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Application of capillary electrophoresis for characterizing interactions between drugs and bile salts. Part I[1]

Maria A. Schwarz, Reinhard H.H. Neubert*, Hans H. Rüttinger

Institute of Pharmaceutics and Biopharmaceutics, Martin-Luther University, Langenbeckstrasse 4, D-06120 Halle/Saale, Germany

Abstract

Capillary electrophoresis offers a new way of characterizing interactions between different bile salts and drugs. The observed interactions were characterized with modified model functions known from affinity capillary electrophoresis (ACE) and micellar electrokinetic capillary electrophoresis (MECC). The methodical background of both methods is the change of the ionic mobility of the drug caused by partition between different phases and aggregation with the bile salt molecules, respectively. This phenomenon is described by two different physicochemical models. A parameter estimation was carried out in order to obtain the partition coefficients K_P as well as constants for the aggregate formation K_A. Furthermore, an expression about the specific molar volume of the micelles and stoichiometric coefficients can be given.

Keywords: Partition coefficients; Aggregation constants; Bile salts; Drugs

1. Introduction

The aim of our investigations was the characterization of the interactions between different bile salts and drugs because the bioavailability of lipophilic drugs following oral administration strongly depends on the presence of food components [1]. Furthermore, the absorption of these drugs strongly depends on the presence of bile salts [2,3]. These surface-active agents 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 of lipophilic drugs can be increased by lowering the surface tension of the gastrointestinal fluid or by micellar solubilization.

The use of bile salts in the background electrolyte of micellar electrokinetic capillary chromatography (MECC) has already been described as an effective method to separate optical isomers [4,5] as well as hydrophobic and uncharged molecules [6,7]. MECC is also important for the separation of ionic compounds [8,9]. Anionic surfactant systems are preferred in MECC since the resultant micelles electrophoretical migrate opposing to the electroosmotic flow and do not interact with the negatively charged walls of the fused-silica capillary columns. Beside its usefulness in separation problems, capillary electrophoresis can also be used for estimation of interactions and complex formation between buffer ingredients and analyte. Avila et al. [10,11] have demonstrated that affinity capillary electrophoresis (ACE) is a powerful method for the measuring of binding constants. A special procedure, called affinity capillary electrophoresis allows also the determination of the binding stoichiometry [12].

The goal of this work was to get information about

*Corresponding author.

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the interactions between bile salts, representing native compounds of the gastrointestinal tract and selected drugs using capillary electrophoresis. The cationic drugs propranolol, atenolol, etilefrine and quinine as well as the nonionic drug chloramphenicol and the anionic drugs tetracycline, diclofenac and salicylic acid were used as model drugs (see Table 1).

The effects of various concentrations of bile salts in the separation buffer on the net retention time of the drugs were used to obtain a quantitative measure of the strength of interactions between the drugs and bile salts. Two different physicochemical models were used to describe these interactions. Each model represents a border type of interaction:

1. Distribution of the drug between the two phases. The equilibrium is described as Nernstian partition.

2. Forming of aggregates between the bile salt and drugs step by step with defined stoichiometric ratios. The equilibrium may be described by the brutto equilibrium constant. Besides the unspecific hydrophobic interaction as the main driving force, columbic, hydrogen bonds, dipolar forces and steric effects influence the interactions.

2. Materials and methods

2.1. Chemicals

Propranolol·HCl, atenolol, quinine·HCl, etilefrine·HCl, chloramphenicol, tetracycline·HCl, Na-diclofenac and salicylic acid were purchased from COM-Pharma-Handels GmbH (Hamburg, Germany) Table 1. The samples (1m*M*) were prepared by dissolving analytical pure substances in bidistilled water. The sodium salts of glycocholic acid (GCA), taurocholic acid (TCA), glycochenodeoxycholic acid (GCDCA) and glycodeoxycholic acid (GDCA) of analytical grade, were obtained from Fluka (Buchs, Switzerland), Table 2.

2.2. Apparatus and methods

A Hewlett–Packard (Waldbronn, Germany) ^{3D}CE system fitted with a 600 (515)×0.05 mm (extended lightpath) fused-silica capillary and an on-column diode array detector (190...600 nm) were used. 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 each

Table 1
Analytical parameters for the drugs

Drug	Ionic mobility ($cm^2/V \cdot s$) (pH=7.4, *10.0*)	pK_a^a *partition coefficient (octanol–water)*
Propranolol·HCl	0.00019	9.42
	0.00005	*5.4*
Quinine·HCl	0.00018	4.2, 8.8
	0.00004	
Atenolol	0.00018	9.6
	0.00009	*0.008*
Chloramphenicol	$8 \cdot 10^{-6}$	5.5
	−0.00007	*12.5*
Etilefrine·HCl	0.00022	9.0
	0.00001	
Tertracycline·HCl	−0.00004	3.1, 7.6, 9.7
	−0.00024	*0.039*
Salicylic acid	−0.00032	2.97, 13.4
	−0.00034	
Diclofenac	*−0.00023*	4.4
		13.4

[a]V. Dinnendahl, U. Fricke (Editors), Arzneistoff-Profile, Govi-Verlag GmbH, Frankfurt/Eschborn (1994).

Table 2
Analytical parameters for the bile salts

Bile acid	Ionic mobility ($cm^2/V\,s$) *micelle* (pH=7.4, *10.0*)	pK_a	CMC (m*M*)*
GCA	−0.00027	3.9	4.2
	−0.00030		
TCA	−0.00027	1.85	4.2
	−0.00028		
GDCA	−0.00034	4.6	0.6
	−0.00031		
GCDCA	−0.00034	4.23	0.8
	−0.00029		

*D.M. Small, in P.P. Nair, D. Krichevsky (Editors), The Bile Acids, Plenum Press, New York (1971) p. 203–226.

following run. The separation conditions were: 30 kV voltage (detection end), 200 mbar s 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.

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

The values of K_P and $\bar{v}$ were obtained by fitting these parameters in Eq. 5 to experimental data. Similar the parameters K_A and m were obtained using Eq. 9. The fitting procedure were carried out by a PC- programm "Modellbank Biotechnologie" (V 5.0, B. Goldschmitt, Martin-Luther University, Halle).

3. Theory

3.1. Partition model

In aqueous solutions amphiphilic bile salts form micelles when their concentration exceeds the critical micelle concentration (CMC). The structure of these micelles is not sufficiently characterized compared to other surfactants such as SDS.

Not all bile salts form the same type of micelle. The structure of micelle depends on bile salts concentration. If the concentration of bile salts slightly exceeds the CMC then the aggregates have a spherical or nearly spherical micellar shape [13]. With increasing concentration of the bile salts the particles grow to rod shaped objects [14]. The CMC of bile salts can change as a function of the pH and of the concentration of other lipids and ions. Trihydroxy bile salts have generally higher CMC than dihydroxy bile salts. At the physiological pH the CMC of most bile salts varies between 2 and 5 m*M*.

For the mathematical handling of the data in terms of the partition model the following simplifications have been introduced:

(a) The drugs (D) distribute between the aqueous and micellar phase according to Nernst's law. The structure and mobility of the micelles are not influenced by the drugs. k'_P represents the capacity factor.

$$K_P = \frac{[D]_{mc}}{[D]_{aq}} = k'_P \frac{V_{aq}}{V_{mc}} \quad (1)$$

(b) The volume of the micellar phase V_{mc} is proportional to the concentration of the bile salt exceeding the critical micelle concentration ($\bar{v}$-partial molar volume of micelle, $[b_0]$-bile salt concentration in the buffer).

$$V_{mc} = \bar{v}([b_0] - CMC) \quad (2)$$

(c) The net mobility μ of the drugs is related to the net mobility of the drug in the aqueous phase responding to its partition in the two phases.

$$\mu = R\mu_D + (1 - R)\mu_{mc} \quad (3)$$

μ_D represents the electrophoretical mobility of the free dissolved drug, μ_{mc} the mobility of the micelle and μ the measured mobility of the drug in dependence of bile salt concentration in the run buffer. The measurement of μ_{mc} is done by measuring the migration time of a neutral, fully solubilized substance e.g. flavonoids.

Taken from the molar ratio R

$$R = \frac{n_{aq}}{n_{aq} + n_{mc}} = \frac{1}{1 + k'_P} \quad (4)$$

k_P' can directly derived from Eq. 3 relating to the experimental retention times. Using Eq. 2 the partition constant K_P can be calculated.

$$k'_P = \frac{\mu_D - \mu}{\mu - \mu_{mc}} = K_P \frac{\bar{v}([b]_0 - CMC)}{1 - \bar{v}([b]_0 - CMC)} \quad (5)$$

This equation has a singularity at $([b]_0 - CMC) = 1/\bar{v}$. Therefore, the product $\bar{v}([b]_0 - CMC)$ has to be smaller than 1.

3.2. Aggregation model

An interpretation of the interactions by a complex formation offers the possibility to characterize the equilibrium at the molecular level. The electrophoretical mobility μ_D of a drug is related to its mass M_D and net charge q_D by a relationship of the approximated form $\mu_D \sim q_D/(M_D)^{2/3}$. If the drug binds on a charged bile salt molecule (b), the change in μ occurs due to the change in mass M_{D+b} and change in the charge q from q_D to q_{D+b}.

The mathematical treatment of aggregation model is based on the following assumption: For the formation of the aggregates by the drug (D) and the bile salt molecule (b) a step by step uptake of single molecules was assumed, because the stoichiometric parameters were unknown. K_{A1-m} are the corresponding aggregation constants.

$$D + b \rightleftharpoons Db \quad K_{A1} \quad (I)$$

$$Db + b \rightleftharpoons Db_2 \quad K_{A2} \quad (II)$$

$$Db_{m-1} + b \rightleftharpoons Db_m \quad K_{Am} \quad (III)$$

Eqs. I–III give the brutto equilibrium constant or stability constant

$$K_A = \prod_{i=1}^{i=m} K_{Ai}$$

$$D + mb \rightleftharpoons Db_m \quad (IV)$$

(a) The complex forming equilibrium between drug and bile salt is described by the stability constant of the complex.

$$K_A = \frac{[Db_m]}{[D][b]^m} = \frac{k_A}{[b]^m} \quad (6)$$

k_A represents the ratio of the bound and free dissolved drug analogously to the capacity factor k'_P.

The α value, the fraction of the unbounded drug, is given by

$$\alpha = \frac{[D]}{[D] + [Db_m]} = \frac{1}{1 + k_A} \quad (7)$$

(b) The net mobility of the drugs is related to the net mobility of the complex and the mobility of the free drug according to its degree of dissociation.

$$\mu = \alpha\mu_D + (1 - \alpha)\mu_{Dbm} \quad (8)$$

μ_{Dbm} is the mobility of the formed aggregation complex and is derived from the maximum peak shift.

The stability constant K_A can be calculated:

$$k_A = \frac{\mu_D - \mu}{\mu - \mu_{Dbm}} = K_A[b_0]^m \quad (9)$$

4. Results and discussions

In this investigation the interactions of four different kinds of bile salts and selected drugs with different lipophilicity and basicity were studied. Except for salicylic acid and diclofenac all other molecules contain a β-hydroxyamine- or a β-hydroxyamide-group as a structural feature, that enables them to exhibit hydrogen acceptor basic as well as hydrogen donor acidic characteristics.

Fig. 1 shows a typical electropherogram when propranolol, quinine and salicylic acid run at different concentrations of GDCA in the buffer. In this

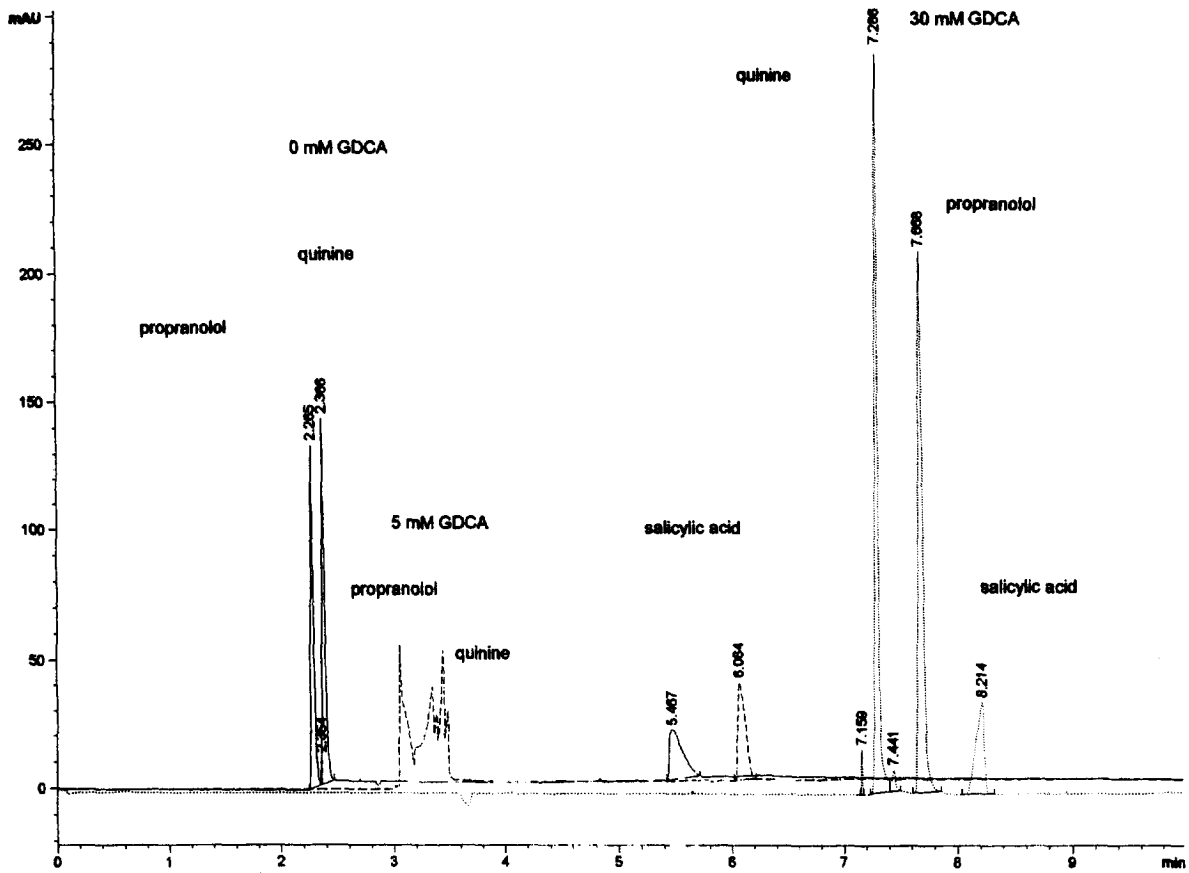

Fig. 1. Electropherogram of propranolol, quinine and salicylic acid in dependence of the concentration of bile salts GDCA (0–5–30 m*M* GDCA), pH = 7.4 (20 m*M* phosphate buffer).

case not only the peak areas, caused by a solvatochromic effect, but also the peak shapes undergo a striking change.

The μ values resulting from the shift of the retention times is shown in Fig. 2 (for different bile salts) and Fig. 3 (for different drugs). The concentration needed for a significant influence of bile salts on μ seems to be lower for the dihydroxy bile salts (GDCA, GCDCA). From concentrations higher than 17 m*M* 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 and the aggregates, respectively, were not changed.

In Fig. 3 comparison of the mobility μ of the used

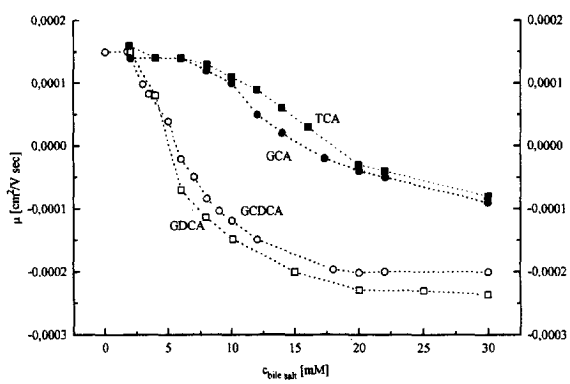

Fig. 2. Electrophoretic ionic mobility μ of propranolol in dependence of various bile salts (GCA, TCA, GCDCA, GDCA), pH = 7.4 (20 m*M* phosphate buffer).

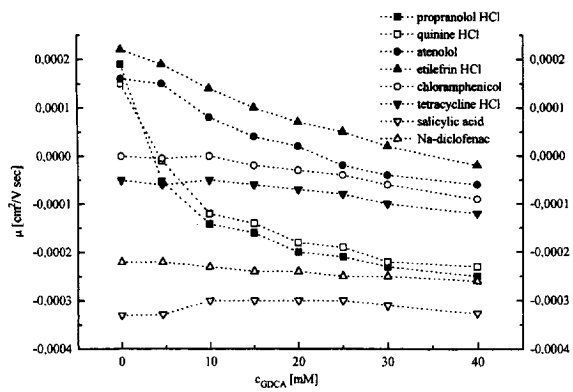

Fig. 3. Electrophoretic ionic mobility μ of the drugs propranolol, quinine, salicylic acid, atenolol, etilefrine, chloramphenicol, diclofenac and tetracycline in dependence of concentration of GDCA (20 m*M* buffer, pH = 7.4–phosphate).

drugs is shown. Quinine had a very similar electrophoretic behaviour compared to propranolol. In contrast, the effective mobility of the anionic drug tetracycline, diclofenac and salicylic acid was not influenced by GDCA. Electrostatic repulsion between the surface of the micelle and negatively charged drugs as well as the hydrophilic nature of tetracycline leads to a retaining in the aqueous phase. Atenolol and etilefrine are only slightly more influenced by bile salts than the nonionic chloramphenicol.

Fig. 4 shows the bile salt concentration dependence on the capacity factor k'_P in the systems propranolol/GDCA and quinine/GDCA at different pH values. Additionally, the fitted curves calculated with the partition model is graphed. They described the dependence $k'_P = f([b_0]$ in a good way.

When the pH was increased from 7.4 to 9.7 the dissociation of the basic drugs propranolol and quinine decreased. Therefore, the capacity factors of both drugs between the micellar and aqueous phase at pH 9.7 were significantly decreased compared with the k'_P at pH 7.4.

The influence of different bile salts on the k_A of propranolol is shown by the calculated data and the fitted curves in Fig. 5. k_A is similar for all three systems up to a bile salt concentration of 20 m*M*. At higher concentrations the binding behaviour of propranolol to the bile salts becomes striking different for the different bile salts. The partition coefficient K_P and the partial molar volumes $\bar{v}$ were estimated by fitting these parameters in Eq. 5 according to experimental data. In general, K_p is significantly higher at a pH of 7.4 than in a basic environment. The capacity factors for propranolol were significantly higher than those for quinine. This effect seems to be caused by steric effects because the molecular volume of quinine is larger than that of propranolol.

Different values for the partial molar volume $\bar{v}$ were obtained. This phenomenon could be caused by a change of the micellar structure due to the dissolution of the drugs in the micellar phase. In the case of strong interactions of the bile salt with the drug and

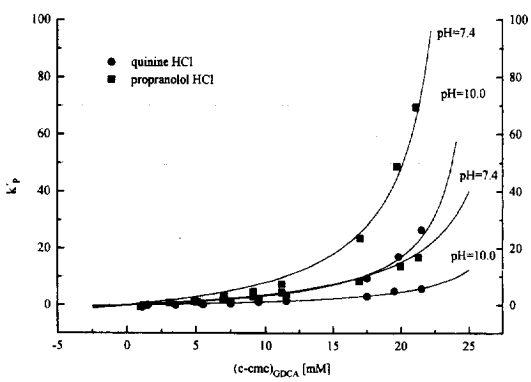

Fig. 4. Dependence of k'_P on c_{GDCA} (20 m*M* buffer, pH = 7.4–phosphate, pH = 10.0–boric acid–NaOH), fitted function of Eq. 5.

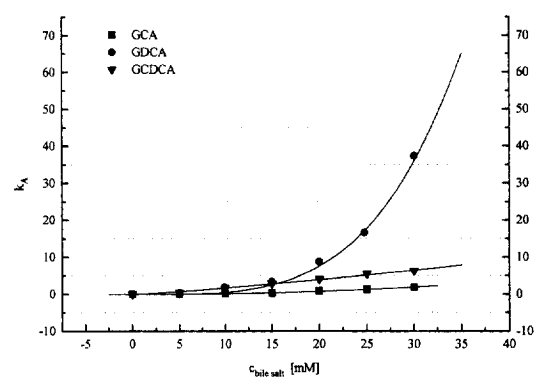

Fig. 5. Dependence of k_A of propranolol on $c_{GDCA,GCDCA,TCA}$ (20 m*M* buffer, pH = 7.4–phosphate), fitted function of Eq. 9.

at higher pH values the values for $\bar{v}$ are remarkable higher. For the second effect higher aggregation numbers of the micelles might be the reason.

For very low interactions between bile salts and drugs it was not possible to calculate the values of K_P because of the deviation of the measurements (TCA, GCA) (Table 3).

Table 4 shows the estimated parameters K_A and m for propranolol and quinine. In principle, very similar results were obtained for the calculation of k_A. It was remarkable that for high interactions ($k_A > 10$) low values for K_A and high values for m were calculated. Except for GDCA a stoichiometric coefficient m of a value between 1.2 and 1.8 was calculated. For the system with very weak interactions GCA/drug ($k_A = 1.87 - 0.44$), we obtained relatively large stoichiometric coefficients in contrary to the assumption.

As shown above, m includes two factors. m is equal to the maximum stoichiometric coefficient which represents an expression according to the affinity of the drugs to the bile salts. On the other hand m is equal to the number of possible equilibria in the used concentration range. The value of the aggregation constants $K_{A1} - K_{Am}$ and thus the state of equilibrium cannot be expressed. A direct comparison between the two systems is only possible for similar values of the stoichiometric coefficients m or aggregation constants related to the same m-values ($K_{m=max}$) (see Table 4).

These results show that there are only interactions between bile acids and drugs with a charge of opposite sign. The strength of interactions depends on the degree of ionization of the counterionic drug. Comparing the behaviour of all drugs it was found that both ionic and hydrophobic interactions play a major role. The cationic and hydrophilic substances atenolol and etilefrine and the anionic substances tetracycline, diclofenac and salicylic acid do not significantly interact with the bile salts.

For both models the following tendency for the strength of the interactions was found:

GDCA ≈ GCDCA >> TCA >> GCA,

propranolol > quinine >> atenolol ≈ etilefrine > chloramphenicol ≈ tetracycline ≈ diclofenac ≈ salicylic acid and pH = 7.4 > pH = 10.0.

Table 3
Comparison of partition coefficients K_P and partial molar volumes $\bar{v}$ estimated by application of the fitted function Eq. 5

GDCA	pH	K_P	$\bar{v}$ [l/mmol]	k'_P (30 m*M*)
Propranolol·HCl	7.4	10.84±0.53	$0.0243 \pm 6.2 \cdot 10^{-5}$	29.16
	10.0	3.00±1.20	$0.0234 \pm 5.1 \cdot 10^{-4}$	6.67
Quinine·HCl	7.4	8.82±1.33	$0.0212 \pm 5.4 \cdot 10^{-4}$	15.07
	10.0	2.09±0.91	$0.0218 \pm 7.0 \cdot 10^{-4}$	3.95
Atenolol		n.d.p.	n.d.p.	n.d.p.
Chloramphenicol				
Diclofenac				
Salicylic acid				
Tetracycline·HCl	7.4	0.301±0.068	0.014±0.012	0.21
	10.0	n.d.p.	n.d.p.	
GCDCA				
Propranolol·HCl	7.4	20.0±1.20	$0.011 \pm 4.2 \cdot 10^{-3}$	9.85
	10.0	3.8±1.21	$0.019 \pm 1.5 \cdot 10^{-3}$	5.03
Quinine·HCl	7.4	16.0±1.00	$0.010 \pm 5.2 \cdot 10^{-3}$	7.46
	10.0	2.27±0.27	$0.017 \pm 3.8 \cdot 10^{-3}$	2.30
TCA				
Propranolol·HCl	7.4	1.8±0.2	$0.013 \pm 4.8 \cdot 10^{-3}$	1.15
	10	0.82±0.32	$0.022 \pm 3.3 \cdot 10^{-3}$	1.59
Quinine·HCl	7.4	1.059±0.3	$0.012 \pm 2.1 \cdot 10^{-3}$	0.59
	10	n.d.p.	n.d.p.	–

n.d.p.-no determination possible

Table 4
Comparison of aggregation constants K_A and stoichiometric coefficients m estimated by application of the fitted function (Eq. 9)

Bile salt	pH	Propranolol·HCl K_A [l/mol]^m m	k_A (30 mM) $K_{m=max}$ [l/mol]$^{3.8}$	Quinine·HCl K_A [l/mol]^m m	k_A (30 mM) $K_{m=max}$ [l/mol]$^{3.8}$
GDCA	7.4	$7.43 \cdot 10-5 \pm 2.1 \cdot 10^{-6}$ 3.85±0.07	36.17 *1*	0.0044±0.0012 2.32±0.02	11.90 *0.00025*
	9.7	0.029±0.004 1.456±0.009	4.10 *0.002*	0.0209±0.0071 1.435±0.012	2.75 $8.7 \cdot 10^{-4}$
GCDCA	7.4	0.099±0.005 1.229±0.007	6.40 *0.0023*	0.083±0.009 1.225±0.017	5.35 *0.0014*
	9.7	0.031±0.001 1.474±0.076	4.70 *0.00027*	0.021±0.008 1.47±0.012	3.11 $1.0 \cdot 10^{-4}$
GCA	7.4	$9.53\ 10^{-4} \pm 9 \cdot 10^{-6}$ 2.23±0.12	1.87 $1.27 \cdot 10^{-5}$	0.0013±0.0001 1.90±0.03	0.90 $2.5 \cdot 10^{-6}$
	9.7	$8.29 \cdot 10^{-5} \pm 1.6 \cdot 10^{-5}$ 2.74±0.27	0.92 $2.95 \cdot 10^{-5}$	n.d.p.	
TCA	7.4	0.0065±0.0012 1.803±0.093	3.0 $5.04 \cdot 10^{-5}$	0.0048±0.0011 1.65±0.034	1.31 $7.9 \cdot 10^{-6}$
	9.7	0.0079±0.0022 1.506±0.098	1.33 $1.2 \cdot 10^{-5}$	0.0803±0.0023 0.613±0.055	0.64 $7.4 \cdot 10^{-5}$

5. Conclusions

The present study shows that it is possible to characterize interactions between bile salts and drugs quantitatively using by MECC and ACE. It was shown that two different physicochemical models are able to describe these interactions. Both models supply expressions concerning the strength of the interactions. In order to compare the discussed drugs it is necessary to take into account both parameters of the two equations, K_P and $\bar{v}$ as well as K_A and m.

Using the partition model a change of the micellar structure was obtained, on the other hand, the application of the aggregation model gives the maximum stoichiometric coefficients of the aggregates. For high interactions it was calculated that the stoichiometric coefficient bile salt/drug was >2.3/1.

The interactions between the selected drugs and bile salts depend both on the lipophilicity of the drugs and on the charge of the components. The highest affinity to the dihydroxy bile salts (GDCA and GCDCA) was found using the cationic, hydrophobic drugs (propranolol and quinine), while anionic hydrophobic (diclofenac) and hydrophilic (tetracycline) drugs do not interact with bile salts.

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