Characterization of Interactions Between Bile Salts and Drugs by Micellar Electrokinetic Capillary Chromatography. Part I.

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Purpose. The general properties of micellar electrokinetic capillary chromatography (MECC) were utilized to characterize the strength of interactions between bile salts and biological active substances.
Methods. For that purpose various bile salts were used as micellar pseudostationary phase in the background electrolyte. Furthermore, a physicochemical model was applied and the effective partition coefficients between micellar and water phase were calculated in order to evaluate the strength of interactions between bile acids and the drugs.
Results. It was found that the interactions between the selected drugs and bile salts depend both on the lipohilicity of the drugs and on the charge of the components. Only hydrophobic, cationic drugs such as quinine and propranolol are able to interact with these surface-active agents.
Conclusions. MECC is a valuable methode to characterize interactions such occurring between drugs and bile salts.

KEY WORDS: MECC; bile salts; partition coefficient; interaction; drug.

INTRODUCTION

The bioavailability of a drug following oral administration strongly depends on the conditions in the gastrointestinal tract such as pH-value and the presence of food components (1). Furthermore, the absorption of lipophilic drugs is influenced by the presence of bile salts (2,3). Bile salts improve the bioavailability of poorly absorbable drugs by increasing the dissolution rate of the drug or by facilitating the transfer of the solute across the intestinal wall. The dissolution rate can be increased by lowering the surface tension of the gastrointestinal fluid or by micellar solubilization.

Therefore, it is necessary to study these interactions.

In aqueous solutions hydrophobic bile salts form aggregates or micelles when their concentration exceeds the critical micelle concentration (CMC). The structure of these micelles is poorly characterized in comparison with more familiar surfactants such as SDS. Not all bile salts form the same type of micelles, also the structure of micelles depends on the concentration. If the concentration slightly exceeds the CMC then the particles have a spherical or nearly spherical micellar shape (4). With increasing concentration of the bile salts, the particles grow to rod shaped objects (5). The CMC of various bile salts can change as a function of the pH and is influenced by the concentration of lipids and cations. Trihydroxy bile salts have generally a higher CMC than dihydroxy bile salts. At the physiological pH the CMC of most bile salts varies between 2 and 5 mM.

The use of bile salts in the "background electrolyte" in micellar electrokinetic capillary chromatography (MECC) has already been described as an effective method to separate optical isomeres (6,7) as well as hydrophobic and uncharged molecules (8,9). The main principle of the measurement is based on the fact that bile salts in the run buffer cause a change of the ionic mobility of the free dissolved drug D by establishing the following equilibrium $[D]_{aq} \rightleftarrows [D]_{mc}$. A change of the migration times is caused by the rate of distribution between those two equilibrium states. It is possible to evaluate interactions between bile salts and drugs by using this principle.

The drug binding to micelles is influenced by hydrophobic interaction as a main driving force, further hydrogen bonding, electrostatic and dipolar interaction or even steric effects.

It was the aim of this study to describe the interactions between bile salts, representing natural compounds of the gastrointestinal tract, and drugs using MECC. The electrokinetic properties of the aggregates formed by bile acids, i.e. ionic mobility, were characterized under presence and absence of drugs in the concentration range of interest (0–30 mM). Furthermore, a physicochemical model was derived in order to calculate partition coefficients of the drugs between the aqueous and the micellar phase. With these coefficients the strength of interactions under discussion were evaluated. Propranolol (pK_a = 9.4), atenolol (pK_a = 4.2, 8.8), etilefrine (pK_a = 9.0), quinine (pK_a = 4.2, 8.8) as well as chloramphenicol (pK_a = 5.5), tetracycline (pK_a = 3.1, 7.6, 9.7), and salicylic acid (pK_a = 2.83, 12.62) were used as model drugs.

EXPERIMENTAL

Chemicals

Propranolol HCl, atenolol, quinine HCl, etilefrine HCl, chloramphenicol, tetracycline HCl and salicylic acid were purchased from COM-Pharma-Handels GmbH (Hamburg, Germany). The model samples (1 mM) were prepared by dissolving analytical pure substances in bidestilled water. The sodium salts of glycocholic acid (GCA), taurocholic acid (TCA), glycochendeoxycholic acid (GCDCA) and glycodeoxycholic acid (GDCA) of analytical grade, were obtained from FLUKA (Buchs, Switzerland).

Micellar Electrokinetic Capillary Chromatography (MECC)

A Hewlett Packard (Waldbronn, Germany) ^{3D}CE system fitted with a 600 (515) × 0.05 mm (extened lightpath) fused silica capillary and an on-column diode array detector (190 . . . 600 nm) was used for MECC. The capillary was preconditioned for 10 min with 1.0 M NaOH before the first run and then for 3 min with 0.1 M NaOH and 3 min with run buffer prior to

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each following run. The separation conditions were: −30 kV voltage (detection end), 200 mbar*sec pressure injection, 25°C capillary temperature. The detection was done on the cathodic side at 200 and 230 nm. All micellar solutions and samples were filtered through a membrane filter of 0.2 μm pore size and degassed by ultrasonic before running.

THEORY

The mathematical description of the interactions starts with the calculation of the capacity factor k′ of the drug in its distribution between the micellar and the aqueous phase (10).

$$k' = \frac{n_{mc}}{n_{aq}} \tag{1}$$

R represents the fraction of active substance in the aqueous phase.

$$R = \frac{n_{aq}}{n_{aq} + n_{mc}} = \frac{1}{1 + k'} \tag{2}$$

The observed electrophoretic mobility μ_P' (also called apparent mobility, see eq.5.) results from the partition of the drug between the aqueous (μ_D-mobility of the free dissolved drug) and the micellar (μ_{mc}-mobility of the micelle) phase, and the electroosmotic flow (EOF). These parameters depend on the concentration of the bile salt and pH-value. The effective mobility, μ_P, can be extracted from the appearent mobility by measuring the EOF using a neutral marker that moves at a velocity equal to the EOF.

$$\mu_D = \mu_D' - \mu_{eof} \tag{3}$$

$$\mu_{mc} = \mu_{mc}' - \mu_{eof} \tag{4}$$

$$\mu_P' - \mu_{eof} = R\,\mu_D + (1\text{-}R)\,\mu_{mc} = \mu_P \tag{5}$$

Equations (2) and (3–5) give R:

$$R = \frac{\mu_P' - \mu_{mc}'}{\mu_D' - \mu_{mc}'} = \frac{v_P' - v_{mc}'}{v_D' - v_{mc}} = \frac{1/t_P' - 1/t_{mc}'}{1/t_D' - 1/t_{mc}'} \tag{6}$$

v_D represents the velocity of the free dissolved drug, v_{mc} the velocity of the micelle and t the migration times taking in consideration the change of EOF when bile salts are added. The determination of t_{mc} was done measuring the migration time of a neutral, completely solubilized substance e.g. flavonoids migrating with the same speed as the micelles.The capacity factor can be calculated by

$$k' = \frac{1/t_D - 1/t_P}{1/t_P - 1/t_{mc}} = \frac{\mu_D - \mu_P}{\mu_P - \mu_{mc}} \tag{7}$$

For the calculation of the partition coefficient $K_P = C_{mc}/C_{aq}$ the partial molar volume $\bar{v}$ of the micelles the CMC (11) and the initial concentration of bile salts c have to be taken into consideration.

$$k' = K_P \frac{V_{mc}}{V_{aq}} = K_P \frac{\bar{v}(c - cmc)}{1 - \bar{v}(c - cmc)} \tag{8}$$

The equation k′ = f(c) has a singularity at (c-cmc) = $1/\bar{v}$. Therefore, the product $\bar{v}c$ has to be smaller than 1. The ratio of micellar to the aqueous phase equals one for K = k′. At this point the slope becomes very steep and the error increases. The calculation of k′ becomes incorrect for the range $K_P > k'$.

RESULTS AND DISCUSSIONS

In this investigation the interactions of four different kinds of bile salts and drugs were studied. In the system bile salt/drug, the hydrophobic interactions play a major role in terms of influencing the electrokinetic migration behaviour. Except salicylic acid all these molecules contain a β-hydroxyamine- or a β-hydroxyamide-group as a structural features, that enables them to exhibit basic hydrogen acceptor as well as acidic hydrogen donor characteristics.

For the calculation of K_P the CMC values of GDCA and GCDCA were determined by means of laser-light-scattering spectrometry (12). The CMC values for TCA and GCA were taken from the literature (13).

Caempferol was used for determining the micellar velocity and the negative water-peak or methanol (detection at 200 nm) for measuring the EOF, respectively.

Figure 1 shows the electrophoretic ionic mobilities of μ_P ($\mu_P = \frac{L_d L_t}{U}(\mu_P' - \mu_{eof})$, L_D-effective capillary length, L_t-total capillary length, U-applied voltage) and $\mu_P = f(R, \mu_{mc}, \mu_D)$) of propranolol in dependence on the concentration of bile salts used at a pH-value of 7.4. The concentration needed for a significant influence of bile salts on μ_P seemed to be lower for the dihydroxy bile salts (GDCA, GCDCA). At a concentration of 25 mM of GDCA and GCDCA no further increase of the ionic mobility was observed because the saturation equilibrium was reached and the charge and size of the micelles was not changed (Fig. 1).

For characterizing the strength of interaction it is necessary to calculate the partition coefficent between aqueous and micellar phase. This is only possible by determining the ionic mobility of the micelles without drugs in the concentration range used. As shown in Figure 2 the aggregates of bile salts have a higher mobility by increasing concentration of the bile salt. An interpretation of this phenomenon could be the formation of aggregates with a higher charge/radius relation. Generally, the trihydroxy bile acids have a smaller ionic mobility. An increase of the pH-value led to similar ionic mobilities of the different bile-salt-micelles. In this case the formation of the aggregates (CMC) takes place at a considerably lower concentration of the bile salt.

With changing the pH-value it is possible to influence the electrophoretic ionic mobility (see Fig. 3) because the partition equilibrium as well as the formation of micelles are influenced. In order to investigate the binding conditions between the drug and the bile salt the measurements were carried out at different pH-values. In particular, the attention was turned to the interaction between the carboxy group of the bile salt and the basic secondary amino group of the drug with its possible electrostatic interaction.

In principle, a change of μ_p caused by different pH-values can influence the micelle velocity as well as the partition between the two phases. When k′ is still influenced at similar micelle velocities and at different pH-values an ionic electrostatic interaction could be the reason.

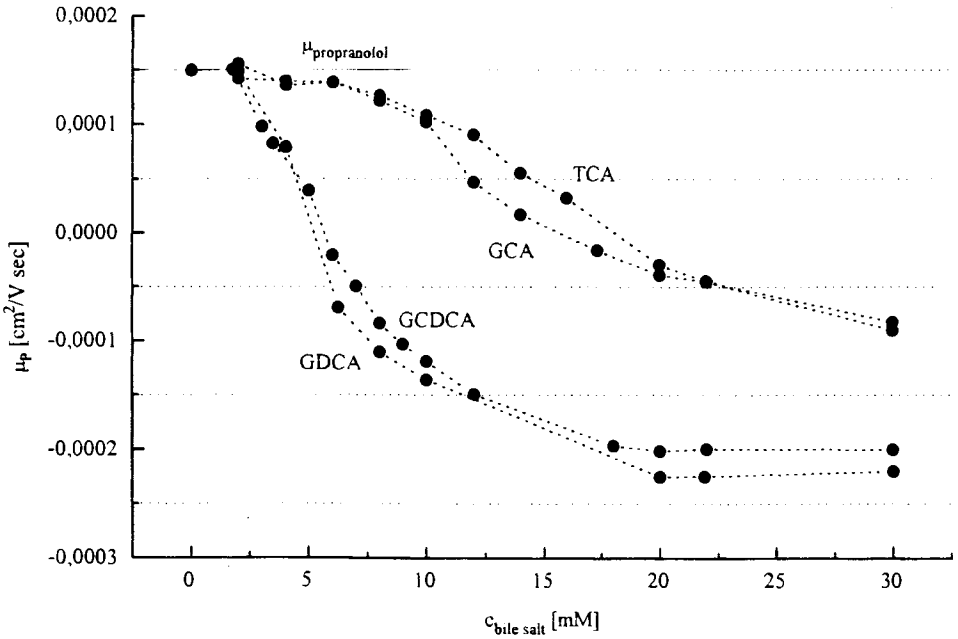

Fig. 1. Electrophoretic ionic mobility of propranolol in dependence of various bile salts, pH=7.4 (20 mM phosphate buffer).

At lower pH-values a reduction of the surface charge and, therefore, a decrease of μ_{mc} and due to decrease of the dissociation of the carboxy group, a reduced interaction of bile salt with the protonated cationic amino groups could be conceived. A similar behaviour could be expected in a strong basic environment due to the reduced dissociation of basic drugs.

In figure 4 a and b μ_P of the drugs used are shown. Quinine had a very similar electrophoretic behaviour compared to propranolol. In contrast, the effective mobility of the anionic salicylic acid was not influenced by GDCA. The salicylate ion was retained in the aqueous phase because of the electrostatic repulsion between the surface of the micelle and the anionic salicylate ion.

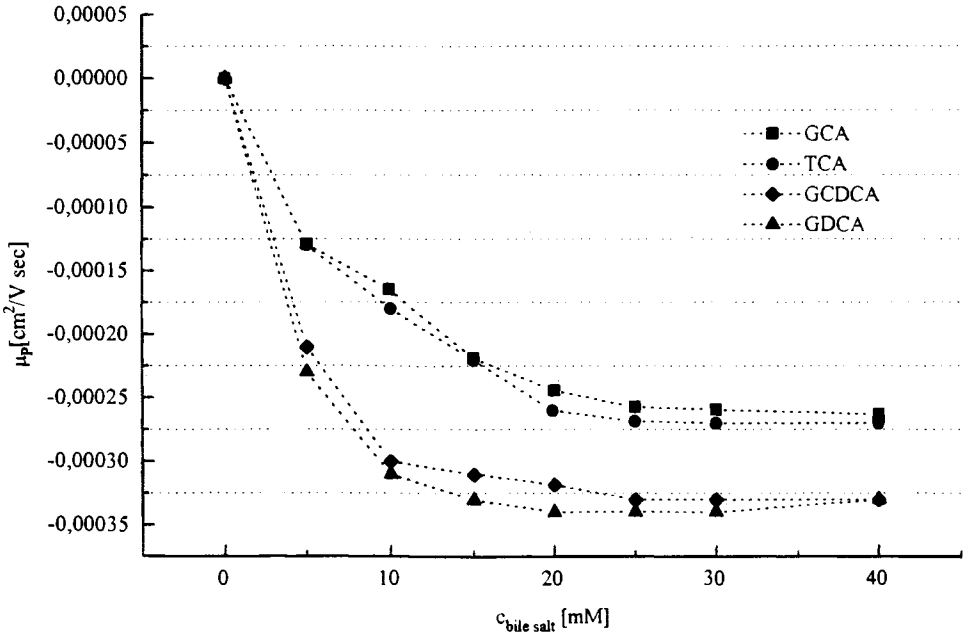

Fig. 2. Electrophoretic ionic mobility of micelles at pH=7.4 marked by caempferol (20 mM phophate buffer).

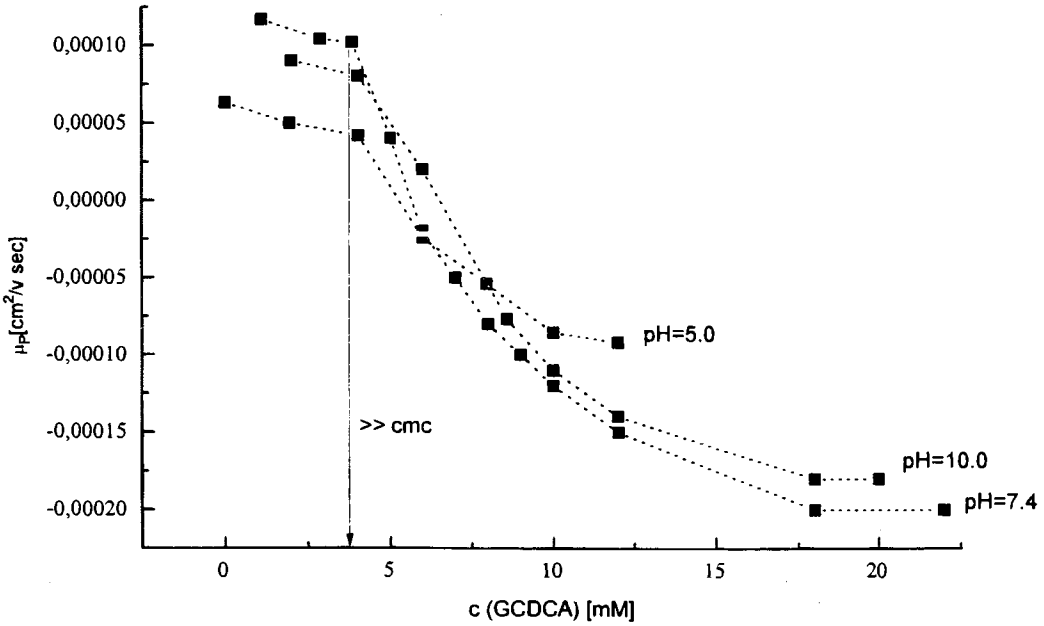

Fig. 3. Electrophoretic ionic mobility of propranolol in dependence of various pH values (20 mM buffer, pH=5.0-citric acid/NaOH/HCl, pH=7.4-phosphate, pH=10.0-boric acid/NaOH).

Atenolol and etilefrine are only slightly more influenced by bile salts than chloramphenicol and tetracycline.

The partition coefficient K_P and the partial molar volume $\bar{v}$ were obtained from the fitted function (equation (8)) (Fig. 5). The physicochemical model can be utilized in order to compare the strength of interactions between bile salt and drugs at different pH-values. For very weak interactions between bile salt (GCA, TCA) and drugs it was not possible to calculate reliable values of K_P because of the standard deviation of the experimental method.

Using this model the partition coefficients between micellar and aqueous phase were calculated. They are listed in Table I.

Equation 8 is only valid if the solute has no influence on the partial molar volume. Therefore, the partial molar volume should be constant for different drugs and the same bile salt. It was obtained that $\bar{v}$ depends on the kind of drug. The parameter K_P and $\bar{v}$ show a strong dependence over the concentration range. These results indicate a change in the shape and the activity of the micelles and probably a change of the number of phases. For that reason, the thermodynamic constants K_P and $\bar{v}$ get an effective character. Despite of this fact the parameters can be used to describe the interaction of the system bile salt/drug in a certain concentration range and represent average values. Because the K_P and $\bar{v}$ are effective parameters the ratio V_{mc}/V_{aq} has also no thermodynamic background although the calculation of k′ gives exact value.

In order to compare different systems it is necessary to take into account both parameters. Particularly, the volume $\bar{v}$ was found to be high for strong interactions between bile salt and drug. The complete description of the parameters should be the next step to get a deeper understanding of this behaviour.

In the partition process the partial molar volume of the micelle (GDCA) $\bar{v}$ for tetracycline is equal 0.0141 l/mmol. An increase of the value for $\bar{v}$ was observed for propranolol and quinine. Possibly, a new structure of the micellar phase is formed when propranolol was added. This phenomenon has already been observed in the case of ionic surfactants when more counterions were added (14). Under these conditions the electrostatic repulsion was decreased.

Additionally, the attractive forces between the micelles increase due to a change of the hydrophobic character of the micelles surface when aromatic compounds are solubilized. Studies by dynamic laser-light-scattering spectrometry confirmed this result, that the volumes of the micelle are increased (12).

As shown in Table I no interactions between bile salts and the acidic drugs such as salicylate ion and tetracycline as well as the hydrophilic drugs were observed. Therefore, k′ was 0 for salicylic acid in the whole concentration range. The other drugs showing only small interactions have a maximum k′ of 1. When the pH was increased from 7.4 to 9.7 the dissociation of the basic drugs propranolol and quinine decreased. Therefore, the partition coefficients of both drugs between micellar and aqueous phase at pH 9.7 were significantly decreased compared to the coefficients at pH 7.4.

Taking these results we can say interactions exist only between bile acids and counterionic drugs. The intensity of these interactions, among others, depends on the degree of dissociation of the counterionic drug.

The partition coefficients for propranolol were significantly higher than for quinine. This seems to be caused by steric effects because the molecular volume of quinine is larger than that of propranolol.

Comparing the behaviour of discussed drugs it was found, that both ionic and hydrophobic interactions play an important role. The cationic and hydrophilic substances atenolol and etilefrine are not remarkably influenced by bile salts. Only the hydrophobic, cationic drugs propranolol and quinine interact with the micellar phase.

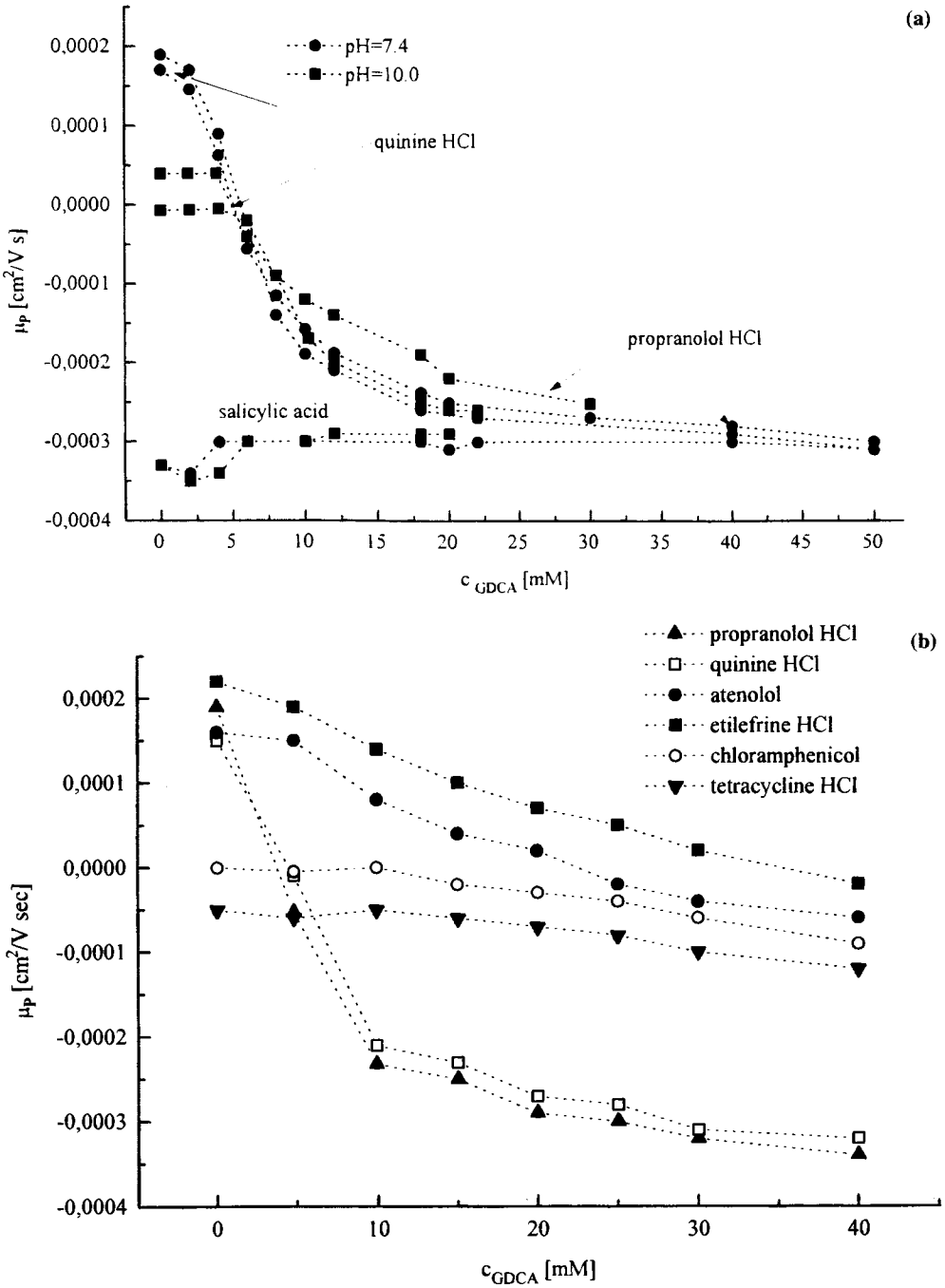

Fig. 4. Electrophoretic ionic mobility of drugs propranolol, quinine and salicylic acid (a) and propranolol, quinine, atenolol, etilefrin, chloramphenicol and teracycline (b) in dependence of concentration of GDCA (20 mM buffer, pH=7.4-phosphate, pH=10.0-boric acid/NaOH (a), 20 mM buffer, pH=7.4-phosphate (b)).

The following tendencies were observed (see Figure 4b and Table I):

GCDCA ≈ GDCA >> TCA > GCA

propranolol > quinine >> atenolol ≈ etilefrine > chloramphenicol ≈ tetracycline ≈ salicylic acid

pH = 3 < pH = 7.4 > pH = 10.0.

CONCLUSIONS

The present study shows that it is possible to characterize interactions between bile acids and drugs using MECC. The physicochemical model could be used to evaluate the intensity of these interactions by calculating partition coefficients between micellar and aqueous phase. The highest partition coefficients in the system GCDCA/buffer were found for proprano-

Table I. Comparison of Partition Coefficients and Partial Molar Volumes Estimated by Application of the Fitted Function (Equation (8)), (N.D.P.-No Determination Possible)

GDCA	pH	CMC	K_P	$\bar{v}$ [l/mmol]	k′ (20 mM)
propranolol HCl	7.4	0.9	12.43 ± 0.35	0.0231 ± 1.38 10^{-3}	9.81
	10.0	0.8	3.00 ± 1.20	0.0234 ± 5.1 10^{-4}	6.67
quinine HCl	7.4	0.9	8.86 ± 0.61	0.0268 ± 4.36 10^{-3}	8.59
	10.0	0.8	2.09 ± 0.91	0.0218 ± 7.0 10^{-4}	3.95
atenolol			n.d.p.	n.d.p.	—
chloramphenicol					
salicylic acid					
tetracycline HCl	7.4	0.9	0.301 ± 0.068	0.014 ± 0.012	0.21
	10.0	0.8	n.d.p.	n.d.p.	
GCDCA					
propranolol HCl	7.4	1.1	20.0 ± 1.20	0.011 ± 4.2 10^{-3}	9.85
	10.0	0.9	3.8 ± 1.21	0.019 ± 1.5 10^{-3}	5.03
quinine HCl	7.4	1.1	16.0 ± 1.00	0.010 ± 5.2 10^{-3}	7.46
	10.0	0.9	2.27 ± 0.27	0.017 ± 3.8 10^{-3}	2.30
TCA					
propranolol HCl	7.4	1.5	1.8 ± 0.2	0.013 ± 4.8 10^{-3}	1.15
	10	1.0	0.82 ± 0.32	0.022 ± 3.3 10^{-3}	1.59
quinine HCl	7.4	1.5	1.059 ± 0.3	0.012 ± 2.1 10^{-3}	0.59
	10	1.0	n.d.p.	n.d.p.	—

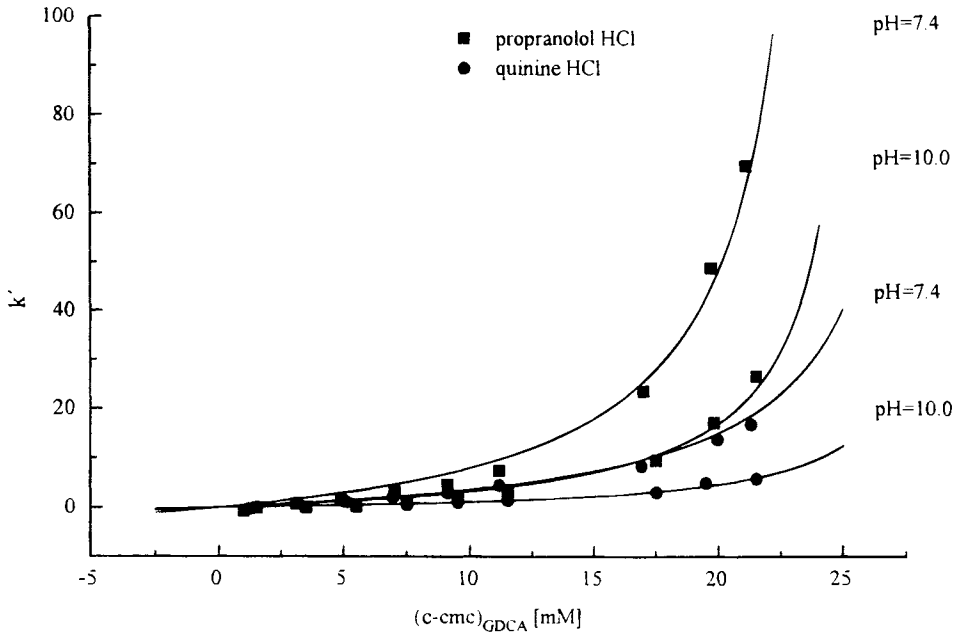

Fig. 5. Capacity factor k′ as a function of concentration c of GDCA (experimental conditions: see Fig. 4), fitted function of equation (8).

lol, followed by quinine. Anionic drugs such as salicylate acid were not bound by bile acids.

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