POTENTIOMETRIC STUDY OF CARBISOCAINE MICELLIZATION AND INCLUSION COMPLEXATION WITH α-CYCLODEXTRIN, β-CYCLODEXTRIN, METHYL-β-CYCLODEXTRIN, AND (HYDROXYPROPYL)-β-CYCLODEXTRIN

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Micellization of the local anesthetic drug carbisocaine hydrochloride (BHCl) was studied by potentiometry with both cation- and anion-selective membrane electrodes in aqueous solution at 25 °C. The found critical micelle concentration 0.022 mol dm^{-3} and the concentration course of the free carbisocaine cation and chloride counterion in the micellar solution corresponded to the characteristic of cationic surfactants. In a more dilute aqueous solution, below critical micelle concentration, the complexation of carbisocaine cation BH^+ with α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and its random substituted methyl (M-β-CD) and hydroxypropyl (HP-β-CD) derivatives was followed using the prepared cation-selective electrodes. The potentiometric data corroborated formation of the carbisocaine–cyclodextrin complexes (BH^+)CD (1 : 1) with all the cyclodextrins and the respective complexation constants K_{11} were estimated using a modified Scatchard method. Slight deviations from 1 : 1 plots were marginally observed with α-CD and HP-β-CD. Bigger K_{11} value of the complexation with α-CD in comparison with β-CD indicated inclusion of the carbisocaine C_7 alkyl chain into the cyclodextrin cavity and the role of the hydrophobic interaction in complexation with β-CDs was emphasized by the increasing magnitude of K_{11} in the order of HP-β-CD < β-CD < M-β-CD.

Keywords: Potentiometry; Ion-selective electrodes; Local anesthetics; Phenylcarbamate; Micelles; Cationic surfactants; Cyclodextrins; Inclusion complexes.

Carbisocaine was designed and prepared as a local anesthetic belonging to the group of hydrochlorides of basic phenylcarbamate derivatives (Chart 1) but, at the same time, its interesting whole-system drug effects were revealed[1]. For its possible use as a system drug, partial suppression or modulation of its local anesthetic properties may be desirable. A viable way in this direction is reversible binding of the amphiphilic carbisocaine cation in an inclusion complex with a suitable cyclodextrin[2–4], such as native α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) or its chemically modified

derivatives, methyl-β-cyclodextrin (M-β-CD) and (hydroxypropyl)-β-cyclodextrin (HP-β-CD), with low degree of substitution. In this work we therefore investigated complexation of carbisocaine with the four stated cyclodextrins in aqueous solution by the direct potentiometric determination of free carbisocaine cation in the presence of a cyclodextrin.

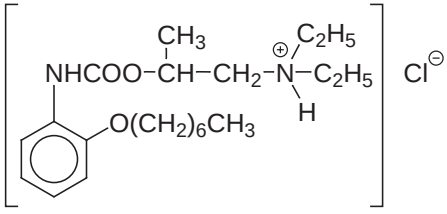

CHART 1

Another motivation of our work has been the actual problem of the complex formation ability of substituted cyclodextrins, such as M-β-CD and HP-β-CD with low degree of substitution[5]. In comparison with the parent β-CD, they exhibit much greater aqueous solubility and in the case of HP-β-CD also better physiological acceptability[2–5]. While the formation of the host-guest type inclusion complexes of the native cyclodextrins utilizing the smaller (α-CD) or bigger (β-CD) central cavity of their macrocycle has been relatively well recognized[6–8], the effect of substituents on the cyclodextrin macrocycle is far from being clear. The methyl or hydroxypropyl substitution on the β-CD macrocycle in some cases facilitates and in others hinders inclusion ability[2,5,9–11]. Obviously more experimental data are needed to elucidate this problem.

With respect to the amphiphilic carbisocaine structure, the micellization and related association phenomena, which are characteristic for surface-active cations[12–14], may be expected in its more concentrated aqueous solution. The formation of micelles or other types of association are not only interesting phenomena, but represent also possible competitive equilibria of the guest compound to its cyclodextrin complexation. Therefor the study of carbisocaine micellization was also included in this work. Because carbisocaine is a salt of a protonated base (hydrochloride), we avoided the usual conductivity measurements and investigated carbisocaine micellization with potentiometric cationic and anionic selective membrane electrodes as well. Such approach can also provide useful information about both submicellar and micellar ionic surfactant solutions[13,15,16].

EXPERIMENTAL

Chemicals

Carbisocaine hydrochloride (BHCl) was a generous gift from L. Beneš (compound BK-95 in ref.[1]) and the purity of the carbisocaine sample used in this work was the same as in the studies of its stability to hydrolysis[17], HPLC and pK_a measurements[18]. α-Cyclodextrin (six glucose units, m.w. 972.9) was bought from Aldrich, β-cyclodextrin (seven glucose units, m.w. 1 135.0) was bought from Aldrich and Merck and no substantial differences between the samples were found. The substituted β-CDs were bought from Aldrich, methyl-β-cyclodextrin with average degree of substitution per one glucose unit 1.8 (average m.w. 1 310) and (hydroxypropyl)-β-cyclodextrin with average degree of substitution per one glucose unit 0.8 (average m.w. 1 460). These chemically modified β-CDs (seven glucose units) are amorphous mixtures of random-substituted regio isomers and related derivatives. The cyclodextrins were used without purification or drying but their water content (α-CD 7–8%, β-CD 13–14%, M-β-CD about 1%, HP-β-CD 9–10%) was always determined in a small sample dried at reduced pressure at 110 °C and proper corrections were made when their aqueous solutions were prepared. Other chemicals were of analytical grade or required purity.

Equipment and procedures

The apparatus for potentiometric measurements with membrane electrodes at 25 °C was described elsewhere[15,16] but in this work, a more recent Microprocessor pH meter (Hanna instruments HI 9017) was used to measure the electromotive voltage (E) of a cell SCE|solution|ISE. In this scheme, SCE was a saturated calomel electrode and ISE was the membrane (ion-selective) electrode, selective either to carbisocaine cations or chloride anions; solution was a standard or investigated solution in which the electrode couple was immersed. For the purpose of the critical micelle concentration[19] (*cmc*) determination, a cell consisting of a couple of membrane electrodes (anionic and cationic) was also measured. The used chloride ISEs were commercial products (Crytur, Theta), they were tested and standardized by measurements of electromotive voltage with electrodes immersed in a KCl solution (c_{KCl} up to 0.1 mol dm^{-3}) and only the electrodes with the potential gradient (slope) $\Delta E/\Delta\log a_{KCl}$ in the range from –58 to –60 mV were selected for the investigation of carbisocaine micellization.

For the potentiometric measurements of free carbisocaine cation, various modifications of membrane electrodes were tediously prepared and tested in this laboratory. These cation selective electrodes were prepared in accordance with other authors[13,20–23] and our previous experience[15,16]. Membrane electrodes with an internal Ag|AgCl reference electrode and internal solution (KCl 0.01 mol dm^{-3}, carbisocaine hydrochloride 0.001 mol dm^{-3}) were the most convenient. Membranes were typically composed of PVC (35%), bis(2-ethylhexyl)phthalate (60%), and an electroactive component (5%), represented by water-insoluble carbisocaine salt, dodecylsulfate or tetraphenylborate. The mixture of the components was carefully kneaded with small amount of anhydrous tetrahydrofuran to form viscous liquid, which was cast on the glass plate and dried overnight. Discs with a diameter *ca* 8 mm were cut off the resulted gel layer and fixed to the electrode body as membranes selective to carbisocaine cations.

The potential of the cationic electrodes prepared by the described procedure was usually not too stable, thus the electrodes had to be carefully checked and repeatedly standardized

in the dilute carbisocaine solutions (c_{BHCl} from 10^{-5} to 0.02 mol dm^{-3}, lower than *cmc*). The electrodes with the potential gradient $\Delta E/\Delta\log a_{BHCl}$ (or approximately $\Delta E/\Delta\log c_{BHCl}$) from 55 to 60 mV were accepted for the investigation of both the carbisocaine micellization and complexation. The gradient $\Delta E/\Delta\log c_{BHCl}$ of the example potentiometric curves in Fig. 1a was 57.3 mV for our carbisocaine cation-selective electrode and –58.8 mV for the commercial chloride ISE Crytur, respectively, in dilute solution below carbisocaine *cmc*. Details of the measurements of the free carbisocaine cation and chloride anion concentrations in the micellar solutions (above *cmc*) were analogous to our previous works[15,16], including the mutual recalculations between activities (a_i) and concentrations of individual ions (i), if necessary.

Before the measurements of the carbisocaine complexation with cyclodextrins, the effect of cyclodextrins on the membrane electrodes was also examined. Only a small and irregular potential response of the prepared cationic electrodes to the concentration changes of the used cyclodextrins in their respective solutions was observed. However, our membrane electrodes deteriorated gradually and their life time was rather short (1–2 months) when they were systematically used for measurements in solutions of cyclodextrins. The deterioration was manifested by their unstable potential, diminishing gradient and erosion of the PVC membrane, apparently due to the slow extraction of its low molecular components into cyclodextrin solutions. Thus the membranes of our carbisocaine cation-selective electrodes had to be often changed. On the other side, the commercial chloride ISEs were more resilient, but they exhibited certain systematic potential responses to cyclodextrins and, for this reason, only the concentration of carbisocaine cation and not the chloride concentration were measured in solutions of carbisocaine with a cyclodextrin.

In the investigation of micellization, aqueous solutions of carbisocaine were measured with the cationic (carbisocaine) or anionic (chloride) electrode against SCE or with cationic electrode against anionic electrode, in the carbisocaine concentration range c_{BHCl} $2.0\cdot10^{-5}$–0.1 mol dm^{-3}. The complexation of carbisocaine with cyclodextrins was investigated by measurements of the free carbisocaine cation concentration with cationic electrode against SCE in solutions of carbisocaine with cyclodextrin. After the preliminary testing, the complexation measurements were done in series with constant total concentration of cyclodextrin (c_{CD}) and varying (decreasing) total carbisocaine concentration (c_{BHCl}) until approximately one tenth of its starting value was reached. In the definite measurements, used for the evaluation, the constant c_{CD} were 0.005 or 0.0045 mol dm^{-3} while the starting values of c_{BHCl} were 0.005 or 0.0025 mol dm^{-3} in various series. In each series, about fifteen measurements of electromotive voltage were done in the course of decreasing c_{BHCl}. The electrodes were always double standardized in the solutions of carbisocaine, before and after the measurements of the solution of carbisocaine with cyclodextrin.

RESULTS AND DISCUSSION

Carbisocaine Micellization

The electromotive voltage measured with the above-mentioned electrode couples in the carbisocaine solutions was plotted against log c_{BHCl} and the obtained potential curves, shown as an example in Fig. 1a, were analyzed as

before[15,16]. At low concentration the curves were linear until a break at a certain c_{BHCl} value, on average 0.022 ± 0.02 mol dm^{-3}, which is regarded as the critical micelle concentration of carbisocaine. The linearity of the potential curves below *cmc* indicated no perceptible premicellar association or substantial effect of proton dissociation[18] from the carbisocaine cation.

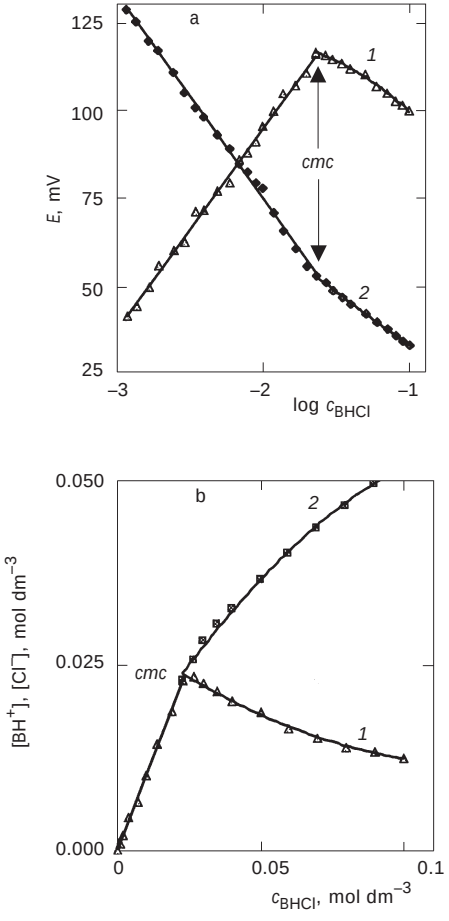

FIG. 1

Potentiometric curves for the determination of critical micelle concentration (*cmc*) of carbisocaine and concentrations of free (monomer) ions in the micellar solution: a potential (*E*) curves for the carbisocaine cation (*1*) and chloride anion (*2*) selective electrodes, c_{BHCl} total carbisocaine concentration; b concentration of the free carbisocaine cation ([BH$^+$], (*1*)) and chloride anion ([Cl$^-$], (*2*)) in the micellar solution ($c_{BHCl} > cmc$)

Measurements of the potential curves in the micellar solution above *cmc* yielded concentrations of free (unimer) carbisocaine cations (BH^+) and free chloride anions (Cl^-) in equilibrium with carbisocaine micelles; they are plotted against total carbisocaine concentration c_{BHCl} in Fig. 1b. Starting from *cmc*, the free cation and anion concentrations have mutually different courses (curves *1*, *2*). The concentration of chlorides increases continuously above *cmc*, though with gradually diminishing slope (curve *2*), but the concentration of free carbisocaine cations actually decreases in the micellar solution (curve *1*), when c_{BHCl} increases above *cmc*. These findings corroborate formation of micelles of carbisocaine cations, associated to a certain degree with chloride counterions. The degree of association (β) of ionic micelles with counterions can be calculated from the concentrations of free (monomer) ions in equilibrium with micelles[19]. In this case it is:

$$\beta = p/n = (c_{BHCl} - [Cl^-])/(c_{BHCl} - [BH^+]) \; . \qquad (1)$$

In Eq. (*1*), *n* is the number of carbisocaine cations forming a micelle, *p* is the number of chloride counterions bound to the micelle, c_{BHCl} is the total carbisocaine concentration, $[BH^+]$ and $[Cl^-]$ are the respective equilibrium concentrations of free carbisocaine and chloride ions.

The evaluated β increases somewhat with c_{BHCl}; for c_{BHCl} 0.04 and 0.1 mol dm^{-3}, the respective β values are 0.4 and 0.55, respectively. Such relatively low β indicates formation of well-ionized micelles with a large positive charge, comparable to the micelles of typical long-chain cationic surfactants[14,19]. Furthermore, *cmc* of cationic surfactants with the same anion is usually a function of the number of alkyl carbons, which are not directly bonded to the positively charged nitrogen of the micellizing cation. The found *cmc* of carbisocaine is nearly equal to that of dodecyl(trimethyl)ammonium chloride[12,14] (0.0228 mol dm^{-3}) and both the considered cations have 11 alkyl carbons not bonded to the charged nitrogen. Such close agreement may be somewhat fortuitous but a comparison with chloride salts of some other micellizing amphiphilic cations[14,19] also suggests that in spite of structural differences, the carbisocaine micellization obeys the general rules for long-chain cationic surfactants and also in this case the value of *cmc* is a suitable measure of hydrophobicity.

Complexation of Carbisocaine with Cyclodextrins in Aqueous Solution

Some characteristic results of potentiometric measurements of free carbisocaine cation concentration ([BH^+]) in aqueous solutions of carbisocaine with cyclodextrin are seen in Fig. 2 as the plots of the fraction [BH^+]/c_{BHCl} against c_{BHCl}. Each curve represents one series of measurements in the solu-

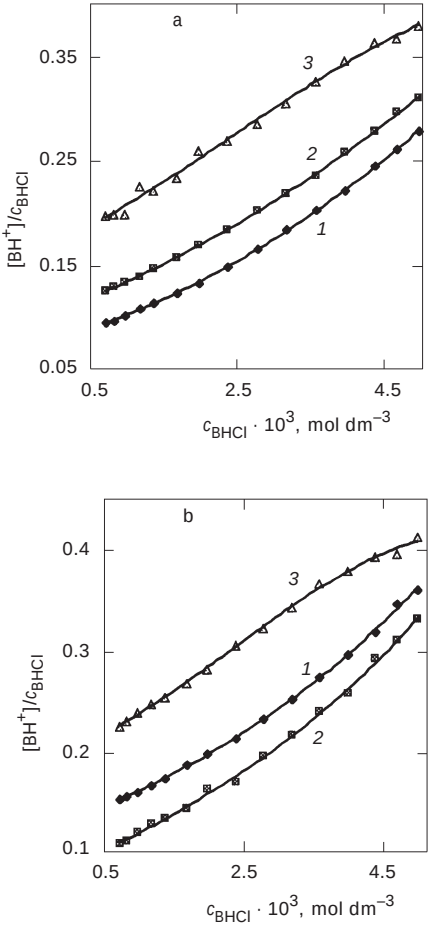

FIG. 2

Fraction of the free carbisocaine cation ([BH^+]/c_{BHCl}) plotted *versus* the total carbisocaine concentration (c_{BHCl}, starting value 0.005 mol dm^{-3}) in solutions of carbisocaine with constant total concentration of cyclodextrin (c_{CD}): a c_{CD} = 0.005 mol dm^{-3}, cyclodextrins α-CD (*1*), β-CD (*2*), HP-β-CD (*3*); b c_{CD} = 0.0045 mol dm^{-3}, cyclodextrins β-CD (*1*), M-β-CD (*2*), HP-β-CD (*3*)

tion with constant cyclodextrin concentration (c_{CD}) and variable total carbisocaine concentration (c_{BHCl}). Since the measurements were done in dilute solutions at $c_{BHCl} < cmc$, where no perceptible premicellar association of carbisocaine or other equilibria were evidenced, the found low fractions of the free carbisocaine cation ($[BH^+]/c_{BHCl} < 1$) indicate various extent of carbisocaine complexation with each of the four cyclodextrins. The measured data were therefore processed by several methods of the association equilibrium analysis, as to estimate the stoichiometry and the equilibrium complexation constants of the carbisocaine–cyclodextrin complexes in solution. Convenient modifications of the Scatchard method[20–23] based on the variable called the binding ratio (r) and on the least squares computation were finally selected for this purpose, while the recently criticized[24,25] double-reciprocal plots (Benesi–Hildebrand) were avoided.

The binding ratio of carbisocaine to cyclodextrin is here defined as

$$r = (c_{BHCl} - [BH^+])/c_{CD} , \qquad (2)$$

where c_{BHCl} and c_{CD} are total concentrations of carbisocaine and cyclodextrin, respectively and $[BH^+]$ is the measured equilibrium concentration of the free carbisocaine cations. Eventual self-association of the used cyclodextrins or their interaction with chloride anion in aqueous solution was disregarded in accordance with other authors[20–23].

The investigated chemical equilibrium was treated as the formation of a single complex with the carbisocaine : CD stoichiometric ratio 1 : 1, 1 : 2 or 2 : 1. The common mode of the cyclodextrin complexation in solution is the reversible formation of an 1 : 1 complex, in this case $(BH^+)CD$,

$$BH^+ + CD \rightleftharpoons (BH^+)CD ,$$

described by the equilibrium complexation constant K_{11}:

$$K_{11} = [(BH^+)CD]_{rel}/([BH^+]_{rel}[CD]_{rel}) . \qquad (3)$$

The symbols in brackets represent equilibrium concentrations of the particular species. Introducing the binding ratio r (Eq. (*2*)) into the relationship for K_{11}, it can be rearranged in the form:

$$r = K_{11}(1 - r)[BH^+]_{rel} \ . \qquad (4)$$

Equation (*4*) is a modified Scatchard plot for the 1 : 1 association, in a diagram it is represented by a straight line passing through origin, with the slope equal to K_{11}. The example curves in Fig. 3 show our experimental re-

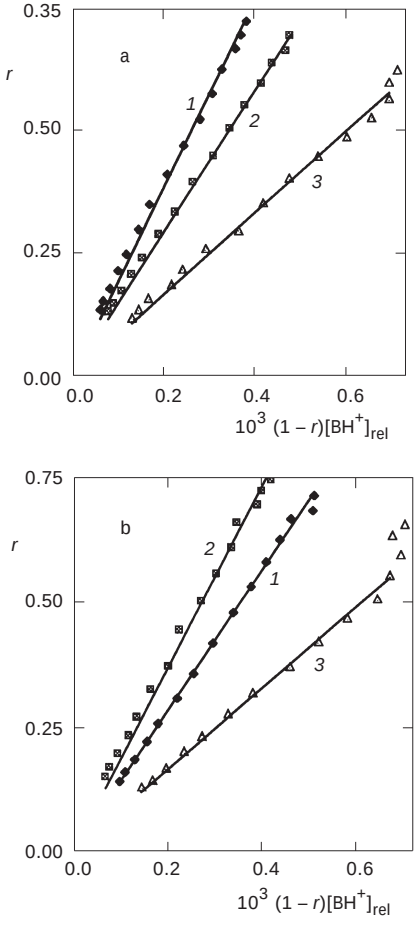

FIG. 3

Binding ratio (*r*) of carbisocaine to cyclodextrin plotted *versus* (1–*r*)[BH^+] for solutions with starting total carbisocaine concentration c_{BHCl} = 0.005 mol dm^{-3} and a constant total concentration of cyclodextrin (c_{CD}): a c_{CD} = 0.005 mol dm^{-3}, cyclodextrins α-CD (*1*), β-CD (*2*), HP-β-CD (*3*); b c_{CD} = 0.0045 mol dm^{-3}, cyclodextrins β-CD (*1*), M-β-CD (*2*), HP-β-CD (*3*) (the same measurements as in Fig. 2)

sults for each of the used cyclodextrins are mostly consistent with Eq. (*4*), at least in a certain range of c_{BHCl} and c_{CD}. In this concentration range, the association of carbisocaine with a respective cyclodextrin may be regarded as the prevalent formation of the 1 : 1 $(BH^+)CD$ complex, in accordance with Eq. (*3*). The values of the complexation constant K_{11}, resulting from the least squares fit of the experimental data with Eq. (*4*), are summarized in Table I. They are averages (and average deviations) from 10–14 selected series of measurements where the regression was characterized by $0.98 < R^2 < 1$.

The best fit with Eq. (*4*) exhibited data measured in the whole studied concentration range of solutions of carbisocaine with β-CD and M-β-CD, respectively. In the case of α-CD and HP-β-CD, partial deflections from the linear Eq. (*4*) were observed in solutions with higher excess of the respective cyclodextrin to carbisocaine. For both α-CD and HP-β-CD, the K_{11} values resulting from the series of solutions with higher relative excess of CD (c_{CD} 0.005 mol dm^{-3} and c_{BHCl} 0.0025–0.0003 mol dm^{-3}) were usually lower than the rest, however within the average deviation stated in Table I. Furthermore, HP-β-CD exhibited also opposite deflection when present in equimolar concentration or at excess of carbisocaine (c_{CD} 0.005 or 0.0045 mol dm^{-3} and starting c_{BHCl} 0.005 mol dm^{-3}), this is seen in Figs 3a and 3b. The highest depicted points, above curves *3*, were therefore excluded from the fit with Eq. (*4*).

In spite of the described imperfections, other models of a single complex formation with the carbisocaine : CD stoichiometry 1 : 2 ($r = K_{12} c_{CD}(1 - 2r)^2 [BH^+]_{rel}$ or 2 : 1 ($r = K_{21}(2 - r)[BH^+]^2_{rel}$) proved to be far less suitable for the description of the investigated complexation of carbisocaine cations with any of the studied cyclodextrins. A possible explanation is a partial formation of the 1 : 2 (α-CD, HP-β-CD) and 2 : 1 complexes (HP-β-CD) at

TABLE I
Complexation constant K_{11} of carbisocaine with cyclodextrins in aqueous solution (25 °C)

Cyclodextrin	Degree of substitution	K_{11}[a]
α-CD	–	1 910 ± 200
β-CD	–	1 400 ± 95
M-β-CD	1.8	1 820 ± 120
HP-β-CD	0.8	750 ± 70

[a] The reference concentration, c_0 = 1 mol dm^{-3}.

the respective marginal conditions, besides of the prevalent 1 : 1 complex. The simultaneous formation of 1 : 1 and 1 : 2 complexes was already considered in analogous potentiometric studies[20–23] of complexation of some long-chain ion surfactants with α-CD and β-CD, however, in the light of the recent critical re-examination of the computation methods of the weak complex equilibria[25] we did not follow this line.

Another similarity with the cyclodextrin complexes of some long-chain ion surfactants[20] is a rather rare feature that the determined K_{11} for the carbisocaine–α-CD complexation is higher than that for the complexation with β-CD (Table I). It suggests the main complexation mechanism is the inclusion of the C_7 alkyl chain (alkoxyl on the benzene ring, Chart 1) of the carbisocaine cation into the α-CD or β-CD cavity. The tight fit of the unbranched alkyl chain into the narrower α-CD cavity ensures stronger hydrophobic interaction with the cavity interior and thus the higher K_{11}, compared with the looser inclusion of the same alkyl into the larger β-CD cavity[6,20]. The 1,2-disubstituted benzene ring of carbisocaine is probably too bulky to be substantially accommodated even into the larger β-CD cavity and the charged amino group does not prefer the cavity interior.

On the other hand, the studied complexes are not too strong, values of K_{11} for α-CD or β-CD are at least by one order of magnitude smaller than the analogous K_{11} of the long-chain cationic surfactants with comparable *cmc* values[22]. As concluded above, the *cmc* of carbisocaine is a suitable measure of its hydrophobicity, because it corelates with the number of hydrophobic alkyl carbons. This discrepancy may be however reconciled, because *cmc* reflects apparently the total hydrophobicity corresponding to all the hydrophobic alkyl carbons, while in the case of carbisocaine, only the C_7 long alkyl takes part in the inclusion.

The determined K_{11} must be anyway considered as a kind of apparent constant, because more types of the complexes with the same 1 : 1 stoichiometry may in principle be formed between the structured carbisocaine cation and each of the cylodextrins. This is especially relevant for the substituted amorphous M-β-CD and HP-β-CD, since these cyclodextrins are not chemical individuals but the populations of the respective isomers and related derivatives. However, in comparison with the parent β-CD, both the higher K_{11} of M-β-CD and the lower K_{11} of HP-β-CD also suggest the major role of hydrophobic interaction in the carbisocaine complexation with β-CDs. More detail conclusions cannot be drawn at this stage for the scarcity of the data on the complexation of other amphiphilic cations with substituted cyclodextrins.

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