Bupivacaine hydrochloride complexation with some α- and β-cyclodextrins studied by potentiometry with membrane electrodes

František Kopecký, Mária Vojteková, Pavol Kaclík, Marek Demko and Zuzana Bieliková

Abstract

Membrane electrodes selective to bupivacaine cations were developed and those with PVC–dibutyl-phthalate membrane containing sparingly soluble bupivacaine phosphotungstate appeared to be the most suitable. Inclusion complexation of bupivacaine cations with cyclodextrins was studied by potentiometric measurements of the free bupivacaine cation concentration in aqueous solutions of bupivacaine hydrochloride with cyclodextrin using the prepared electrodes. Native α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD), as well as their random-substituted derivatives hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and methyl-β-cyclodextrin (M-β-CD), were chosen for the study. The measured potentiometric data processed both by a linear and non-linear regression corroborated the formation of weak 1:1 bupivacaine cation–cyclodextrin complexes and the corresponding complexation constants $K_{11} \sim 50$–$155\,\mathrm{M}^{-1}$ were evaluated by the non-linear least-squares method. The mutual order of K_{11} values, especially α-CD > β-CD, suggested that the bupivacaine butyl group was mainly responsible for the inclusion complexation; the highest K_{11} was exhibited by M-β-CD followed by α-CD. The observed complexation may substantially modify properties of bupivacaine hydrochloride dosage forms with sufficient concentration of cyclodextrin but bupivacaine cations are readily released from the weak cyclodextrin complexes by dilution.

Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University, SK-83232 Bratislava, Slovak Republic

František Kopecký, Mária Vojteková, Pavol Kaclík, Marek Demko, Zuzana Bieliková

Correspondence: F. Kopecký, Faculty of Pharmacy, Comenius University, Odbojárov 10, SK-83232 Bratislava, Slovak Republic. E-mail: kopecky@fpharm.uniba.sk

Introduction

Bupivacaine is regarded as a standard long-acting local anaesthetic despite its certain drawbacks (Mather & Chang 2001; Whiteside & Wildsmith 2001; Halpern & Walsh 2003). In this connection, several workers studied the complexation of bupivacaine with β-cyclodextrin and its derivatives in-vitro (Dollo et al 1996a, b, 1998a), as well as the effect of these cyclodextrins on bupivacaine action in-vivo (Meert & Melis 1992; Fréville et al 1996; Dollo et al 1998b), with the aim of modulating the biopharmaceutic properties of bupivacaine and improving the quality of its injection solutions. Bupivacaine hydrochloride is a well-soluble salt but the aqueous solubility of unionized bupivacaine base is poor (Shah & Maniar 1993). Thus the addition of cyclodextrin into solutions of bupivacaine hydrochloride was also used to avoid precipitation of the base at higher pH and to eliminate turbidity on mixing the solutions with body fluids (Miyoshi et al 1998).

Native and derivatized cyclodextrins are recognized as pharmaceutical excipients suitable in similar circumstances due to the reversible formation of the soluble drug–cyclodextrin inclusion complexes (Burnette & Connors 2000) but only few cyclodextrins (e.g. hydroxypropyl-β-cyclodextrin (HP-β-CD)) are acceptable in parenteral solutions until now (Thompson 1997; Zhang & Rees 1999). However, other cyclodextrins may be used as stabilizers or penetrants in topical formulations (Loftsson & Masson 2001) and it is therefore also desirable to study their interaction with local anaesthetics like bupivacaine. Furthermore, native cyclodextrins, α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD), and their derivatives have been employed as chiral selectors in electrophoretic enantioseparation of racemic cationic local anaesthetics, including bupivacaine (Amini & Paulsen-Sorman 1997; Amini et al 1998).

The formation of the drug–cyclodextrin inclusion complex in aqueous solution is a reversible process, characterized by the equilibrium between the free and complex-bound drug, and the biopharmaceutic effect of the complexation depends primarily on the variable ratio between the complexed and free drug. In the studied case, the free drug in solution is represented by bupivacaine cations since their deprotonization is not actual in acidic or neutral solution with regard to bupivacaine, pK_a 8.2 (Strichartz et al 1990), and, furthermore, the free bupivacaine base is only poorly soluble in water (Shah & Maniar 1993; Dollo et al 1998a, b). The important concentration of the free bupivacaine cations thus follows from the respective total concentrations of bupivacaine hydrochloride and cyclodextrin, the stoichiometry of the formed complex and the corresponding complexation constant (equilibrium or binding constant), supposing that no other competitive processes occur. Complexation constants of bupivacaine cations or base with some β-cyclodextrins have already been estimated from the phase solubility diagrams (Dollo et al 1996b, 1998a, b; Fréville et al 1996) but determination of the free bupivacaine cations in solution with cyclodextrin has not yet been reported.

In this work, inclusion complexation of bupivacaine cations with three β-cyclodextrins (β-CD, HP-β-CD and methyl-β-cyclodextrin (M-β-CD)) and two α-cyclodextrins (α-CD and hydroxypropyl-α-cyclodextrin (HP-α-CD)) was therefore investigated by a direct potentiometric determination of the concentration of free bupivacaine cations in aqueous solution of bupivacaine hydrochloride with a respective cyclodextrin at 25°C. Racemic bupivacaine hydrochloride was chosen for this study because of its still prevalent acceptance in clinical anaesthesiology along with the enantiopure levobupivacaine and other local anaesthetics of similar type (Mather & Chang 2001; Whiteside & Wildsmith 2001, Halpern & Walsh 2003). Potentiometric determination was carried out with membrane electrodes selective to bupivacaine cations, following the construction and testing of suitable membrane electrodes for the purpose of this work.

Materials and Methods

Materials

Bupivacaine hydrochloride (MW 324.9, Figure 1) was supplied by Pharmatrade-Slovakia and cyclodextrins by Aldrich. Two of the cyclodextrins were native (parent) crystalline cyclodextrins in the form of hydrates: well-soluble α-CD (six glucose units, MW 972.9) and less-soluble β-CD (seven glucose units, MW 1135.0). Three others were amorphous random-substituted cyclodextrin derivatives with non-ionic substituents, very-well-soluble in water (Thompson 1997; Zhang & Rees 1999); they were characterized by average degree of substitution (per one glucose unit) and average molecular weight, both given, respectively, in parentheses: HP-α-CD (0.6, 1180), HP-β-CD (0.8, 1460) and M-β-CD (1.8, 1310), known also as RAMEB. All the stated molecular weights are valid for anhydrous substances, although
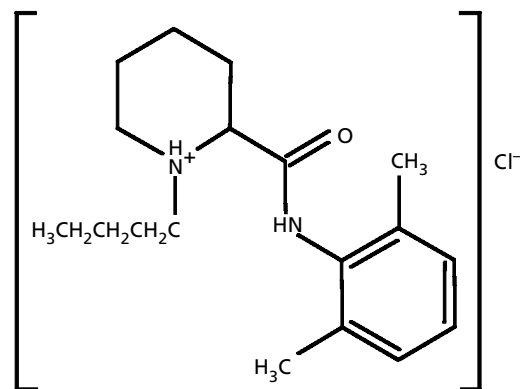

Figure 1 Structure of bupivacaine hydrochloride.

commercial bupivacaine hydrochloride is a defined mono-hydrate and cyclodextrins also contain a certain amount of crystalline water or moisture (Zhang & Rees 1999). The water content of cyclodextrins was therefore determined in a small sample dried at 110°C and proper corrections were made when solutions of the substances were prepared in de-ionized water. The stock solutions of bupivacaine hydrochloride were slightly acidified to pH 5–6 with HCl, to prevent deprotonization of bupivacaine cations. PVC Selectophore (high molecular weight, Fluka) was a material for electrode membranes.

### Potentiometric method and preparation of membrane electrodes

Microprocessor pH meter (Hanna Instruments 9017) provided with the on-line data acquisition system was used for measuring the potential E (electromotive force) of a cell SCE |measured solution| ISE, where SCE was the saturated calomel electrode and ISE was the prepared membrane (ion-selective) electrode, selective to the bupivacaine cations (BuH^+). The electrode couple was immersed in the measured aqueous solution and the cell was placed in a vessel tempered at 25°C. For the purpose of the electrode testing and standardization the measured solution was that of bupivacaine hydrochloride. In actual potentiometric measurements of the free bupivacaine cation concentration, the measured solution contained two solutes, bupivacaine hydrochloride and cyclodextrin.

Membrane electrodes selective to bupivacaine cations were constructed in accordance with our previous experience (Kopecký et al 2002). They consisted of a tube electrode body, inside which was the internal reference Ag/AgCl electrode immersed in the internal solution of KCl 3.5 M (mol dm^{-3}) and bupivacaine hydrochloride 0.001 M, acidified to pH 6, and the tube body was closed at the bottom by a membrane. Membranes were composed of PVC, dibutylphthalate (DBP) and an electroactive ingredient in various ratios. Several electroactive ingredients were tried. These were sparingly soluble bupivacaine salts, namely tetraiodobismuthate, phosphomolybdate and phosphotungstate, prepared by precipitation from solutions of bupivacaine hydrochloride.

For the membrane preparation, PVC was dissolved in anhydrous tetrahydrofuran, the solution mixed with DBP and the selected electroactive ingredient and the homogenized mixture was cast on the glass plate. After evaporation of tetrahydrofuran, the disc membranes (approx. 8 mm diameter, mass of 200–300 mg) were cut off and fixed to the electrode body. Membrane parameters were checked and optimized. The optimal membranes were composed of PVC with DBP (in the weight ratio 2:3) and bupivacaine phosphotungstate (1–2%); such plastic gel membranes were used for the final measurements. The PVC purity, membrane thickness (controlled by weight) and homogeneity were important factors as well.

The prepared membrane electrodes were stored dry but they were conditioned for 24 h in the bupivacaine hydrochloride solution (0.001 M) before use. Then they were combined with an external reference (calomel) electrode into the electrode couples and tested by measurements in bupivacaine hydrochloride solutions in the concentration range of c_{BuHCl} 10^{-1} to 10^{-7} M. Measured potential curves of three electrodes are in shown in Figure 2. Selected membrane electrodes exhibited increasing potential curves of E vs log c_{BuHCl}, which were linear at least in the range of c_{BuHCl} 10^{-1} to 10^{-4} M and the slope $\Delta E/\Delta$log c_{BuHCl} of the linear section was not significantly different from the theoretical Nernst value of 59.16 mV. Potential curves were measured repeatedly and deteriorated electrodes with diminished slopes were discarded. The well-prepared electrodes with bupivacaine phosphotungstate membrane ingredient thus showed practically Nernstian potential response to bupivacaine cations over the required concentration range.

The potential response of the electrodes was not significantly affected by the pH changes of the measured
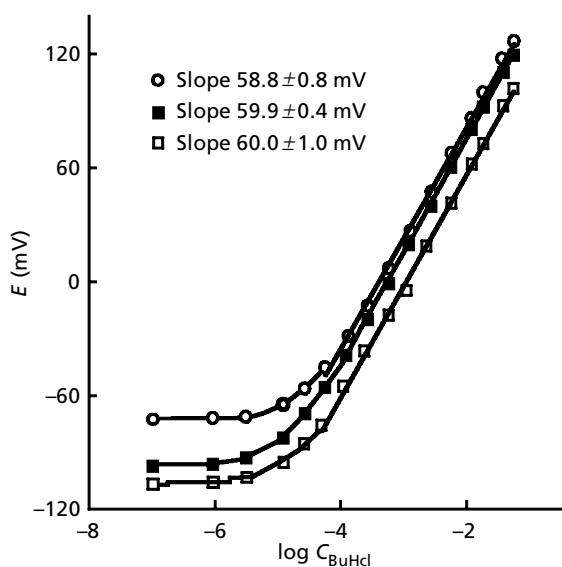
solutions in the range of 5–6.5 or by the cyclodextrin itself. In the dilute solution of a cyclodextrin (up to 0.005 M), without bupivacaine hydrochloride, the measured potential was not significantly different from that in pure water and it corresponded to the lower end of the potential curves shown in Figure 2. Selected membrane electrodes withstood several hundreds hours of potentiometric measurements but in solutions with cyclodextrins their membranes deteriorated faster.

## Measurements of the solutions of bupivacaine hydrochloride with cyclodextrin

Before and after each series of potentiometric measurements of the investigated solution of bupivacaine hydrochloride with cyclodextrin, the electrode couple was always standardized by measurements in the standardization solution of bupivacaine hydrochloride without cyclodextrin. Both standardization and investigated solutions were prepared from the acidified stock solution of bupivacaine hydrochloride so that their pH was 6–6.5, which was suitable for the prevention of deprotonization of bupivacaine cations (pK$_a$ 8.2) (Strichartz et al 1990). The concentration of the free bupivacaine cations, [BuH$^+$], in the dilute standardization solution was thus assumed to be equal to the respective total bupivacaine hydrochloride concentration, c_{BuHCl}. The linear standardization potential curves of E vs log [BuH$^+$] obtained by this way (linear sections of the curves in Figure 2) were processed by the least-squares fitting and the found parameters of the standardization curves were used for calculations of the concentration of the free bupivacaine cations, [BuH$^+$], in the investigated solution of bupivacaine hydrochloride with cyclodextrin from the potentials measured therein.

Potentiometric measurements of the investigated solutions were mostly made in series with constant total cyclodextrin concentration, c_{CD}, and varying (decreasing) total bupivacaine hydrochloride concentration, c_{BuHCl}. In each of the series, about 15 potential values were measured. Since the studied bupivacaine complexation appeared to be rather weak, it was necessary to work with a certain excess of cyclodextrin in the investigated solutions. In the final series of measurements, the concentration, c_{CD}, in the investigated solutions was in the range 0.002–0.005 M and the starting value of c_{BuHCl} was always either equal to, or lower than, c_{CD}. In the course of one series of potential measurements, c_{BuHCl} decreased gradually to about one-tenth of its starting value, while c_{CD} remained unchanged. The ratio of total concentrations covered by all the series of measurements, c_{CD}/c_{BuHCl}, was in the range of approximately 1–15. Examples of some series of measurements are shown in Figure 3A–C.

Figure 2 Potential curves of three membrane electrodes measured in solutions of bupivacaine hydrochloride (c_{BuHCl}, M); electrode membranes composed of PVC–DBP (2:3) and bupivacaine phosphotungstate (1%).

Evaluation of complex formation of bupivacaine cations with cyclodextrin

In a simple case, bupivacaine cation, BuH$^+$, and cyclodextrin, CD, may form a 1:1 inclusion complex (BuH$^+$)CD:

$$BuH^+ + CD \rightleftharpoons (BuH^+)CD \qquad (1)$$
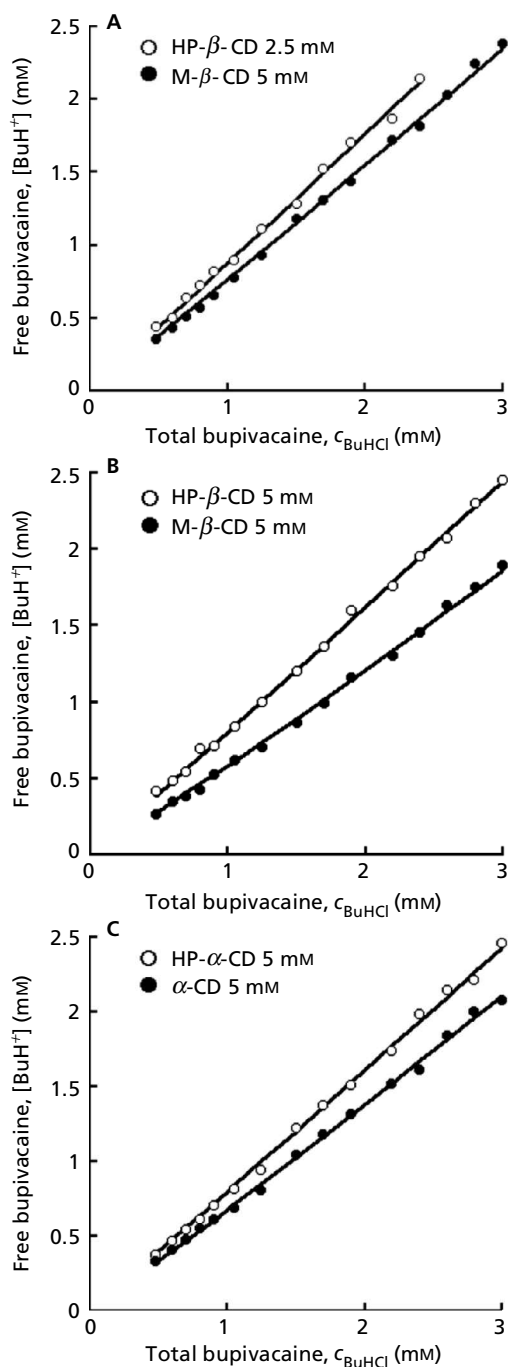
Figure 3 A–C   Free bupivacaine cation concentration ([BuH$^+$]) measured in solutions with variable total concentration of bupivacaine hydrochloride (c_{BuHCl}) and constant total concentration of cyclodextrin (c_{CD} 0.0025 or 0.005 M). Each curve represents one series of measurements; points are experimental values and solid lines represent non-linear fitting ($1 > R^2 > 0.997$) with equation 3; the curves appear nearly linear because of the weak complexation.

that is characterized by the equilibrium complexation constant (binding constant) K_{11}:

$$K_{11} = [(BuH^+)CD]/([BuH^+][CD]) \qquad (2)$$

A relationship for the equilibrium concentration of bupivacaine cations (free BuH$^+$ cations) can be derived from equation 2:

$$[BuH^+] = (c_{BuHCl} - c_{CD} - 1/K_{11})/2 + \\ (((c_{CD} - c_{BuHCl} + 1/K_{11})/2)^2 + c_{BuHCl}/K_{11})^{1/2} \quad (3)$$

Square brackets denote equilibrium concentrations and c represents the total concentrations of the corresponding species. Equation 3 is suitable for evaluation of the complexation constant K_{11} by a non-linear least-squares method (iterative procedure) from potentiometrically measured concentrations of the free bupivacaine cation [BuH$^+$] in the presence of cyclodextrin.

Since eventual formation of bupivacaine cation–cyclodextrin complexes with more complicated stoichiometry (*i:j*) cannot be a-priori excluded, experimental data were at first analysed with a linear Scatchard method for the evaluation of association equilibria, modified for the purpose of potentiometric investigation of weak cyclodextrin complexes (Mwakibete et al 1994; Kopecký et al 2002). The applied linear method operates with an auxiliary variable, r, representing a binding ratio of bupivacaine cations to cyclodextrin:

$$r = (c_{BuHCl} - [BuH^+])/c_{CD} \qquad (4)$$

Linear relationships of the type of $r = K_{ij} \, x$ were then derived for bupivacaine cation–cyclodextrin complexes with various stoichiometry (*i:j*) and the corresponding complexation constants K_{ij}. For example:

Complex 1:1, $r = K_{11}(1 - r)[BuH^+]$ $\qquad$ (5)

Complex 2:1, $r = K_{21}(2 - r)[BuH^+]^2$ $\qquad$ (6)

Complex 1:2, $r = K_{12}(1 - 2r)^2[BuH^+]c_{CD}$ $\qquad$ (7)

The linearity, or deviations from linearity, of the plots corresponding to equations 5–7 indicated which complex is predominantly formed and the approximate complexation constants estimated from the linear plots were used as the first guesses for the iterative non-linear least-squares method.

Statistical methods

Mean values $\pm$ s.d. of the data are presented when appropriate. The slopes of the linear sections of the measured potential curves (Figure 2) were tested for mutual equality and for equality with the theoretical Nernst value by the Student's *t*-test. A value of $P < 0.05$ was considered to be significant in all cases. The goodness of fit of the regression models represented by equations 5–7 was compared using the extra sum of squares F test based on the difference between the sum of squares of the two compared models and using the corrected Akaike's information criteria (AICc).

Results and Discussion

The drug–cyclodextrin inclusion complexation might be competitively affected by various kinds of the drug

self-association, thus the solutions of bupivacaine hydrochloride as a single solute were at first checked from this point of view. In this connection, the Nernstian slopes of the measured potentiometric curves (Figure 2) indicated the proper function of the prepared membrane electrodes as well as the absence of self-associations of bupivacaine cations at concentrations up to 0.1 M (mol dm^{-3}). Other authors also found no self-association or micellization in dilute aqueous solutions of bupivacaine hydrochloride (Attwood & Fletcher 1986; Thoma & Herzfeldt 1988).

Examples of the potentiometrically measured concentration of free bupivacaine cations, [BuH$^+$], in solutions of bupivacaine hydrochloride with individual cyclodextrins are shown in Figure 3A–C. In the absence of self-association or deprotonization (pH adjustment to 6–6.5), the systematically lower measured [BuH$^+$], in comparison with total bupivacaine hydrochloride, c_{BuHCl}, indicates a certain degree of complexation of bupivacaine cations with the individual cyclodextrins. The experimental data were therefore analysed using equations 5–7 and an example of corresponding plots for M-β-CD is shown in Figure 4. With all the used cyclodextrins, the linear plot corresponding to equation 5 (i.e. corresponding to the formation of the 1:1 complex) appeared to be the best model for the description of the experimental data.

The investigated complexation of bupivacaine cations with the used cyclodextrins was therefore regarded as a prevalent formation of the 1:1 complex (BuH$^+$)CD, in accordance with equation 1, and the first guesses of the complexation constants, K_{11}, were obtained from the linear least-squares calculations applied to equation 5. The definite K_{11} values were then evaluated by the iterative non-linear least-squares method using equation 3; from each series of about 15 potentiometric measurements (example curves in Figure 3A–C), one K_{11} value was obtained and the means of K_{11} are summarized in Table 1.

The weak complexation of bupivacaine cations with the used cyclodextrins is reflected by the relatively small K_{11} values (Table 1). However, yet lower respective complexation constants of 1.7 M^{-1} and 65.7 M^{-1} were reported (apparent stability constants, Dollo et al 1998a) for HP-β-CD with the degree of substitution 0.47 per one glucose unit (recalculated) and for (2,6-di-O-methyl)-β-cyclodextrin (so-called DIMEB), analogous to cyclodextrins used in this work. The two mentioned complexation constants were derived from the phase solubility diagram (i.e. from the small solubility increase of the well-soluble salt bupivacaine hydrochloride (Shah & Maniar 1993), brought about by the respective cyclodextrin as a co-solute). The phase solubility method is indeed quite essential for the determination of the cyclodextrin complexation of poorly soluble hydrophobic compounds, such as bupivacaine base (Dollo et al 1996b, 1998a, b; Fréville et al 1996). However, it is in principle less suitable for the system of a well-soluble electrolyte like bupivacaine hydrochloride with cyclodextrin, since neutral organic co-solutes mostly suppress the electrolyte solubility in aqueous medium and this effect may at least partially cancel the small increase in solubility due to the complexation. For this reason we
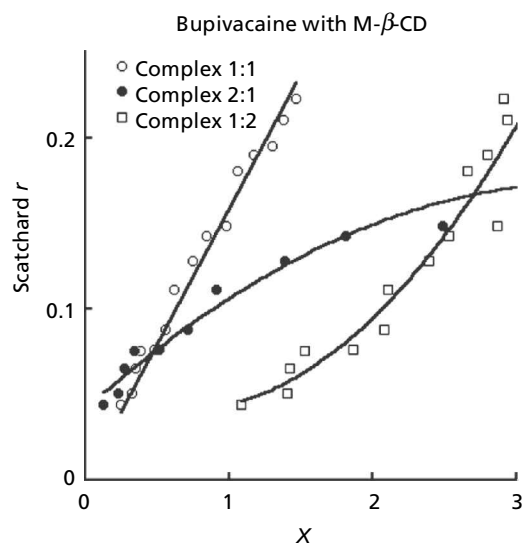
Figure 4 Plots of the Scatchard binding ratio r ($r = (c_{BuHCl} - [BuH^+])/c_{CD}$) against x for the formation of various BuH$^+$–CD complexes in solutions of bupivacaine hydrochloride with M-β-CD. The plots for complex 1:1 ($x = (1 - r)[BuH^+]$), complex 2:1 ($x = (2 - r)[BuH^+]^2$) and complex 1:2 ($x = (1 - 2r)^2[BuH^+]c_{CD}$) correspond to the same measured data (in mM) as the curve for M-β-CD shown in Figure 3B.

Table 1 Complexation constants, K_{11}, of bupivacaine cations with cyclodextrins in aqueous solution of bupivacaine hydrochloride–cyclodextrin at 25 °C.

Cyclodextrin	Degree of substitution	K_{11} (M^{-1})	No. of series of measurements
β-CD	–	63.0 ± 5.2	9
HP-β-CD	0.8	50.4 ± 7.5	8
M-β-CD	1.8	155 ± 11	10
α-CD	–	102 ± 9.5	8
HP-α-CD	0.6	54.9 ± 5.6	8

K_{11} values are means $\pm$ s.d. from individual series of measurements.

preferred potentiometric measurements of the free bupivacaine cation concentration in solutions of bupivacaine hydrochloride with cyclodextrin accompanied by adequate pH control.

The most informative feature of the K_{11} values (Table 1) with respect to the mechanism of the studied inclusion complexation is the conspicuously higher K_{11} found for the α-CD complex than that for the β-CD complex. Several hundred complexation constants had already been published and statistically processed and the mean value of K_{11} for α-CD complexes was found to be only one-quarter of the value for β-CD complexes (Burnette & Connors 2000). This is explained by a small diameter of the α-CD macrocyclus cavity in comparison with the larger size of the β-CD cavity (Thompson 1997; Zhang & Rees 1999). A few

known exceptions, characterized by higher (or not too different) K_{11} values for α-CD complexes than for β-CD complexes (Mwakibete et al 1994; Kopecký et al 2002), are represented by the structures with a longer unbranched alkyl chain, which can penetrate into the narrow α-CD cavity with optimum action of intermolecular forces. The measured K_{11} values thus suggest that the bupivacaine butyl group (Figure 1) is mainly responsible for the inclusion complexation with the cyclodextrins studied.

However, because the butyl group is rather short and linked to the positively charged nitrogen, the investigated inclusion complexation of bupivacaine cations is relatively weak and all the K_{11} values in Table 1 are relatively small in comparison with corresponding K_{11} values of organic cations (or anions) with a longer unbranched alkyl group (Mwakibete et al 1994; Kopecký et al 2002) or in the general context of cyclodextrin complexes (Burnette & Connors 2000). Higher K_{11} values for M-β-CD and lower K_{11} values for HP-β-CD and HP-α-CD, in comparison with the corresponding non-substituted cyclodextrin, reflect the effect of cyclodextrin substitution (Thompson 1997; Zhang & Rees 1999). Methyl substitution extends the cyclodextrin cavity and reinforces its non-polar character while the bulky and partially polar hydroxypropyl groups hinder penetration into the cavity.

All the determined K_{11} values must be regarded as macroconstants, since the used random-substituted cyclodextrins are in fact mixtures of a huge number of isomers and related derivatives (Thompson 1997) and also because of the racemic character of the studied bupivacaine. The chiral centre of the bupivacaine cation is located between the charged nitrogen and the polar carbonyl group (Figure 1), which are not attracted into the low-polar cyclodextrin cavity so that no strong effect of the cyclodextrin chiral recognition on the studied complexation can be expected (Rekharsky & Inoue 2000). However, the distribution of two bupivacaine enantiomers between the complexed and free fraction is probably somewhat unequal.

Experimental measurements were carried out in solutions of various concentration but, using the determined K_{11}, it is possible to calculate concentration of the free bupivacaine cations from equation 3 at required rounded total concentrations or concentration ratios. The free bupivacaine cation fraction ($100[BuH^+]/c_{BuHCl}$, %) calculated by this way for solutions of bupivacaine hydrochloride with ten-fold concentration of HP-β-CD or M-β-CD ($c_{CD}/c_{BuHCl} = 10$) is shown in Figure 5. The two curves in Figure 5 also illustrate, in logarithmic concentration scale ($-\log c_{BuHCl}$), the release of bupivacaine cations from the corresponding cyclodextrin complex when the solution of bupivacaine hydrochloride with ten-fold concentration of the given cyclodextrin is diluted with water. The data for other cyclodextrins studied in this work correspond to the region between the two curves for HP-β-CD (the lowest K_{11}) and M-β-CD (the highest K_{11}) shown in Figure 5.

According to Figure 5, the free bupivacaine fraction can be effectively suppressed in more concentrated solutions by any of the studied cyclodextrins but bupivacaine cations are readily released from the complexes if the
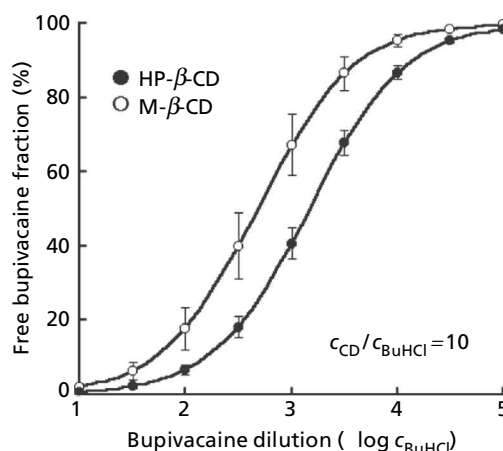
Figure 5   Release of bupivacaine cations from the inclusion complex in solutions of bupivacaine hydrochloride (c_{BuHCl}, M) with tenfold excess of HP-β-CD or M-β-CD ($c_{CD} = 10c_{BuHCl}$) in the course of dilution calculated by means of equation 3. The s.d. bars follow from the corresponding s.d. of K_{11}.

solution of bupivacaine hydrochloride with a given cyclodextrin is diluted with water, even when the cyclodextrin is in ten-fold excess. The weakest complexation is seen in the frequently studied system bupivacaine–HP-β-CD (Dollo et al 1996a, b, 1998a; Meert & Melis 1992; Fréville et al 1996) and the strongest complexation is exhibited by M-β-CD. Practically, a yet more effective agent for the bupivacaine cation complexation (if required) appears to be the well-soluble native α-CD with a not-so-large K_{11} value but with the lowest molecular weight of all the cyclodextrins.

In slightly acidic or roughly neutral body fluids, bupivacaine remains predominantly in the form of cations, with respect to pK_a 8.2 (Strichartz et al 1990). The bupivacaine release from the cyclodextrin complexes on dilution with body fluids has, therefore, to be similar as it is seen in Figure 5, or rather more progressive due to the competitive cyclodextrin complexation with various organic components, especially lipids, and due to bupivacaine binding to proteins.

Conclusions

The observed weak complexation of bupivacaine cations with native cyclodextrins α-CD, β-CD, and their non-ionic derivatives can substantially modify the properties of bupivacaine hydrochloride dosage forms containing a sufficiently high concentration of the selected cyclodextrin. However, with respect to the ready release of bupivacaine from the complexes on dilution, it deserves further investigation, if the reported favourable effect of the weakly complexing HP-β-CD on the bupivacaine anaesthetic activity (Fréville et al 1996; Meert & Melis 1992) is indeed due to the complex formation or due, at least in part, to another kind of cyclodextrin effect.

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