Expert Opinion

informa
healthcare

Promising complexes of acetazolamide for topical ocular administration

Gladys E Granero† & Marcela R Longhi
Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, Argentina

Importance of the field: Acetazolamide (ACZ), a carbonic anhydrase inhibitor (CAI), and other oral CAIs have been an integral part of antiglaucoma therapy for > 40 years. ACZ is used orally for the reduction of intraocular pressure in patients suffering from glaucoma. However, this treatment leads to unpleasant systemic side effects. The answer to the undesirable effects of ACZ is the topical delivery of this drug into the eye, where it could elicit its physiological action. However, the development of a topical formulation of ACZ is limited by its poor ocular bioavailability, which can be largely attributed to its poor penetration coefficient and poor biphasic solubility.

Areas covered in this review: This review offers an overview of different approaches to delivering ACZ to the eye, highlighting the potential of the ternary system ACZ:HP-β-CD:TEA as a tool for formulating aqueous ACZ eye drop solutions.

What the reader will gain: A critical analysis is provided to highlight the key issues to design formulations containing hydrophilic cyclodextrins.

Take home message: The ACZ:HP-β-CD:TEA complex is an important new approach to improve the ocular bioavailability of this drug. This approach may be applied to other CAIs in the future.

Keywords: acetazolamide, cyclodextrins, multicomponent inclusion complexes, ophthalmic drug delivery system

Expert Opin. Drug Deliv. (2010) 7(8):943-953

1. Introduction

Despite numerous scientific efforts, efficient ocular drug delivery remains a challenge for pharmaceutical scientists. At present, typical ophthalmic dosage forms are preferred ways to achieve therapeutic levels of pharmacological agents used to treat ophthalmic diseases.

The topical ocular administration of drugs has two different purposes: to treat superficial eye diseases, such as infections (e.g., conjunctivitis, blepharitis, keratitis sicca); and to provide intraocular treatment through the cornea for diseases such as glaucoma or uveitis.

Most ocular diseases are treated by topical drug application in the form of solutions, suspensions and ointments. These conventional dosage forms suffer from the problems of poor ocular bioavailability because of various anatomical and pathophysiological barriers prevailing in the eye [1].

Although most topical ophthalmic preparations available today are in the form of aqueous solutions, the bioavailability of these ophthalmic drug formulations is usually low owing to rapid elimination after mucosal instillation, a consequence of reflex blinking and tear drainage, as well as of the presence of the cornea barrier.

Very little of the topically applied drug reaches the posterior segment of the eye because the cornea is characterized by lipophilic and hydrophilic structures, which

10.1517/17425247.2010.497536 © 2010 Informa UK Ltd ISSN 1742-5247

Article highlights.

- The development of a topical formulation of ACZ is limited by its poor ocular bioavailability, which can be largely attributed to its poor penetration coefficient and poor biphasic solubility.
- Sulfonamides are the best known inhibitors of carbonic anhydrase enzyme, now used for the treatment of glaucoma in clinical medicine. Topical CAIs were developed to minimize systemic side effects. However, compared with acetazolamide, dorzolamide, the first commercially available topical CAI for glaucoma treatment, has been reported to be equally or less effective.
- Cyclodextrins are a group of homologous cyclic oligosaccharides, consisting of six, seven and eight units, namely, α-, β-, γ-cyclodextrin (α-, β-, γ-CD). As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity. Owing to their complexation ability and other versatile characteristics, CDs have a wide range of applications in different areas of drug delivery and pharmaceutical industry.
- Although CDs are promising drug delivery systems, their effects on ocular delivery and bioavailability remain conflicting, as only the free fraction of drug that is in equilibrium with the complexed fraction may be available for ocular absorption. Liberation of the drug from the CDs' cavity is achieved by modifying the thermodynamic parameters that determine the binding.
- The ternary system ACZ:HP-β-CD:TEA produced an important reduction in intraocular pressure in normotensive rabbits compared with the binary system ACZ:HP-β-CD or the commercially available topical brinzolamide formulation (AZOPT®; AlconMR, Montevideo-Uruguay). The extended ocular hypotensive effect produced by this ternary system ACZ:HP-β-CD:TEA was attributed to the faster dissociation of the ternary complex because the addition of TEA made the inclusion strength of HP-β-CD weaker, because the interaction between HP-β-CD and TEA might prevent complexation of ACZ with HP-β-CD, allowing a larger amount of free drug available for absorption. Thus, this ternary system could be a very interesting alternative for delivery ACZ in the form of eye drops.

This box summarizes key points contained in the article.

represent an effective barrier to the absorption of both hydrophilic and lipophilic molecules, resulting in low absorption of ophthalmic drugs [2].

Physicochemical drug properties, such as lipophilicity, solubility, molecular size and shape, charge and degree of ionization affect the route and rate of permeation in the cornea. The rate limiting barrier for ocular penetration of highly hydrophilic drugs is the lipophilic corneal epithelium, whereas for highly lipophilic drugs partitioning from the epithelium to the hydrophilic stroma is rate limiting and, for the most part, determines corneal permeability [3].

Permeation of an ionizable drug (weak acids and weak bases) depends on the chemical equilibrium between the ionized and unionized drug in the eye drop and eventually in the lachrymal fluid. The unionized species usually penetrates the lipid membranes more easily than the ionized form. Corneal penetration enhancement can be achieved best by increasing drug concentration, increasing contact time in the pre-corneal packet, selecting the drug with appropriate pK_a and offering optimal lipid solubility.

Acetazolamide (ACZ), a carbonic anhydrase inhibitor (CAI), and other oral CAIs have been an integral part of antiglaucoma therapy for > 40 years. Glaucoma is the term used for a group of ophthalmic disorders. Most forms of glaucoma are accompanied by an increase in intraocular pressure (IOP), which results in damage to the optic disc and visual field disturbances. IOP increases through an imbalance between the production and drainage of aqueous humor. Agents used to treat glaucoma are designed to decrease IOP [4].

ACZ is used orally (no topical formulation being available on the market) for the reduction of IOP in patients suffering from glaucoma. However, this treatment leads to unpleasant systemic side effects such as CNS depression, renal failure, diuresis, vomiting, anorexia and metabolic acidosis [5]. The answer to the systemic side effects of ACZ is the topical delivery of this drug into the eye, where it could elicit its physiological action. However, the development of a topical formulation of ACZ is limited by its poor ocular bioavailability, which can be largely attributed to the poor penetration coefficient and the poor biphasic solubility of this drug [5].

Scientists throughout the world have attempted to formulate topical formulations of ACZ by adapting several approaches to enhance its ocular bioavailability. Topical formulations of ACZ solution (in the form of sodium salt) were initially unsuccessful because of its limited ocular penetration, which caused an insufficient amount of the drug to reach the ciliary body. Other attempts included the use of a high concentration of the drug, its combination with viscosity enhancing of polymers, multiple dosing, use of a modified form of the drug, or different drug delivery systems, including impregnated contact lenses, gels, cyclodextrins, or liposomes [4].

2. Advantages of topical administration of carbonic anhydrase inhibitors

Carbonic anhydrase (CA) inhibitor medications are very reliable for lowering intraocular pressure. Carbonic anhydrase, an enzyme present in the eye, reversibly catalyzes the reaction of H_2O and CO_2 to form carbonic acid and subsequently the bicarbonate ion, which is responsible for the movement of sodium and water into the eye to form aqueous humor, within the ciliary body [6].

Sulfonamides are the best known inhibitors of CA enzyme, used at present for the treatment of glaucoma in clinical medicine. The ocular effects of sulfonamides possessing CA inhibitory properties such as acetazolamide, brinzolamide or

dorzolamide result from inhibition of at least two CA isozymes present within ciliary processes of the eye, that is, CA-II and CA-IV, followed by a diminished secretion of bicarbonate and a 25 – 30% reduction of aqueous humor secretion. The main drawback of such agents is constituted by side effects such as augmented diuresis, fatigue, paresthesias, anorexia, and so on, due to CA inhibition in tissues/organs other than the target one (this enzyme has 16 different isoenzymes presently known in human). The above-mentioned side effects could be absent in the case of inhibitors having topical activity and being applied directly into the eye.

The systemic carbonic anhydrase inhibitor (CAI) acetazolamide was introduced in 1954 into ophthalmology as a treatment for glaucoma and, since then, has been widely used for this purpose. Topical CAIs were developed to minimize systemic side effects. In 1995, 2% dorzolamide became the first commercially available topical CAI for glaucoma treatment. In 1998, the US Food and Drug Administration (FDA) approved the use of Cosopt (Merck Sharp & Dohme, USA), a combination topical medication containing 2% dorzolamide and 0.5% timolol. Compared with acetazolamide, dorzolamide has been reported to be equally or less effective. Centofanti *et al.* [7] compared the efficacy of dorzolamide 1% eye drops with 250 mg acetazolamide tablets in a double-blind crossover study on the ocular pressure diurnal curve in patients with maximal medical therapy. They found that dorzolamide 1% eye drops is as effective as acetazolamide tablets in reducing the IOP curve. On the contrary, Portellos *et al.* [8] compared, in a crossover design, the hypotensive effect of oral acetazolamide (Diamox; Duramed Pharms Barr, USA) and topical dorzolamide (Trusopt; Merck Sharp & Dohme, USA) in patients with pediatric glaucoma, finding that the mean IOP was reduced to 18.5 ± 4.3 mmHg on acetazolamide (mean per cent IOP reduction 35.7 ± 15.6%, $p < 0.01$) and to 22.2 ± 5.4 mmHg on dorzolamide (mean per cent IOP reduction 27.4 ± 17.1%, $p < 0.01$). Also, Maus *et al.* [9] compared, in a randomized double-masked, placebo-controlled study of 40 human subjects, the efficacy of topical 2% dorzolamide hydrochloride (Trusopt) as a suppressor of aqueous humor flow in the human eye with the efficacy of systemically administered acetazolamide (Diamox). They found that the topically applied 2% dorzolamide hydrochloride was not as effective as systemically administered acetazolamide. Dorzolamide suppressed flow by 17%, approximately half as much as acetazolamide did in the same subjects (30% suppression), although there was no obvious explanation for the lower effect of dorzolamide, which *in vitro* is an effective inhibitor of CA isozyme II. Similar results were obtained by Larsson and Alm [10] in a randomized, double-masked, placebo-controlled study of 20 human subjects, who found that treatment with dorzolamide reduced aqueous flow by 17%, and a maximum dose of acetazolamide alone reduced flow by 29%. In this study it was concluded that the smaller effect of dorzolamide, as compared with acetazolamide, was a result of insufficient inhibition of at least one of the two carbonic anhydrase isozymes involved in aqueous humor production. Recently, Al-Barrag *et al.* [11] compared the effect of oral acetazolamide and topical 2% dorzolamide in the prevention of ocular hypertension after scleral tunnel cataract surgery. In this study it was concluded that acetazolamide offers better IOP control than topical 2% dorzolamide in preventing ocular hypertension after scleral tunnel cataract surgery.

As oral acetazolamide is reported to be more physiologically effective than 2% dorzolamide hydrochloride administered topically, even though in isolated tissues dorzolamide appears to be most active as it shows the lowest IC_{50} values for CA-II and CA-IV [12], there exists considerable scope for the development of more/equally effective and inexpensive topically effective formulations of acetazolamide.

Brinzolamide and dorzolamide are now being marketed as topical medications. These topically acting antiglaucoma sulfonamides have incorporated secondary amine moieties (**Figure 1**), so the water solubility needed for effective topical action is achieved using their hydrochloride salts. Still, this represents an undesired problem because the pH of such solutions is rather acidic (pH 5.5) and consequently produces eye irritation after the topical administration of the drug, as already reported for many patients treated with dorzolamide. In fact, the most common adverse effects after topical dorzolamide treatment are local burning, stinging and reddening of the eyes, blurred vision and bitter taste, but also more serious side effects, such as contact allergy, nephrolithiasis, anorexia, depression and dementia, as well as irreversible corneal decompensation in patients already presenting corneal problems [13].

Acetazolamide (the most widely prescribed carbonic anhydrase inhibitor) and methazolamide are given orally for the reduction of IOP in patients suffering from glaucoma because of their inability to penetrate the eye easily (no topical formulation being available in the market). However, several side effects of these drugs are experienced, such as numbness and tingling in the fingers and toes, taste alterations, blurred vision, kidney stones, and an increase in urination after oral administration. A randomized, double-masked, multi-center, active-controlled, parallel group study of 215 patients with primary open-angle glaucoma (POAG) or IOP was conducted to evaluate the safety and efficacy of dorzolamide versus acetazolamide when added to 5% w/v timolol once a day. Systemic adverse events were statistically greater in the acetazolamide group (75%) versus the dorzolamide group (50%). In addition, adverse events associated with CAI therapy were higher in the acetazolamide group (53%) versus the dorzolamide group (26%); discontinuations due to CAI adverse experiences were lower in the dorzolamide group (8%) versus the acetazolamide group (24%) [14]. This indicates that there is a greater incidence of systemic and CAI adverse experiences and discontinuations with oral acetazolamide compared with topical dorzolamide.

It is generally recognized that topical acetazolamide formulation possessing efficacy similar to that achieved on oral administration would be a significant advancement in the

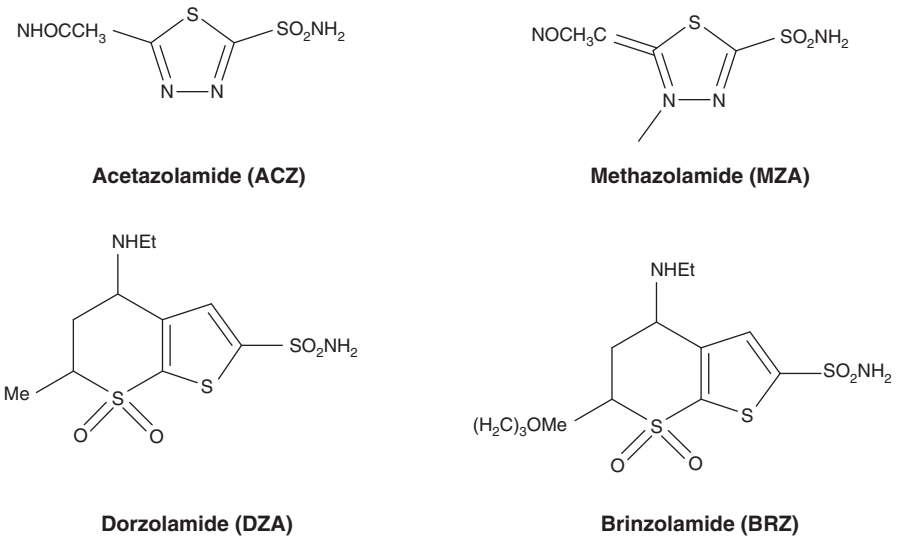

Figure 1. Structure of several carbonic anhydrase inhibitors used in glaucoma treatment.

treatment of glaucoma to minimize the systemic adverse reactions associated with its use.

3. Cyclodextrins as ocular delivery systems

Cyclodextrins (CDs) are a group of homologous cyclic oligosaccharides consisting of six, seven and eight units, namely, α-, β-, γ-cyclodextrin (α-, β-, γ-CD). Cyclodextrins are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface. Owing to the lack of free rotation around the bonds connecting the glucopyranose units, the CDs are not perfectly cylindrical molecules but are toroidal or cone shaped. They are water-soluble because all the hydroxyl groups of the glucopyranose units are located on the outside surface of the rings. However, the internal cavity of the CD molecule is relatively nonpolar. As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity [15].

In an aqueous environment, CDs form inclusion complexes with many lipophilic molecules through a process in which water molecules located inside the central cavity are replaced by either a whole molecule or, more frequently, by some lipophilic structure of the molecule. Importantly, as no covalent bonds are formed or broken during the guest–host complex formation, the complexes are in dynamic equilibrium with free drug and CD molecules [16].

Owing to their complexation ability and other versatile characteristics, CDs have a wide range of applications in different areas of drug delivery and pharmaceutical industry. The most common pharmaceutical application of CDs is to enhance solubility, stability, safety and bioavailability of drug molecules [17].

Although CDs are promising drug delivery systems primarily because of their ability to increase the amount of drug dissolved in the pre-corneal area, in ophthalmology their effects on ocular delivery and bioavailability remain conflicting. It would appear that the amount of CD used must be optimized so that only the drug that is in excess of its solubility is complexed. Overcomplexation by the addition of excess CD appears to reduce ocular bioavailability [18].

The binding of guest molecules within the host CD is not fixed or permanent but rather is a dynamic equilibrium. Binding strength depends on how well the 'host–guest' complex fits together and on specific local interactions between surface atoms [15].

The liberation of the drug from the CDs' cavity is achieved by modifying the thermodynamic parameters that determine the binding. The use of molecules or parts of molecules with better binding affinity towards the CDs' cavity will destabilize the several existing equilibria, shifting them in the direction of the free drug [19]. Several authors have proposed that some co-solvents act as a space-regulating molecule so that the drug molecule can fit better into the CD cavity [20].

Depending on their structure and conformation, the use of low-molecular-mass organic acids can enhance the solubility of weak basic compounds by stabilizing the drug:CD complex, increasing its stability constant, but also increasing the solubility of the basic drug by salt formation [21]. A similar approach has been applied for improving the properties of weak acidic drugs [22]. In some cases, this is reflected in a higher bioavailability (rate and extent of absorption). On the other hand, simple salt formation often favors only the preparation of the pharmaceutical formulation without involving a real advantage in terms of absorption.

In vivo, the drug release from the CD complex is mainly caused by dissociation resulting from dilution in fluids. In

the case of topical applications, such as ocular, with minimal or impossible dilution mechanism, the potential mechanism of drug release from the CD complex is preferential drug uptake by tissue [18].

Epithelial pores in the cornea having dimensions of ~ 6.0 nm would be potential pathways for the movement of CD into the tissue. However, hydroxypropylated CDs are sterically hindered because of their bulky hydroxyalkyl side chains and they probably have less ability to permeate corneal pores. Hence, only the free fraction of drug that is in equilibrium with the complexed fraction may be available for ocular absorption [23]. Thus, CDs are able to increase bioavailability by delivering the drug substance to the absorption site and by minimization of drug hydrophobicity rather than by permeation themselves.

4. A promising ocular formulation of ACZ by the combination of hydroxypropyl-β-cyclodextrin with triethanolamine

The authors' research group has investigated the effect of incorporating the basic compound triethanolamine (TEA) as a ternary component in drug–complexant systems of acidic drugs with hydroxypropyl-β-cyclodextrin (HP-β-CD). For example, they demonstrated that the solubility capacity of the HP-β-CD is significantly enhanced when TEA is incorporated as a ternary component in the complexes of the acid drug sulfisoxazole [24]. Moreover, it was found that the simultaneous complexation and salt formation with TEA significantly increased the HP-β-CD solubilizing power for ACZ by forming a drug:HP-β-CD:TEA multicomponent system [25]. Therefore, it was hypothesized that the higher ACZ solubility in aqueous HP-β-CD solutions might result in a more effective drug delivery to the cornea surface. Accordingly, this formulation was evaluated in terms of its *in vitro* permeability and physiological effectiveness to lower IOP of normotensive rabbits, and it was found that this ternary system produced an ~ 30% reduction of IOP compared with the control blank saline. This lowering effect was observed during at least the following 4 h (n = 6). In comparison with the binary system ACZ:HP-β-CD, which produced an ~ 6% reduction on IOP compared with the control blank saline during ~ 3 h of action (n = 6), the ternary system ACZ:HP-β-CD:TEA was most efficient in ACZ permeation according to percentage of permeation, steady-state flux and apparent permeability coefficient (P_{app}) values. The extended ocular hypotensive effect produced by the ternary system ACZ:HP-β-CD:TEA was attributed to the faster dissociation of the ternary complex because the addition of TEA made the inclusion strength of HP-β-CD weaker, because the interaction between HP-β-CD and TEA might prevent complexation of ACZ with HP-β-CD, allowing a larger amount of free drug to be available for absorption owing to the generation of a supersaturated solution of ACZ, and thus favoring the ocular absorption of this drug [26]. Also, a potential contributing mechanism for the ACZ release from CD is the access of this drug to the corneal tissue, which is not available to the CD or the complex, which acts as a 'sink', causing dissociation of the complex based on simple mass action principles owing to complexation of molecules being a dynamic process whereby the guest molecule continuously associates and dissociates from the host CD. **Figure 2** shows a schematic representation of all processes involved in the absorption of ACZ from its multicomponent system containing HP-β-CD and TEA through the corneal epithelium.

Several approaches have been adapted to develop an effective topical formulation of ACZ. Gloster and Perkins [27] used various routes of administration for ACZ. They administered 5% solution of ACZ-Na by subconjunctival injection and also by intraocular injection. Both these routes of administration, however, failed to show any reduction in IOP. Similar findings were reported by Grant and Trotter [28].

Later, different group of workers investigated several drug delivery systems in order to enhance the bioavailability of acetazolamide by the topical ocular route and to improve the corneal permeability of the drug. However, topical formulations of acetazolamide solution (in the form of sodium salt) were initially unsuccessful because of its limited ocular penetration, which caused an insufficient amount of the drug to reach the ciliary body.

Other significant attempts have been made to formulate effective acetazolamide topical preparations. A statistically significant lowering of IOP was observed by using high water content soft contact lenses soaked in acetazolamide [29]. Following 2.5 h application of + 37 D lenses soaked in 5% acetazolamide, a significant reduction in IOP was observed up to 5 h. Similar results were obtained for lenses soaked in 2.5% acetazolamide. However, no IOP reduction was observed with 1% acetazolamide solution. It is important to note that these formulations had a pH ranking from 8.2 to 8.5 (acetazolamide has been reported to degrade at a very fast rate in an alkaline pH ≥/8.0; the pH of maximum stability being 4 – 5). As acetazolamide concentrations were far beyond the aqueous solubility of the free acid (0.7 mg/ml), acetazolamide solutions, where the contact lenses were soaked, were prepared by dissolving the acetazolamide in water with 5% polysorbate 80 (Tween 80) at concentrations of 1 – 5% with the pH titrated to 8.5 by sodium hydroxide. It is probably in these conditions that the sodium salt of acetazolamide was formed. It is possible that the small IOP lowering effect of the aqueous 10% acetazolamide solutions is a result of the inability of the anionic form to penetrate the eye.

In other attempts to increase the ocular bioavailability of acetazolamide, suspensions of this drug were formulated using several viscolyzers and penetration enhancers [30]. A reduction in IOP was observed in normotensive rabbits by incorporating 2% polyvinyl alcohol (PVA) and 0.5% ethylenediaminetetraacetic acid (EDTA) into a suspension of acetazolamide (10%) (**Table 1**).

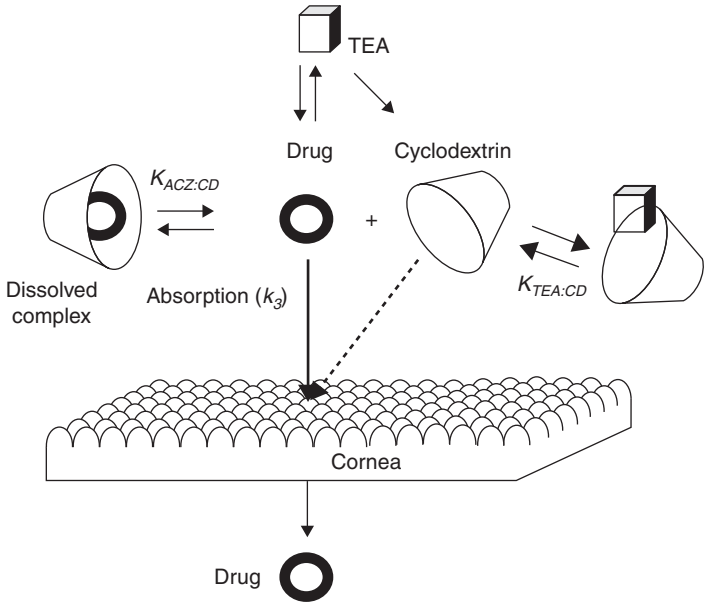

Figure 2. Schematic representation of the ocular absorption of ACZ from through its cyclodextrin complex in the presence of TEA.

ACZ: Acetazolamide; TEA: Triethanolamine.

Topically effective formulations of acetazolamide using cyclodextrins in combination with bioadhesive polymers, penetration enhancers and co-solvents were developed by Kaur *et al.* [4]. Using these agents in varying combinations, nine formulations were prepared (**Table 1**). The physiological effectiveness of these formulations was determined in terms of their IOP lowering effect in normotensive rabbits. It may be pointed out that the formulations obtained had acetazolamide concentrations of 0.5 or 2% w/v, which are fivefold and 20-fold higher than in the case of the authors' formulation, which showed a reduction in IOP in the range 17 – 28%.

Recently, a bioadhesive niosomal (non-ionic surfactant vesicles using span 60 and cholesterol obtained by the reverse-phase evaporation method coated with Carbopol 934P) preparation of acetazolamide (0.5% w/v) was reported to show a 33% reduction of IOP in normotensive rabbits [31]. It is interesting to note that the effect of the authors' formulation compared well with this five times higher concentration bioadhesive niosomal formulation of acetazolamide. However, surfactants (the chief constituents of these niosomes) act as penetration enhancers as they can remove the mucus layer and break junctional complexes, causing light reversible irritation.

The preparation of reverse-phase evaporation (REVs) and multilamellar (MLVs) acetazolamide liposomal formulations has also been reported [32]. Among the different liposomal formulations obtained, the positive multilamellar liposomes composed of egg phosphatidylcholine:cholesterol:stearylamine (7:4:1) molar ratio showed the maximal response, which produced a lowering in IOP on normotensive rabbits of ~ 40%. By comparing the IOP lowering activity of this formulation with the authors' formulation an important observation was the fact that the acetazolamide concentration in the liposomal formulation was 10 times higher than in the case of the authors' formulation. Although the various drug delivery systems mentioned above offer numerous advantages over conventional drug therapy, they are not devoid of pitfalls, including poor patient compliance and difficulty of insertion, as in contact lenses, and tissue irritation, as well as damage and toxicological complications caused by penetration enhancers.

In the case of the ternary system ACZ:HP-β-CD:TEA, the potential ocular irritancy and/or damaging effects of this formulation were evaluated using a slightly modified version of the Draize test [26]. This ternary system has been shown to be non-irritant. Between the excipients used to prepare this system are HP-β-CD and TEA. The most commonly used CD for aqueous eye drop formulations is HP-β-CD. Numerous studies in animals as well as in humans have shown that this β-CD derivative is well tolerated from aqueous solutions, even at concentrations as high as 45% [18]. One cyclodextrin (hydroxypropyl-γ-cyclodextrin [HP-γ-CD]) is used as solubilizer for diclofenac sodium in Voltaren Ophtha CD eye drops (Novartis Ophthalmics AG, Switzerland). Alkaline products such as TEA are commonly used in cosmetic preparations as neutralizing agents for acid-functional raw materials, cosmetic ingredients that are not ocular irritants [33]. Therefore, it is expected that this formulation will had a safety profile for its use in the long term.

Table 1. Effect of topically administrated ACZ formulations on the change on IOP in normotensive rabbits.

ACZ (%)	Type of formulation	Composition of formulations	ΔIOP_{max} (%)	T_{max} (h)	Ref.
5	ACZ-soaked + 37 lens	5% polysorbate 80 (Tween 80) at	~ 30	NR	[29]
2.5		concentrations of 1 – 5% with	~ 30	NR	
		the pH titrated to 8.5 by sodium hydroxide			
10		PVA (2% w/v):EDTA disodium (0.5% w/v)	46.4	1	[30]
0.5		HP-β-CD (10% w/v):PVP (0.05% w/v)	17	1.5	[4]
0.5		HP-β-CD (10% w/v):PEG (30% v/v)	23	1	
0.5		HP-β-CD (10% w/v):PVP (0.05% w/v):PEG (30% v/v)	23	1	
0.5		HP-β-CD (10% w/v):EDTA disodium (0.5 w/v)	26	1	
0.5		HP-β-CD (10% w/v):PVP 0.05% w/v:Sodium deoxycholate (0.05% w/v)	25	2	
0.5		HP-β-CD (10% w/v):PVP (0.05% w/v):Chitosan (0.5% w/v)	24	1.5 – 2	
0.5		HP-β-CD (10% w/v):PVP^h:EDTA disodium (0.5 w/v):Chitosan (0.5% w/v)	26	1.5	
2		HP-β-CD (10% w/v):PVP (0.05% w/v): Tween 80 (1% w/v)	20	2	
2		HP-β-CD (10% w/v):PVP (0.05% w/v): Chitosan (0.5% w/v)	28	1.5	
0.5	Bioadhesive coated niosomes	Span 60:CH (7:4)	33	1.5	[31]
1	Positive liposomes	PC:CH:SA (7:4:1)	~ 40	3	[32]
0.1	Ternary inclusion complex	ACZ:HP-β-CD:TEA (1:1:1)	~ 30	2	[26]

ΔIOP_{max}: Maximum reduction in IOP; ACZ: Acetazolamide; CH: Cholesterol; EDTA disodium: Disodium ethylenediaminetetraacetic acid; IOP: Intraocular pressure; NR: Not reported; PC: Phosphatidylcholine; PVA: Polyvinyl alcohol; PVP: Polyvinylpyrrolidone; SA: Stearylamine; T_{max}: Time required to reach the peak effect.

5. Expert opinion

Cyclodextrins play an important role in improving the therapeutic efficacy of drugs with poor solubility and/or stability problems. However, the results achieved by simple encapsulation are not always satisfying.

Davis *et al.* [34] have reported that the ocular bioavailability following topical administration to rabbits of a 1% hydrocortisone solution formulation of the complex hydrocortisone/HP-β-CD was lower than that of a 1% hydrocortisone suspension formulation. This result is in contrast to that reported by Bary *et al.* [35], who found that a formulation of hydrocortisone as a solution with HP-β-CD increased the bioavailability of this drug in the aqueous humor by ~ 55% and in the cornea by 75% when compared with suspension. In this later report, the ability of the CD to increase the ocular bioavailability of the hydrocortisone was ascribed to only a minimal amount of CD added to solubilize the drug, which is in excess of its saturated solubility.

A decrease in the relative bioavailability compared with suspension of 10% was reported also for the flurbiprofen complex [36], whereas a decrease of between 25 and 50% was reported for O′O-dipropionyl-(1,4-xylylene)-bispilocarpate depending on the amount of CD added to the system [37]. Burgalassi *et al.* [38] evaluated the aqueous humor pharmacokinetics of rufloxacin in rabbits after topical administration of six formulations (two pH 7.2 suspensions of non-salified rufloxacin base, or zwitterion, one of which was viscosized with tamarind seed polysaccharide [TSP]; two pH 7.2 solutions of rufloxacin obtained using HP-β-CD (8.6% w/v), one of which was viscosized with TSP; and two pH 5.0 solutions of rufloxacin hydrochloride, one of which was viscosized with TSP). They found that the CD formulations resulted in decreased drug availability with respect to standard formulations. The decreased drug availability of the rufloxacin:HP-β-CD complex was ascribed to the complex not being able to release the drug before its clearance from the precorneal area and to insufficient dilution of the complex, which contributed further to decreased drug availability. On the other hand, the further drug bioavailability decrease observed after TSP addition to rufloxacin:HP-β-CD complex was attributed to hindered dilution and to slow diffusion of the drug from CD complex, caused by the viscous vehicle.

On the contrary, a mydriatic activity test in rabbits showed an improved bioavailability and maximal mydriatic response for two pH 7.4 formulations containing tropicamide solubilized by HP-β-CD (alone or associated with hydroxypropyl methylcellulose [HPMC]) when compared with standard

1% (w/v) tropicamide eye drops, buffered at pH 5.0. The improved effect of the CD formulations with respect to the reference solution was attributed to their physiological pH value (7.4 versus 5.0 for the reference solution), resulting in reduced irritation and lacrimation, and hence prolonged retention at the absorption site. However, the *in vivo* data indicated a slightly superior performance (shorter time to peak) for the 4% (w/v) HP-β-CD formulation, when compared with the 1% (w/v) one containing 0.1% (w/v) HPMC. The delayed peak time was ascribed to the higher stability constant of the formulation containing HP-β-CD and HPMC, resulting in a slower release mode of tropicamide from the tropicamide-HP-β-CD-HPMC aggregate or co-complex [39].

The relatively lipophilic corneal epithelium has a low affinity for the hydrophilic CD molecules or the hydrophilic drug/CD complexes, which therefore remain in the aqueous membrane exterior, that is, the tear fluid. This is why it is thought that such CDs act as true carriers by keeping the hydrophobic drug molecules in solution, and by delivering them to the surface of the biological membrane, for example, the corneal epithelium, where they partition from the CD cavity into the lipophilic membrane [18]. Attention should be paid to creating better delivery and drug release of sparingly water-soluble drugs from CDs; one must understand how drugs bind CDs to rational formulation, design formulations containing hydrophilic CDs, as the free fraction (i.e., the uncomplexed drug) is the only form capable of diffusion across the biological membranes.

Thermodynamically, the formation of cyclodextrin–guest complexes is influenced by various factors, namely: i) penetration of the hydrophobic part of the guest molecule into the cyclodextrin cavity; ii) dehydration of the organic guest; iii) hydrogen-bonding interactions; iv) conformational changes in the cyclodextrin molecule on complexation; and v) release of water molecules originally included in the cyclodextrin cavity to the bulk water. Moreover, the complexes are in dynamic equilibrium with free guest and host molecules, and they are exchanging one another with very rapid speeds. The presence of a third component in the system might affect the dynamic characteristics for the complexation reaction, stabilizing or destabilizing the inclusion complex.

The simple and familiar idea of CDs as rigid buckets with a hydrophobic cavity easily led to the role of major driving force for the inclusion process being ascribed to the transfer of a (possibly) hydrophobic guest from the water pool into a more 'friendly' environment, with the simultaneous transfer of 'high energy' water molecules from the host cavity into the aqueous bulk. However, CDs are not rigid, but fairly flexible systems, as accounted for by both computational and experimental evidence [40]. The possible partial rotation around the glycosidic bridge bonds allows a dynamic rearrangement of the different glucose units, up to a certain extent, which affects the optical activity of the macrocycle, as well as its binding properties ('induced fit' effect). Thus, it can be reasonably expected that the binding properties of CDs should be significantly affected by the presence of other components.

Lo Meo *et al.* [41] studied the overall contribution of methanol in affecting the structure and the intimate features of the inclusion complex between the native β-cyclodextrin (β-CD) and a set of suitably selected *p*-nitroaniline derivatives. They found that, in general, the values of the binding constants between β-CD and the *p*-nitroaniline derivatives decreased exponentially on increasing the amount of methanol, with the exception of two compounds of the series, for which almost no effect was detected. Methanol increasingly tended to destabilize the inclusion complex on increasing the intrinsic stability of the complex itself. On the contrary, on decreasing the intrinsic stability of the complex, the unfavorable effect of methanol addition becomes weaker and weaker, the effect of methanol on the dynamics of the inclusion complex being ascribed to the occurrence of a continuous swap of solvent molecules between the solvent bulk and the residual space within the complex, without requiring the formation of any individual structure/stoichiometry defined species, suggesting that this dynamic co-inclusion process is able to affect the time-averaged conformational dynamics of the complex, as well as the inner interplay of molecular interactions, the methanol being able to compete successfully with water molecules for this dynamic process because of the occurrence of more effective hydrophobic interactions within the host cavity. As a consequence, the complex tends to become on average more rigid owing to the occurrence of multiple host–guest hydrogen bond interactions decreasing the binding constant of the complex. From a thermodynamic point of view, it was suggested that in general the addition of methanol decreases the inclusion entropies because of the stiffening of the inclusion complex, with a stronger relative effect for intrinsically less rigid and therefore more stable complexes.

In the case of the ternary system ACZ:HP-β-CD:TEA, the results obtained by the authors' group showed that the association equilibrium of ACZ with HP-β-CD is strongly influenced by TEA. It was found that the stability constant values for the binding of HP-β-CD to ACZ decreased exponentially on increasing the amount of TEA, which promotes the dissociation of the complex favoring the drug bioavailability. The decrease of the binding constant of the inclusion complex between ACZ and HP-β-CD with an increase of the TEA concentration suggests a weaker interaction of ACZ with HP-β-CD in the presence of TEA. On the other hand, it was demonstrated that there is little formation of a ternary supramolecular complex between HP-β-CD, TEA and ACZ in the system and it can be neglected, and the association constant value binding of TEA to HP-β-CD is small (approximately eight orders of magnitude lower than the association constant value binding of ACZ to HP-β-CD). So, it could be hypothesized that TEA may have an unbeneficial

effect similar to that observed with methanol on the inclusion process of ACZ with HP-β-CD, resulting in the exclusion of ACZ from the HP-β-CD cavity.

Depending on its structure and conformation, the use of a low-molecular-mass organic acid can either stabilize the formation of complexes by ternary inclusion structure complex or extract the drug from the CD cavity where the acid plays a competitive role, hindering the CD cavity, or destabilizing the drug complex.

For acidic drugs, the basic agent can increase the stability constant of the complex when there is a good match among the three inclusion complex components. In the case of strongly bound complexes, the extent of drug release from the multicomponent CD complexes can be considered a crucial parameter, as the free drug molecules are the only species able to cross the biological membranes.

In other cases the release of the drug from the CD cavity is favored by a modification of the complexation thermodynamics in such a way that the binding of any competitive compound to CDs is favored and the drug's inclusion in the CDs' cavity is not favored.

During the formation of miconazole/CD complex using supercritical carbon dioxide, low-molecular-mass acids generally improve the inclusion yield. This improvement depends on several factors. The structure and also the conformation of the acidic ternary compound are very important and manage the interactions between the host and the guest. The interactions between miconazole, CDs and acids depend on the nature of both the acid and the CD. With β-CD, maleic and fumaric acids stabilize the formation of complexes by ternary inclusion structure. With the same CD, the situation is totally different with L-tartaric acid. Indeed, this acid extracts miconazole from the β-CD cavity. Conversely, for HP-β-CD and HP-γ-CD, L-tartaric acid stabilizes the miconazole complexes [42].

On the other hand, frequently pharmaceutical formulations contain the combination of two or more drugs that possess different physicochemical properties, including different affinities for the formulation excipients such as CDs.

Eye drops are usually delivered in multidose containers and thus they should contain a suitable antimicrobial preservative. The addition of preservatives to eye drops, artificial tears, or contact lens solutions is aimed at destroying microorganisms (bactericidal effect), or at least preventing their growth (bacteriostatic effect). When selecting a preservative for an ophthalmic formulation containing a cyclodextrin complex, it must be taken into account that the delivery and efficacy of the complex are highly dependent on the dynamic equilibrium of the active pharmaceutical ingredient (API) and excipients involved.

The interactions between several commonly used preservatives, that is, benzalkonium chloride, chlorhexidine gluconate, chlorobutanol, methylparaben and propylparaben, and 2-hydroxypropyl-β-cyclodextrin were investigated [43]. The interactions were shown to be twofold. First, the preservative molecules can displace the drug molecules from the cyclodextrin cavity, thus reducing the solubilizing effects of the cyclodextrin. Second, the antimicrobial activity of the preservatives was reduced by the formation of preservative-cyclodextrin inclusion complexes. In consideration of this, preservatives might be avoided with the use of some promising alternatives that have been proposed recently in the form of original new packaging systems that are now available, which consist of packaging in multidose bottles with a special filter device.

Consequently, the amount of cyclodextrin included in the aqueous eye drop formulation has to be based on availability studies performed on the actual eye drop formulation, which must contain all necessary excipients (e.g., preservatives, polymers and buffer salts).

Although CD formulation studies should always be performed in media that closely resemble the final drug formulation, the ternary system ACZ:HP-β-CD:TEA could be a very interesting alternative for delivery of ACZ in the form of eye drops.

Declaration of interest

The HP-β-CD used in this research was donated by Ferromet SA, agent of Roquette in Argentina.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Sahoo SK, Dilnawaz F, Krishnakumar S. Nanotechnology in ocular drug delivery. Drug Discov Today 2008;13(3/4):144-51
2. Ali M, Byrne ME. Challenges and solutions in topical ocular drug-delivery systems. Expert Rev Clin Pharmacol 2008;1(1):145-61
3. Chen C, Yang J. MI-QSAR models for prediction of corneal permeability of organic compounds. Acta Pharm Sin 2006;27(2):193-204
4. Kaur IP, Smitha R, Aggarwal D, Kapil M. Acetazolamide: future perspective in topical glaucoma therapeutics. Int J Pharm 2002;248(1-2):1-14

• **A good summary of different approaches for ocular drug delivery of acetazolamide.**

5. Granero GE, Longhi MR, Becker C, et al. Biowaiver monographs for immediate release solid oral dosage forms: acetazolamide. J Pharm Sci 2008;97(9):3691-9

• **An important monograph that discusses the biopharmaceutical and biological properties of acetazolamide.**

6. Andrea Scozzafava A, Luca Menabuoni L, Mincione F, Supuran CT. Carbonic anhydrase inhibitors. A general approach for the preparation of water-soluble sulfonamides incorporating polyamino-polycarboxylate tails and of their metal complexes possessing long-lasting, topical intraocular pressure-lowering properties. J Med Chem 2002;45:1466-76
7. Centofanti M, Manni GL, Napoli D, Bucci MG. Comparative effects on intraocular pressure between systemic and topical carbonic anhydrase inhibitors: a clinical masked, cross-over study. Pharmacol Res 1997;35:481-5
8. Portellos M, Buckley E, Freedman SF. Topical versus oral carbonic anhydrase inhibitor therapy for pediatric glaucoma. J AAPOS 1998;2:43-7
9. Maus TL, Larsson LI, McLaren JW, Brubaker RF. Comparison of dorzolamide and acetazolamide as suppressors of aqueous humor flow in humans. Arch Ophthalmol 1997;115:45-9
10. Larsson LI, Alm A. Aqueous humor flow in human eyes treated with dorzolamide and different doses of acetazolamide. Arch Ophthalmol 1998;116:19-24
11. Al-Barrag A, Al-Shaer M, Al-Matary N, Bamashmous M. Oral versus topical carbonic anhydrase inhibitors in ocular hipertension alter scleral tunnel cataract surgery. Clin Ophthalmol 2009;3:357-62
12. Sugrue MF. Review the preclinical pharmacology of dorzolamide hydrochloride, a topical carbonic anhydrase inhibitor. J Ocul Pharmacol Ther 1996;12:363-76
13. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168-81
14. Stewart WC, Halper LK, Johnson-Pratt L, et al. Tolerability and efficacy of dorzolamide versus acetazolamide added to timolol. J Ocul Pharmacol Ther 2002;18(3):211-20
15. Del Valle EMM. Cyclodextrins and their uses: a review. Process Biochem 2004;39:1033-46

• **An excellent summary on the characteristics of cyclodextrins.**

16. Stella VJ, He Q. Cyclodextrins. Toxicol Pathol 2008;36:30-42

• **An excellent summary on the characteristics of cyclodextrins.**

17. Carrier RL, Miller LA, Ahmed I. The utility of cyclodextrins for enhancing oral bioavailability. J Control Release 2007;123:78-99
18. Cal K, Centkowska K. Use of cyclodextrins in topical formulations: practical aspects. Eur J Pharm Biopharm 2008;68:467-78

• **An excellent review summarizing applications of cyclodextrins in topical formulations.**

19. Jansook P, Loftsson T. CDs as solubilizers: effects of excipients and competing drugs. Int J Pharm 2009;379:32-40
20. Wan J, Yuan S, Mak K, et al. Enhanced washing of HCB contaminated soils by methyl-beta-cyclodextrin combined with ethanol. Chemosphere 2009;75:759-64
21. Barillaro V, Dive G, Bertholet P, et al. Theoretical and experimental investigations of organic acids/cyclodextrin complexes and their consequences upon the formation of miconazole/cyclodextrin/acid ternary inclusion complexes. Int J Pharm 2008;347:62-70
22. Redenti E, Szente L, Szejtli L. cyclodextrin complexes of salts of acidic drugs. Thermodynamic properties, structural features, and pharmaceutical applications. J Pharm Sci 2001;90:979-86

• **This mini-review summarizes the findings concerning the physicochemical properties and the pharmaceutical applications of acidic drugs whose performances have been modified by simultaneous complexation with cyclodextrins and salt formation.**

23. Siefert B, Keipert S. Influence of alpha-cyclodextrin and hydroxyalkylated beta-cyclodextrin derivatives on the in vitro corneal uptake and permeation of aqueous pilocarpine-HCl solutions. J Pharm Sci 1997;86:716-20
24. Granero G, Garnero C, Longhi M. The effect of a basic substance and pH on cyclodextrin complexation of sulfisoxazole. Eur J Pharm Sci 2003;20:285-93
25. Granero GE, Maitre MM, Garnero C, Longhi MR. Synthesis, characterization and in vitro release studies of a new acetazolamide-HP-beta-CD-TEA inclusion complex. Eur J Med Chem 2008;43:464-70
26. Palma SD, Tartara LI, Quinteros D, et al. An efficient ternary complex of acetazolamide with HP-beta-CD and TEA for topical ocular administration. J Control Release 2009;138:24-31

• **Studies of the *in vitro* permeability and physiological effectiveness to lower IOP of normotensive rabbits of different formulations of acetazolamide with HP-beta-CD and TEA.**

27. Gloster J, Perkins ES. Effect of a carbonic anhydrase inhibitor (Diamox) on intra-ocular pressure of rabbits and cats. Br J Ophthalmol 1955;39:647-58
28. Grant WM, Trotter RR. Diamox (acetazolamide) in treatment of glaucoma. Arch Ophthalmol 1954;51:735-9

29. Friedman Z, Allen RC, Raph SM. Topical acetazolamide and methazolamide delivered by contact lenses. Arch Ophthalmol 1985;103:963-6

30. Kaur IP, Singh M, Kanwar M. Formulation and evaluation of ophthalmic preparations of acetazolamide. Int J Pharm 2000;199:119-27

31. Aggarwal D, Pal D, Mitra AK, Kaur IP. Study of the extent of ocular absorption of acetazolamide from a developed niosomal formulation, by microdialysis sampling of aqueous humor. Int J Pharm 2007;338:21-6

32. Hathout RM, Mansour S, Mortada ND, Guinedi AS. Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies. AAPS PharmSci Tech 2007;8:E1-E11

33. Kurishita A, Katoh T, Ohsawa H, et al. Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (5) Skin2TM ZK1100 and Tissue Equivalent Assay. Toxicol In Vitro 1999;13:139-51

34. Davis NM, Wang G, Tucker IG. Evaluation of a hydrocortisone/ hydroxypropyl-beta-cyclodextrin solution for ocular drug delivery. Int J Pharm 1997;156:201-9

35. Bary AR, Tucker IG, Davis NM. Considerations in the use of hydroxypropyl-beta-cyclodextrin in the formulation of aqueous ophthalmic solutions of hydrocortisone. Eur J Pharm Biopharm 2000;50:237-44

36. Masuda K, Tohno M, Konishi Y, et al. Ocular penetration of flurbiprofen, an anti-inflammatory drug, in rabbits after topical instillation. Atarashii Ganka 1985;2(3):441-3

37. Jarho P, Jarvinen K, Urtti A, Stella VJ. Modiefied beta-cyclodextrin (SBE7-beta-CYD) with viscous vehicle improves the ocular delivery and tolerability of pilocarpine prodrug in rabbits. J Pharm Pharmacol 1996;48:263-9

38. Burgalassi S, Cappello B, Chetoni P, et al. Rufloxacin eyedrops: effect of different formulations on ocular pharmacokinetics in rabbits. Eur J Ophthalmol 2006;16(2):311-7

39. Cappello B, Carmignani C, Iervolino M, et al. Solubilization of tropicamide by hydroxypropyl-b-cyclodextrin and water-soluble polymers: in vitro:in vivo studies. Int J Pharm 2001;213:75-81

40. Raffaini G, Ganazzoli F. Hydration and flexibility of alpha-, beta-, gamma- and delta-cyclodextrin: a molecular dynamics study. Chem Phys 2007;333:128-34

41. Lo Meo P, D'Anna F, Riela S, et al. Binding equilibria between b-cyclodextrin and p-nitro-aniline derivatives: the first systematic study in mixed water–methanol solvent systems. Tetrahedron 2009;65:2037-42

- **An excellent report on the effect of methanol on the thermodynamic binding equilibrium constant of beta-CD to suitable selected drugs.**

42. Barillaro V, Dive G, Bertholet P, et al. Theoretical and experimental investigations of organic acids/ cyclodextrin complexes and their consequences upon the formation of miconazole/cyclodextrin/acid ternary inclusion complexes. Int J Pharm 2008;347:62-70

43. Loftsson T, Stefansdottir O, Frioriksdottir H, Guomundsson O. Interactions between preservatives and 2-hydroxypropyl-beta-cyclodextrin. Drug Dev Ind Pharm 1992;18(13):1477-84

Affiliation

Gladys E Granero[†] & Marcela R Longhi
[†]Author for correspondence
Departamento de Farmacia,
Facultad de Ciencias Químicas,
Universidad Nacional de Córdoba,
Ciudad Universitaria,
X5000HUA Córdoba, Argentina
Tel: +54 351 4334163; Fax: +54 351 4334127;
E-mail: glagra@fcq.unc.edu.ar