ELSEVIER

International Journal of Pharmaceutics 171 (1998) 147–156

international journal of pharmaceutics

Physicochemical properties, NMR spectroscopy and tolerance of inclusion complexes of antazoline and tetracaine with hydroxypropyl-β-cyclodextrin

L. Van Santvliet [a], K. Caljon [a], L. Pieters [b], A. Ludwig [a,*]

[a] *Laboratory of Pharmaceutical Technology and Biopharmacy, University of Antwerp (UIA), Department of Pharmaceutical Sciences, Universiteitsplein 1, Antwerpen, B-2610, Belgium*

[b] *Laboratory of Pharmacognosy, University of Antwerp (UIA), Department of Pharmaceutical Sciences, Universiteitsplein 1, Antwerpen, B-2610, Belgium*

Received 14 November 1997; received in revised form 1 April 1998; accepted 12 May 1998

Abstract

To improve the tolerance of antazoline and tetracaine ophthalmic solutions, inclusion complexes of the free bases of both drugs with hydroxypropyl-β-cyclodextrin (HP-β-CD) were prepared. The physicochemical properties of the drug:HP-β-CD solutions were determined and the inclusion complexes were characterised by ^{1}H and ^{13}C NMR spectroscopy. The apparent complex constants were calculated from the phase-solubility diagram and were estimated at 403 M^{-1} and 1308 M^{-1} for the antazoline:HP-β-CD complex and tetracaine:HP-β-CD complex, respectively. ^{1}H and ^{13}C NMR analysis showed that in the 1:1 complexes the total antazoline fraction was present as an inclusion complex, whereas tetracaine was only partly included in spite of a similar phase solubility diagram. NMR spectroscopy also revealed the site of interaction of the drugs with the HP-β-CD molecule. A solution acceptability test was carried out on volunteers. A relationship between the surface tension of the solutions and the tolerance was observed.

Keywords: Tetracaine; Antazoline; Hydroxypropyl-β-cyclodextrin; Surface tension; NMR spectroscopy; In vivo tolerance

1. Introduction

Antazoline is an antiallergenic agent used in the treatment of irritation of the ocular mucosa. Tetracaine is a local anaesthetic for diagnostic or therapeutic use. Both drugs have an amphiphilic

* Corresponding author. Tel.: + 32 3 8202716; fax: + 32 3 8202734; e-mail: ludwig@uia.ua.ac.be

0378-5173/98/$ - see front matter
PII: S0378-5173(98)00171-9

structure and consequently exhibit surface active properties with an irritating potential (Hugues and Le Jeunne, 1993). Instillation of irritating substances into the cul-de-sac induces lachrymation and consequently rapid drainage, which can lead to a very poor therapeutic response. These substances may have a deleterious effect on the barrier function of the corneal epithelium, depending on their CMC value and ionic nature (Marsh and Maurice, 1971; Burstein, 1980; Etter and Wildhaber, 1984). Various prodrugs developed to enhance the ocular absorption possess an amphiphilic structure. In animal studies, they showed strong eye irritation which may hinder their clinical usefulness (Suhonen et al., 1995; Jarho et al., 1996). Saarinen-Savolainen et al. (1996) have demonstrated the relationship between amphiphilic structure, surface tension and animal tolerability.

The use of cyclodextrins was proposed to encapsulate the drug molecules and reduce eye irritation (Järvinen et al., 1995; Suhonen et al., 1995; Jarho et al., 1996). Cyclodextrins are cyclic oligosaccharides consisting of six, seven or eight D-glucopyranose units (α, β and γ cyclodextrin) linked by (1–4) glycosidic bonds. They have a torus-shaped cavity into which hydrophobic molecules can be entrapped. Due to their ability to form inclusion complexes, which are readily dissociated upon instillation, cyclodextrins are widely used to increase drug solubility or stability as well as ocular bioavailability (Freedman et al., 1993; Van Doorne, 1993; Loftsson and Brewster, 1996; Rajewski and Stella, 1996). The hydroxypropyl derivative has been investigated to increase the safety, aqueous solubility and pharmaceutical usefulness of β-cyclodextrin (Pitha et al., 1986; Jansen et al., 1990; Freedman et al., 1993; Loftsson and Brewster, 1996).

The present study aimed at the evaluation of the use of HP-β-CD to improve the tolerance of antazoline and tetracaine, two model amphiphilic ophthalmic drugs previously used in this laboratory (Ludwig and Van Ooteghem, 1987; Ludwig, 1990). The characteristics of the inclusion complexes, the influence of HP-β-CD on the surface tension of the drug solutions and the results of a solution acceptability test performed on human volunteers will be discussed.

2. Materials and methods

2.1. Materials

Pharmaceutical grade antazoline HCl was supplied by Pharmachemic (Antwerp, Belgium) and tetracaine HCl was purchased from Federa (Brussels, Belgium). The free bases of the drugs were obtained after alkalisation of an aqueous salt solution with diluted ammonia. The precipitate formed was washed with water until a neutral pH filtrate. The powder was dried overnight at 40°C. The chemical structure of the drugs is given in Fig. 1. Hydroxypropyl-β-cyclodextrin (HP-β-CD) (degree of substitution 0.47; relative molecular mass 1379) was obtained from Janssen Biotech (Olen, Belgium). All other chemicals were of analytical grade. Deionised freshly double-distilled water was used throughout the study.

2.2. Preparation of inclusion complexes and phase-solubility analysis

The hydrochloride salts of both drugs are readily soluble in water (1:50 for antazoline HCl and 1:100 for tetracaine HCl); because the free bases are practically insoluble, they were complexed with HP-β-CD.

The required amount of antazoline (33.1 mM) or tetracaine (16.6 mM) was added to aqueous

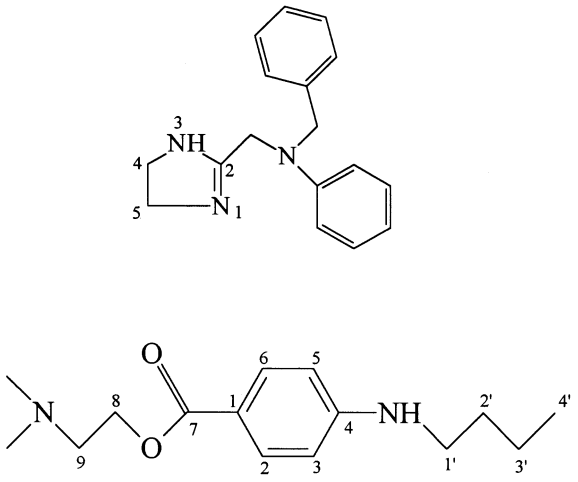

Fig. 1. Chemical structure of antazoline and tetracaine.

solutions of HP-β-CD with an increasing concentration ranging from a 0:1 to 3:1 HP-β-CD:drug ratio. The dispersions were agitated with a magnetic stirrer during 7 days at room temperature (approximately 22°C) and protected from light to equilibrate.

After equilibration, the dispersions were filtered through a cellulose nitrate membrane filter with pore size 0.45 μm (Sartorius, Göttingen, Germany). The filtrate was suitably diluted with double-distilled water and analysed spectrophotometrically at the appropriate wavelength (Uvikon 810, Kontron, Italy). All experiments were carried out in triplicate. Apparent stability constants were calculated from the slope and intercept of the straight portion of the phase solubility diagram, according to the method of Higuchi and Connors (1965).

As reference solutions for the solution acceptability test a 33.1-mM HP-β-CD, a 33.1-mM antazoline HCl and a 16.6-mM tetracaine solution were prepared by dissolving the required amount of powder in double-distilled water. Sodium chloride was added to obtain iso-osmotic conditions.

2.3. Physical measurements

2.3.1. Physicochemical properties

The pH of the solutions was determined at room temperature with a CG840 pH meter (Schott-Geräte, Mainz, Germany). The osmolality (mOsm/kg) of the filtered solutions was measured using a vapour pressure osmometer (model 5500, Wescor, Logan, UT). The mean of three measurements was calculated. The surface tension of the filtered solutions was calculated from the mean weight of drops dispensed from an officinal dropper of the Eur. Ph. III (1997) for six determinations. Double-distilled water was used as a reference with 20 drops equalling 1 g or 1 ml, having a surface tension of 72 mN/m.

2.3.2. Drop delivering method and drop weight determination

The drop size of the filtered solutions was characterised by its weight, which was determined using an apparatus developed in our laboratory to dispense drops separately under standard conditions (Van Santvliet and Ludwig, 1995). A flexible dropper bottle filled with 10-ml solution and fitted with a dropper tip was fixed in the upright position (90° angle) in the apparatus. A motor-driven pusher compressed the bottle at a constant rate, 30 or 100 rpm, until a drop was delivered. The drop was weighed immediately on an analytical balance (Sartorius model 2462, readability 0.1 mg, Sartorius, Göttingen, Germany). The mean weight of ten drops was calculated for each solution at both speeds.

2.3.3. NMR analysis

^{1}H and ^{13}C NMR spectra were recorded at 30°C on a Bruker DRX-400 instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (TMS) (internal reference, 0 ppm). Samples were prepared by dissolving freeze-dried powder in CD_3OD (99.8% D). ^{1}H and ^{13}C NMR spectra of the following samples were recorded: HP-β-CD (10 mM in CD_3OD), antazoline (10 mM in CD_3OD) antazoline:HP-β-CD 1:1 complex (10 mM in CD_3OD), antazoline:HP-β-CD 1:1 mixture (10 mM in CD_3OD), tetracaine (20 mM in CD_3OD), tetracaine:HP-β-CD 1:0.75, 1:1, 1:2 and 1:3 complex (20 mM in CD_3OD), and tetracaine:HP-β-CD 1:1 mixture (20 mM in CD_3OD). The corresponding 1:1 samples (complexes and mixtures) of antazoline HCl and tetracaine HCl were prepared and analysed in the same manner. Samples of complexes or mixtures containing HP-β-CD were prepared at least 48 h before running the NMR spectra.

2.4. Solution acceptability test

A solution acceptability test was performed on human volunteers. Each volunteer gave informed consent prior entering the study. As the human conjunctival sac can only retain about 30 μl fluid without overflow onto the lids and cheek, and assuming a normal tear volume of 7 μl, a 10-μl drop of the test solutions was instilled into one eye of the volunteer using a sterile Eppendorf pipette (Mishima et al., 1966). After instillation, the volunteers were asked to give their own evaluation by answering a standard questionnaire

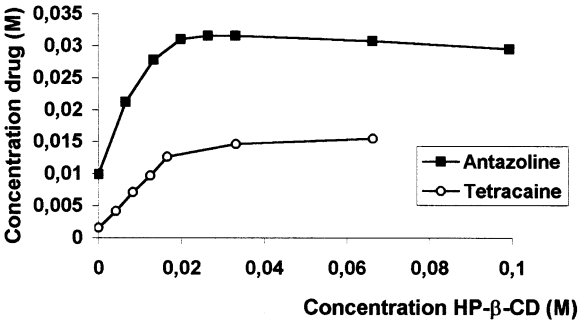

Fig. 2. Phase-solubility diagrams for antazoline and tetracaine.

(Ludwig and Van Ooteghem, 1992). The mean irritation, lachrymation, sensation, vision and pain score were calculated for each solution.

Moreover, the overflow of the lachrymal fluid due to lachrymation was quantified. When the instillation caused irritation resulting in overflow of tears, the lachrymal fluid was absorbed by preweighed cotton-wool tips. The volume of the overflow was determined by the weight increase of the cotton-wool tip.

In order to evaluate if rapid topical anaesthesia was elicited by test solutions containing tetracaine:HP-β-CD complexes, a drop of 5% (w/w) sterile NaCl was instilled 10 min after the application of the test solution. The volunteers were asked if this very hypertonic solution (1534 mOsm/kg) caused a sensation of pain or not.

3. Results and discussion

3.1. Characterisation of drug:HP-β-CD complexes

The phase solubility diagrams of antazoline and tetracaine are given in Fig. 2. The apparent complex constants of the free base of the drugs were calculated from the straight part of the curve. The value of the complex constant of the antazoline:HP-β-CD complex amounted 403 M^{-1}, while for the tetracaine:HP-β-CD complex a higher value of 1308 M^{-1} was measured. To determine the interaction of the drugs with HP-β-CD, the antazoline and tetracaine inclusion complexes were studied by NMR spectroscopy.

^{1}H as well as ^{13}C NMR spectroscopy indicated that in the antazoline:HP-β-CD 1:1 inclusion complex the heterocyclic ring of antazoline is located inside the hydrophobic cavity of the HP-β-CD molecule. This was evident from the ^{1}H and ^{13}C chemical shift of H-4/H-5 and C-4/C-5, respectively, and the multiplicity of H-4 and H-5 in ^{1}H NMR. ^{1}H and ^{13}C NMR spectra of antazoline, and of an equimolar mixture of antazoline and HP-β-CD, showed that in antazoline positions 4 and 5 are equivalent (i.e. N-1 and N-3 are equivalent due to rapid exchange). H-4 and H-5 appear as a four-proton singlet at δ 3.552 in the ^{1}H NMR spectrum of antazoline (masked by the solvent in ^{13}C NMR). The ^{1}H and ^{13}C NMR spectra of the 1:1 mixture of antazoline and HP-β-CD are the sum of the spectra of the individual compounds. Chemical shift differences observed for antazoline in the presence or absence of an equimolar amount of HP-β-CD are in the range of 0.01 ppm or below for ^{1}H NMR (e.g. antazoline δ 3.552 for H-4/H-5, antazoline:HP-β-CD 1:1 mixture δ 3.560), and in the range of 0.1 ppm or below for ^{13}C NMR.

However, in the antazoline:HP-β-CD inclusion complex apparently the symmetry of the dihydroimidazole moiety is lost, and positions 4 and 5 are not equivalent any more, obviously because in the hydrophobic cavity exchange between N-1 and N-3 is too slow on the NMR time scale. H-4 and H-5, each having two neighbouring hydrogens, each produce a triplet with a different chemical shift, due to their different chemical environment (δ 3.263 and δ 2.684, t each, $J = 6.2$ Hz).

Also in ^{13}C NMR two signals are observed for C-4 and C-5 (δ 42.473 and δ 41.873). Therefore, it can be concluded that the heterocyclic moiety of antazoline is located inside the torus of the HP-β-CD molecule.

Also the tetracaine:HP-β-CD inclusion complexes were studied by ^{1}H and ^{13}C NMR spectroscopy. In the NMR spectra of the inclusion complexes two sets of signals are observed for tetracaine, indicating that the exchange rate between free and included tetracaine is slow on the NMR time scale. ^{1}H NMR assignments for tetracaine, for tetracaine in a 1:1 mixture with HP-β-CD, and in a tetracaine:HP-β-CD 1:0.75

Table 1
^{1}H NMR assignments (δ, ppm) and multiplicity for tetracaine (CD_3OD, 400 MHz)

H No.	A	B	C		
			C1	C2	$\Delta(\delta_{C2}-\delta_{C1})$
2,6	7.783, d, $J=8.9$ Hz	7.779	7.782	7.753	−0.029
3,5	6.567, d, $J=8.9$ Hz	6.564	6.564	6.548	−0.016
8	4.352, t, $J=5.6$ Hz	4.351	4.367	4.367	0.0
9	2.730, t, $J=5.6$ Hz	2.729	2.785, m	2.785	[a]
N-methyl	2.336, s	2.334	2.378	2.537	+0.159
1′	3.136, t, $J=7.1$ Hz	3.133	3.134, m	3.134	[a]
2′	1.608, m	1.605	1.605	1.605	0.0
3′	1.431, m	1.428	1.428	1.428	0.0
4′	0.968, t, $J=7.3$ Hz	0.964	0.965	0.965	0.0

A, tetracaine (free); B, tetracaine in a 1:1 mixture with HP-β-CD; C, tetracaine: HP-β-CD 1:0.75 inclusion complex; C1, tetracaine (free); C2, tetracaine (inclusion complex); Δ, chemical shift difference between free tetracaine and tetracaine in the inclusion complex ($\delta_{C2}-\delta_{C1}$).
[a] Overlapping triplets.

inclusion complex are listed in Table 1. Only minor differences are observed between chemical shifts of pure tetracaine or in a 1:1 mixture with HP-β-CD. In the ^{1}H NMR spectrum of the 1:0.75 tetracaine:HP-β-CD inclusion complex, however, two sets of signals are observed for several hydrogens, obviously because tetracaine occurs only partly as an inclusion complex with HP-β-CD. The chemical shifts which are most close to those of tetracaine in the 1:1 mixture with HP-β-CD can be assigned to free tetracaine; these are also the more intense signals. The minor signals can be assigned to tetracaine in the inclusion complex. These assignments were confirmed by adding an amount of tetracaine to the 1:0.75 inclusion complex, and recording the ^{1}H and ^{13}C NMR spectra again; the relative intensity of all resonance signals assigned to free tetracaine increased, compared to those of tetracaine in the inclusion complex. The largest chemical shift difference between free tetracaine and tetracaine in its complex is observed for the *N*-methyl group: from δ 2.378 (free) to δ 2.537 (inclusion complex) ($\Delta + 0.159$ ppm) in ^{1}H NMR. Using the integration values of these two singlets, it could be calculated that only about one third (32%) of tetracaine is present as an inclusion complex. Chemical shift differences were also observed for H-2/H-6 ($\Delta - 0.029$ ppm), H-3/H-5 ($\Delta - 0.016$), and for H-9 and H-1′. For H-9 and H-1′ a chemical shift difference in the range of the coupling constant resulted in a complex multiplet (two overlapping triplets), and the chemical shift difference cannot exactly be calculated (≈ 0.02 ppm).

The same approach was followed to study the ^{13}C NMR spectra of tetracaine containing samples. ^{13}C NMR assignments are listed in Table 2. Chemical shift differences observed between tetracaine in a 1:1 mixture with HP-β-CD, and in the 1:0.75 inclusion complex, are shown in Fig. 3. In the inclusion complex, all carbons of the dimethylaminoethyl chain are shielded (up to 1.178 ppm for H-8 and 0.886 ppm for the *N*-methyl groups), and all carbons of the *N*-butyl group are slightly deshielded (< 0.3 ppm). Also C-4 of the aromatic ring shows a large shielding effect in the inclusion complex. Taking into account that in ^{1}H as well as ^{13}C NMR spectra major chemical shift effects are observed for the aromatic ring, and especially the dimethylaminoethyl chain, and only minor effects for the butyl chain, it can be concluded that the carboxylic acid ester side chain, and not the butylamino chain, is the site of interaction between tetracaine and HP-β-CD in the inclusion complex, or in other words that the dimethylaminoethyl chain is located inside the cavity of HP-β-CD.

Table 2
^{13}C NMR assignments (δ, ppm) and multiplicity for tetracaine (CD_3OD, 100 MHz)

C No.	A	B	C		
			C1	C2	$\Delta(\delta_{C2}-\delta_{C1})$
1	117.421	117.341	117.276	117.276	0.0
2,6	132.651	132.578	132.607	132.437	−0.170
3,5	112.061	111.990	112.002	112.043	+0.041
4	154.940	154.844	154.879	153.724	−1.155
7	168.825	168.718	168.680	168.680	0.0
8	62.874	62.794	62.567	61.389	−1.178
9	58.865	58.779	58.722	58.639	−0.083
N-methyl	45.866	45.805	45.691	44.805	−0.886
1′	43.730	43.661	43.668	43.945	+0.277
2′	32.361	32.279	32.287	32.411	+0.124
3′	21.330	21.253	21.262	21.298	+0.036
4′	14.247	14.185	14.192	14.224	+0.032

A, tetracaine (free); B, tetracaine in a 1:1 mixture with HP-β-CD; C, tetracaine: HP-β-CD 1:0.75 inclusion complex; C1, tetracaine (free); C2, tetracaine (inclusion complex); Δ, chemical shift difference between free tetracaine and tetracaine in the inclusion complex ($\delta_{C2}-\delta_{C1}$).

NMR studies of α-CD inclusion complexes with simple aromatic compounds such as benzoic acid, para-hydroxy-benzoic acid, etc., have shown that the included ('head') carbons are shielded, and that the corresponding para-carbons ('tail') are deshielded. For para-OH-benzoic acid it was concluded that the carboxyl groups were directed into the α-CD cavity (Gelb et al., 1981; Inoue et al., 1985; Inoue, 1993). In a larger molecule such as tetracaine the dimethylaminoethyl chain can be considered as the 'head', which is shielded, and the butylamino group as the 'tail', which is deshielded.

Apparently the chemical shift differences of the middle aromatic part in tetracaine are more difficult to rationalise; steric effects may also play a role.

Also the tetracaine:HP-β-CD 1:1, 1:2 and 1:3 inclusion complexes were studied by ^{1}H and ^{13}C NMR spectroscopy. All these spectra showed the same pattern as the 1:0.75 inclusion complex, i.e. doubling of signals due to free tetracaine, and tetracaine in the inclusion complex. Surprisingly, the relative amount of free tetracaine, calculated from the integration values of the *N*-methyl groups as described above, increased from 68% in the 1:0.75 complex, over 87% (1:1 complex) and 91% (1:2 complex), to 94% (1:3 complex). Increasing the amount of HP-β-CD when preparing the tetracaine:HP-β-CD inclusion complexes under the conditions as described in Section 2.2 does not result in including more tetracaine. This implies that the increase in solubility of tetracaine in the presence of HP-β-CD, as illustrated in the phase solubility diagram (Fig. 2), is not due to the formation of an inclusion complex, but to a solubilising effect of HP-β-CD (formation of an 'external' complex). Apparently, increased solubility of a drug in the presence of cyclodextrins observed in a phase solubility analysis cannot be considered as a proof for the formation of inclusion complexes.

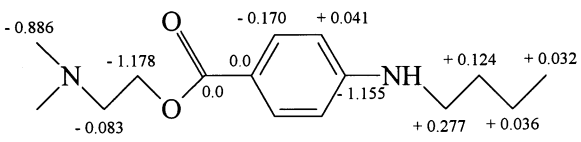

Fig. 3. Chemical shift differences (ppm) observed in ^{13}C NMR for free tetracaine and tetracaine in an inclusion complex with HP-β-CD (see Table 2).

On the other hand, samples containing antazoline HCl or tetracaine HCl and HP-β-CD, and prepared in the same manner to produce inclusion complexes, were also analysed by ^{1}H and ^{13}C

Table 3
Physicochemical properties of the solutions

Solution	PH	σ (mN/m)	Osmolality (mOsm/kg)
HP-β-CD 33.1 mM (NaCl)	6.4	69.4	268
Antazoline HCl 33.1 mM (NaCl)	6.2	58.5	262
Antazoline:HP-β-CD 1:1	9.6	49.4	120
Antazoline:HP-β-CD 1:2	9.7	55.9	138
Antazoline:HP-β-CD 1:3	9.7	59.9	156
Tetracaine HCl 16.6 mM (NaCl)	6.1	52.9	269
Tetracaine:HP-β-CD 1:1	8.4	44.4	114
Tetracaine:HP-β-CD 1:2	8.8	54.8	103
Tetracaine:HP-β-CD 1:3	8.6	61.3	109

NMR. No chemical shift differences compared to the corresponding mixtures were observed, indicating that the hydrochlorides do not form inclusion complexes. Apparently, there is no interaction between the ionic forms of the drugs and HP-β-CD.

3.2. Surface tension and tolerance of the drug solutions containing HP-β-CD

As the tolerance of ophthalmic solutions depends in part on the surface tension of the preparation instilled, the surface tension of the solutions where the drug molecule is complexed with HP-β-CD were examined (Marsh and Maurice, 1971; Etter and Wildhaber, 1984). The results of the surface tension measurements determined with the officinal dropper are summarised in Table 3.

The pure HP-β-CD solution did not exhibit surface active properties, in contrast to the antazoline HCl and tetracaine HCl solutions, where the surface tension was about 10 and 15 mN/m lower, respectively. The surface tension of the antazoline:HP-β-CD and tetracaine:HP-β-CD 1:1 complexes was even lower, but increasing amounts of HP-β-CD (up to 1:3) reduced the surface active properties. A 1:3 molar ratio (for antazoline) or a 1:2 molar ratio (for tetracaine) yielded solutions with about the same surface tension as the HCl salt solutions. Martini et al. (1996) demonstrated the clear CMC shift to higher values when CD was added to aqueous solutions of amphiphilic molecules.

These changes in surface tension were confirmed by the mean drop weight of the test solutions dispensed from flexible dropper bottles. The surface tension of the solutions was calculated by comparing its drop weight with that of solutions with known surface tension, at both speeds, at 30 rpm and 100 rpm. The results of the drop weight determination and the surface tension of the solutions calculated are given in Table 4.

As according to Tate's law (Equation 1):

Table 4
Drop weight determination and surface tension calculation

Solution	Mean drop weight (mg)		σ (mN/m)
	30 rpm	100 rpm	
HP-β-CD 33.1 mM (NaCl)	37.26	39.56	60.1
Antazoline HCl 33.1 mM (NaCl)	35.92	37.95	57.5
Antezoline:HP-β-CD 1:1	30.28	31.48	46.6
Antazoline:HP-β-CD 1:2	33.34	35.46	52.5
Antazoline:HP-β-CD 1:3	34.34	36.14	54.5
Tetracaine HCl 16.6 mM (NaCl)	32.29	33.99	50.5
Tetracaine:HP-β-CD 1:1	28.85	30.89	43.8
Tetracaine:HP-β-CD 1:2	30.82	32.40	47.6
Tetracaine:HP-β-CD 1:3	32.20	34.28	50.3

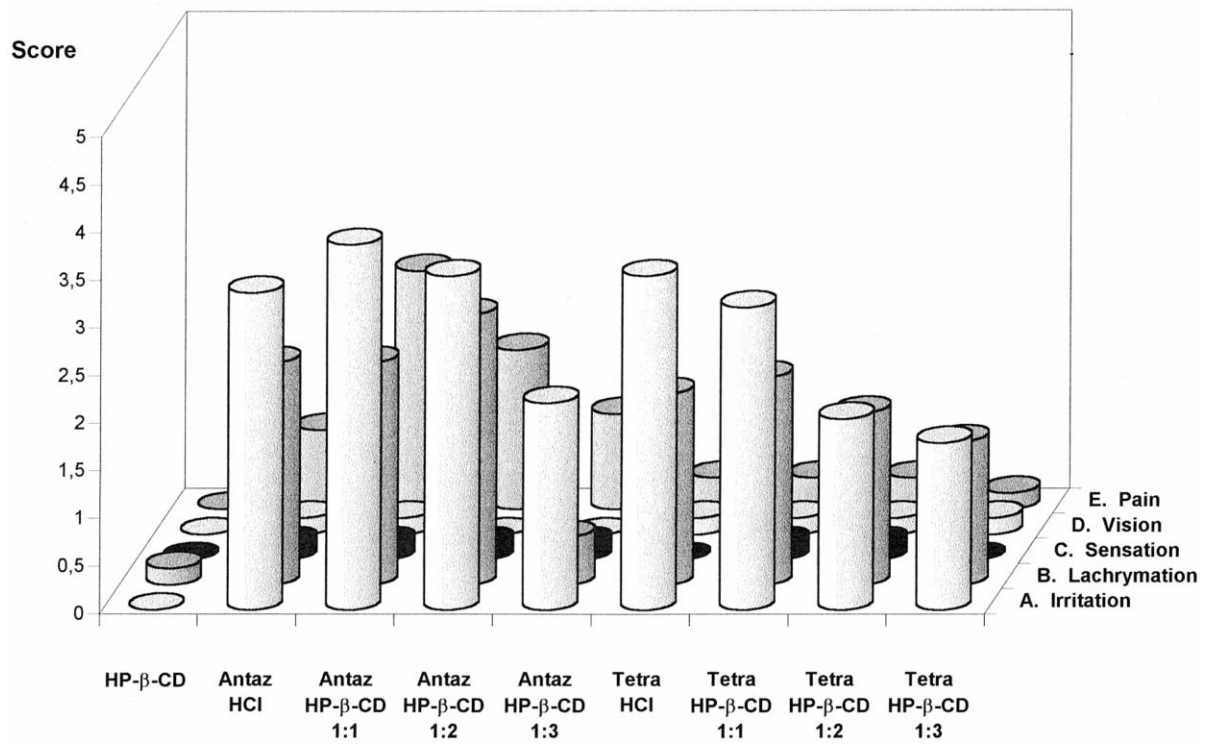

Fig. 4. Results of the solution acceptability test.

$$W = 2 \cdot \pi \cdot \sigma \cdot r \quad (1)$$

where W is the weight of the drop, σ is the surface tension of the solution and r is the radius of the tip. The lower the surface tension of the solution, the lower the weight of the drop dispersed. Squeezing the bottle more slowly resulted in a decrease in drop weight. At the instant of breaking away of the drop from the outer orifice of the dropper tip, an extra impulse of liquid is injected into the falling drop when the drop formation is high.

Here also, the HP-β-CD solution did not exhibit surface active properties and a decrease in surface tension for both salt solutions was observed. Similar changes of the surface tension as measured in the first experiment, were observed. The values of the surface tension deviated from those of the method with the officinal dropper because the formation of drops at the outer orifice of the tip and the speed of dispensing drops were slightly different in both methods.

Considering the potential ocular irritancy of the amphiphilic drugs and to determine the influence of HP-β-CD on this discomfort, the tolerance of the test solution was evaluated. The six volunteers (three men and three women) gave their own evaluation about the discomfort elicited by the sterile filtered solutions instilled. The following questions were asked and a 0–5 grading was used:

(a) Does your eye hurt? Irritation score: no irritation (0), mild (1), hurting (2), stinging (3–5);

(b) Does your eye feel watery? Lachrymation score: eye watery (0–2), lachrymation (3–4), overflow onto the cheek (5);

(c) How does the eye drop feel? Sensation score: smooth (0), thick (1), sticky (2), gritty (3), sandy (4–5);

(d) Does the drop cause blurring of vision? Vision score: clear (0), blurred vision (1–5);

(e) Does the drop cause a sensation of pain at the inner canthus? Pain score: 0–5.

After the test, the addition of the grading value or scores given by the six volunteers was made and the mean value for each question was calculated. The results of the solution acceptability test are presented in Fig. 4.

The HP-β-CD solution was well tolerated by the volunteers as indicated by the low score values. For the reference antazoline HCl and tetracaine HCl solutions, on the other hand, ocular irritancy with lachrymation and pain at the inner canthus was observed. The antazoline:HP-β-CD complex in a 1:1 molar ratio hurt even more than the reference antazoline HCl solution. This could be explained by the lower surface tension (49.4 mN/m) of this solution. To reduce the irritancy potential of antazoline base a 1:3 molar ratio was necessary. The tetracaine:HP-β-CD 1:1 complex exhibited a similar irritation as the tetracaine HCl solution. The higher molar complexes had a slightly reduced ocular irritancy. No difference in tolerance between the tetracaine HCl solution and a tetracaine HCl:HP-β-CD solution was observed (results not shown). Although the surface tension properties may not be the only source of the irritancy of the drugs, apparently the tolerance of the test solutions is related to their surface tension.

Even if the solutions are not isohydric and iso-osmotic as can be seen from the results in Table 3, the pH and osmolality values should not cause irritation as several clinical studies demonstrated that hypotonic and weak alkaline solutions are well tolerated after instillation to human volunteers (Ludwig and Van Ooteghem, 1987).

During this test, the overflow of lachrymal fluid caused by irritation after instillation of the solution was also quantified. The results are summarised in Table 5. The overflow measured after instillation of the antazoline:HP-β-CD 1:1 complex was larger compared to that of the antazoline HCl solution. Improvement was only observed when a 1:3 molar ratio was used. In the case of the tetracaine preparations, no important lachrymal overflow could be measured.

In order to evaluate if rapid topical anaesthesia was elicited by test solutions containing HP-β-CD complexes a drop of 5% (w/w) NaCl was instilled 10 min after application of the tetracaine preparation. The volunteers sensed no pain. The rapid therapeutic effect, even at a 1:3 molar ratio, is probably due to the high percentage of free drug as seen in the NMR study.

Table 5
Determination of the overflow of lachrymal fluid after instillation of the test solutions and evaluation of the topical anaesthesia of the tetracaine solutions

Solution	Overflow (mg)	Instillation of 5% NaCl
HP-β-CD 33.1 mM (NaCl)	0.008	
Antazoline HCl 33.1 mM (NaCl)	0.018	
Antazoline:HP-β-CD 1:1	0.037	
Antezoline:HP-β-CD 1:2	0.015	
Antazoline:HP-β-CD 1:3	0.006	
Tetracaine HCl 16.6 mM (NaCl)	0.006	No pain
Tetracaine:HP-β-CD 1:1	0.006	No pain
Tetracaine:HP-β-CD 1:2	0.004	No pain
Tetracaine:HP-β-CD 1:3	0.004	No pain

4. Conclusions

Whereas in a 1:1 complex the total antazoline fraction was present as an inclusion complex, tetracaine was only partly included, although the phase solubility diagram might also suggest the formation of an inclusion complex. This can be explained by a solubilising effect of HP-β-CD. By increasing the amount of HP-β-CD, solutions with a higher surface tension and a better tolerance could be prepared. The ratio of drug to HP-β-CD required has to be determined for each drug separately. Moreover the therapeutic activity of the complex prepared should be evaluated, especially when high amounts of CD are used.

Acknowledgements

This work was supported by the FWO (Fund for Scientific Research) - Flanders (Belgium) (grant no. G.0119.96).

References

Burstein, N.L., 1980. Corneal cytotoxicity of topically applied drugs, vehicles and preservatives. Surv. Opthalmol. 25, 15–30.

Etter, J.C., Wildhaber, A., 1984. Développement d'un test objectif d'irritation oculaire sur la souris: intérêt en pharmacie galénique et biopharmacie. Première partie: les tensioactifs. Pharm. Acta Helv. 59, 8–15.

Freedman, K.A., Klein, J.W., Crosson, C.E., 1993. β-Cyclodextrins enhance bioavailability of pilocarpine. Curr. Eye Res. 12, 641–647.

Gelb, R., Schwartz, L., Cardelino, B., Fuhrman, H., Johnson, R., Layer, D., 1981. Binding mechanisms in cyclohexa-amylase complexes. J. Am. Chem. Soc. 103, 1750–1757.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. In: Reilly, C.N. (Ed.), Advances in Analytical Chemistry and Instrumentation, vol. 4. Interscience, New York, pp. 117–212.

Hugues, J.-C., Le Jeunne, C., 1993. Systemic and local tolerability of ophthalmic drug formulations, an update. Drug Saf. 8, 365–380.

Inoue, Y., 1993. NMR studies of the structure and properties of cyclodextrins and their inclusion complexes. Annu. Rep. NMR Spectrosc. 27, 63–101.

Inoue, Y., Hoshi, H., Sakurai, M., Chûjô, R., 1985. Geometry of cyclohexaamylose inclusion complexes with some substituted benzenes in aqueous solution based on carbon-13 NMR chemical shifts. J. Am. Chem. Soc. 107, 2319–2323.

Jansen, T., Xhonneux, B., Mesens, J., Borgers, M., 1990. β-Cyclodextrins as vehicles in eye-drop formulations: an evaluation of their effects on rabbit corneal epithelium. Lens Eye Toxicity Res. 7, 459–468.

Jarho, P., Järvinen, K., Urtti, A., Stella, V.J., Järvinen, T., 1996. Modified β-cyclodextrin (SBE7-β-CyD) with viscous vehicle improves the ocular delivery and tolerability of pilocarpine prodrug in rabbits. J. Pharm. Pharmcol. 48, 263–269.

Järvinen, T., Järvinen, K., Urtti, A., Thompson, D., Stella, V.J., 1995. Sulfobutyl ether β-cyclodextrin (SBE-β-CD) in eyedrops improves the tolerability of topically applied pilocarpine prodrug in rabbits. J. Ocul. Pharmacol. Therap. 11, 95–106.

Loftsson, T., Brewster, M., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilisation and stabilisation. J. Pharm. Sci. 85, 1017–1025.

Ludwig, A., 1990. Study on administration and physical properties of ophthalmic solutions on the precorneal retention of a tracer. Thesis. University of Antwerp.

Ludwig, A., Van Ooteghem, M., 1987. The influence of the osmolality on the precorneal retention of ophthalmic solutions. J. Pharm. Belg. 42, 259–266.

Ludwig, A., Van Ooteghem, M., 1992. Influence of viscolyzers on the residence of ophthalmic solutions evaluated by slitlamp fluorophotometry. Stp Pharma Sci. 2, 81–87.

Marsh, R.J., Maurice, D.M., 1971. The influence of non-ionic detergents and other surfactants on human corneal permeability. Exp. Eye Res. 11, 43–48.

Martini, A., Artico, R., Civaroli, P., Muggetti, L., De Ponti, R., 1996. Critical micellar concentration as a simple tool for evaluating cyclodextrin/enhancer interactions. Int. J. Pharm. 127, 239–244.

Mishima, S., Gasset, A., Klyce, S.D., Baum, J.L., 1966. Determination of tear volume and tear flow. Invest. Ophthalmol. 5, 264–276.

Pitha, J., Milecki, J., Fales, H., Pannel, L., Uekama, K., 1986. Hydroxyl-β-cyclodextrin: preparation and characterisation; effects on solubility of drugs. Int. J. Pharm. 29, 73–82.

Rajewski, R.A., Stella, V.J., 1996. Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery. J. Pharm. Sci. 85, 1142–1169.

Saarinen-Savolainen, P., Järvinen, T., Suhonen, P., Urtti, A., 1996. Amphiphilic properties of pilocarpine prodrugs. Int. J. Pharm. 127, 171–178.

Suhonen, P., Järvinen, T., Lehmusssaari, K., Reunamaki, T., Urtti, A., 1995. Ocular absorption and irritation of pilocarpine prodrug is modified with buffer, polymer, and cyclodextrin in eyedrop. Pharm. Res. 12, 529–533.

Van Doorne, H., 1993. Interactions between cyclodextrins and ophthalmic drugs. Eur. J. Pharm. Biopharm. 39, 133–139.

Van Santvliet, L., Ludwig, A., 1995. Statistical analysis of the weight of drops delivered from flexible dropper bottles. In: Proceedings of the 14th Pharmaceutical Technology Conference, Barcelona, Spain, pp. 486–494.