolution process.

Summing up, although the best results were therefore obtained with the lyophilized binary system (1:1 ACZ:HP-β-CD_L), the 1:1:1 ACZ:HP-β-CD:TEA_L also achieved satisfactory rates of dissolution.

3.4.2. *In vivo intraocular pressure-lowering efficacy study*

Improving the bioavailability of poorly dissolving drugs in biological fluids is one of the greatest challenges faced by scientists in pharmaceutical research, since increasing the dissolution velocity is the primary and prerequisite step by which solid formulations can achieve an enhanced bioavailability.

Results of the IOP measurements are displayed in Fig. 8A and B. It can be seen from Fig. 8A that in less than 90 min (approximately), the change in IOP was greater for the binary and ternary lyophilized ACZ complexes with HP-β-CD alone or with TEA than those of the commercial and lyophilized ACZ powders, although the general shape of the profile was similar.

The maximum IOP-lowering effect (∼4 mmHg, ∼33%) obtained with these binary and ternary lyophilized ACZ systems occurred at approximately 90 min, although the ternary lyophilized system exhibited a maximum IOP-lowering effect peak at a later time compared with that of the binary lyophilized system. These results are in line with those obtained from the dissolution studies, where the ternary system exhibited longer dissolution times compared to the binary one.

Results obtained from the dissolution studies also showed that freeze-drying the native crystalline ACZ significantly improved the dissolution rate of ACZ, thus leading to a better the IOP-lowering effect for this compound.

4. Conclusion

The currently used poorly water-soluble model drug (acetazolamide) exhibits a clear solid-state dependent oral absorption in rabbits. Amorphous ACZ was shown to have an improved IOP-lowering effect compared to its crystalline counterparts, which was in line with a much faster release rate of the ACZ lyophilized powder in comparison with the crystalline ACZ. In vivo IOP studies have also demonstrated that the binary and ternary complexes of ACZ with HP-β-CD alone or with TEA obtaining by lyophilization showed a pronounced improvement of the dissolution profile of ACZ compared with those of ACZ powders, which was accompanied by a significant enhancement in the IOP-lowering effect of ACZ.

The positive results demonstrate that binary and ternary complexes of ACZ with HP-β-CD alone or with TEA prepared by freeze-drying may be a promising method for the enhancement of the IOP-lowering effect of the poorly soluble drug ACZ.

Acknowledgements

The authors thank FONCyT Préstamo BID 1728/OC-AR, PICT 1376, the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT), and the Consejo Nacional de Investigaciones Científicas y Tecnológicas de la Nación (CONICET) for their financial support. We also thank Ferromet S.A. (agent of Roquette in

Argentina) for their donation of hydroxypropyl-β-cyclodextrin. We thank Dr. Paul Hobson, native speaker, for revision of the manuscript.

References

Amidon, G. L., Lennernas, H., Shah, V. P., & Crison, J. R. (1995). A theoretical basis for a biopharmaceutic drug classification – The correlation of in-vitro drug product dissolution and in-vivo bioavailability. *Pharmaceutical Research*, *12*, 413–420.

Baraldi, C., Gamberini, M. C., Tinti, A., Palazzoli, F., & Ferioli, V. (2009). Vibrational study of acetazolamide polymorphism. *Journal of Molecular Structure*, *918*, 88–95.

Borka, L. (1991). Review on crystal polymorphism of substrates in the European Pharmacopoeia. *Pharmaceutical Acta Helvetiae*, *66*, 16–22.

Brewster, M. E., & Loftsson, T. (2007). Cyclodextrins as pharmaceutical solubilizers. *Advanced Drug Delivery Reviews*, *59*, 645–666.

Chatyrvedu, D., Gupta, V., Tandon, P., Sharma, A., Baraldi, C., & Gamberine, M. C. (2012). Intermolecular charge transfer and vibrational analysis of hydrogen bonding in acetazolamide. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *99*, 150–159.

Chufán, E. E., Pedregosa, J. C., Ferrer, S., & Borrás, J. (1999). Spectroscopic behavior of metal drug complexes. Infrared spectra of Cu(II) dimer complexes with acetazolamide (H2acm) and an analogue sulfonamide (B-H2ats). *Vibrational Spectroscopy*, *20*, 35–45.

El-Nahhas, S. A. (1996). Physico-chemical characteristics of carbamazepine-β-cyclodextrin inclusion compounds and carbamazepine-PEG solid dispersions. *Pharmazie*, *51*, 960–963.

Fernandes, C. M., Vieira, M. T., & Veiga, F. J. B. (2002). Physicochemical characterization and in vitro dissolution behavior of nicardipine–cyclodextrins inclusion compounds. *European Journal of Pharmaceutical Sciences*, *15*, 79–88.

Granero, G. E., Longhi, M. R., Becker, C., Junginger, H. E., Kopp, S., Midha, K. K., et al. (2008). Biowaiver monographs for immediate release solid oral dosage forms: Acetazolamide. *Journal of Pharmaceutical Sciences*, *97*, 3691–3699.

Granero, G. E., Maitre, M. M., Garnero, C., & Longhi, M. R. (2008). Synthesis, characterization and in vitro release studies of a new acetazolamide-HP-β-CD-TEA inclusion complex. *European Journal of Medicinal Chemistry*, *43*, 464–470.

Griesser, U. J., Burger, A., & Mereiter, K. (1997). The polymorphic drug substances of the European pharmacopoeia. Part 9. Physicochemical properties and crystal structure of acetazolamide crystal forms. *Journal of Pharmaceutical Sciences*, *86*, 352–358.

Hirayama, F., Usami, M., Kimura, K., & Uekama, K. (1997). Crystallization and polymorphic transition behavior of chloramphenicol palmitate in 2-hydrozypropyl-β-cyclodextrin matrix. *European Journal of Pharmaceutical Sciences*, *5*, 23–30.

Hirayama, F., Wang, Z., & Uekama, K. (1994). Effect of 2-hydroxypropyl-b-cyclodextrin on crystallization and polymorphic transition of nifedipine in solid state. *Pharmaceutical Research*, *11*, 1766–1770.

Iohara, D., Yoshida, K., Yamaguchi, K., Anraku, M., Motoyama, K., Arima, H., et al. (2012). Cyclodextrin-induced change in crystal habit of acetylsalicylic acid in aqueous solution. *Crystal Growth & Design*, *12*, 1985–1991.

Junginger, H. (1976). Spray drying polymorphic drugs. Part 3. Spray drying of chloramphenicol palmitate. *Pharmaceutical Zeitung*, *121*, 1107–1110.

Kaur, I. P., Smitha, R., Aggarwal, D., & Kapil, M. (2002). Acetazolamide: Future perspective in topical glaucoma therapeutics. *International Journal of Pharmaceutics*, *248*, 1–14.

Khan, K. A., & Rhodes, C. T. (1972). Effect of compaction pressure on the dissolution efficiency of some direct compression systems. *Pharmaceutica Acta Helvetiae*, *47*(10), 594–607.

Leuner, C., & Dressman, J. (2000). Improving drug solubility for oral delivery using solid dispersions. *European Journal of Pharmaceutics and Biopharmaceutics*, *50*, 47–60.

Lindenberg, M., Kopp, S., & Dressman, J. B. (2004). Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, *58*, 265–278.

Loftsson, T., & Duchene, D. (2007). Cyclodextrins and their pharmaceutical applications. *International Journal of Pharmaceutics*, *329*, 1–11.

Marques, H. C., Hadgraft, J., & Kllaway, I. (1990). Studies of cyclodextrin inclusion complexes I: The salbutamol–cyclodextrin complex studies by phase solubility and DSC. *International Journal of Pharmaceutics*, *63*, 259–266.

Martindale. The complete drug reference. Electronic version. (2005). In S. Sweetman (Ed.). London, UK: Pharmaceutical Press, Thomson. MICROMEDEX, Greenwood Village, Colorado.

Miller, L. A., Carrier, R. L., & Ahmed, I. (2007). Practical considerations in development of solid dosage forms that contain cyclodextrin. *Journal of Pharmaceutical Sciences*, *96*, 1691–1707.

Palma, S. D., Tartara, L. I., Quinteros, D., Allemandi, D. A., Longhi, M. R., & Granero, G. E. (2009). An efficient ternary complex of acetazolamide with HP-β-CD and TEA for topical ocular administration. *Journal of Controlled Released*, *138*, 24–31.

Seliger, J., Zagar, V., & Katisubsja, J. N. (2010). 14N NQR, 1H NMR and DFT/QTAIM study of hydrogen bonding and polymorphism in selected solid 1,3,4-thiadiazole derivatives. *Physical Chemistry Chemical Physics*, *12*, 13007–13019.

Taub, M. E., Kristensen, L., & Frokjaer, S. (2002). Optimized conditions for MDCK permeability and turbidimetric solubility studies using compounds representative of BCS classes I–IV. *European Journal of Pharmaceutical Sciences*, *15*, 331–340.

Wu, C-Y., & Benet, L. Z. (2005). Predicting drug disposition via application of BCS: Transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharmaceutical Research*, *22*, 11–23.

Yilmaz, V. T., Topcu, Y., & Karadag, A. (2002). Thermal decomposition of triethanolamine. *Thermochimica Acta*, *383*, 129–135.