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# Investigation of the interactions between drugs and mixed bile salt/lecithin micelles
## A characterization by micellar affinity capillary electrophoresis (MACE). Part III

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**Abstract**

Mixed micelles, which mimic the bile in the gastrointestinal tract, were used as a pseudostationary phase in capillary electrophoresis. The mixed micellar system studied contained sodium glycodeoxycholate (NaGDC) or sodium taurocholate (NaTC) as bile salt and lecithin (LC) or dipalmitoylphosphatidylcholine (DPPC) as phospholipid. The determination of the changing mobilities of ionic analytes in the presence of mixed micelles reflected interactions between the used drugs and the mixed micelles. These were estimated according to the lipid concentration in the bile salt (BS)/phosphatidylcholine (PC) micelles and the capacity factors $k$ for the partition were calculated whenever possible. For the calculation of $k$ it is important to characterize the formation of the mixed micellar phase and to estimate the ionic mobility of the mixed micelles. In doing so two different methods were applied in order to determine this micellar phase. The partition equilibrium of basic and acidic drugs depends considerably on size and shape of the micelles as well as on the lipophilicity of the drug. This paper also outlines the use of electrospray mass spectrometry and MS–MS for the characterization of mixed micelle composition.


*Keywords:* Micelles, mixed; Pharmaceutical analysis; Affinity capillary electrophoresis; Drugs; Bile salts; Lecithin

## 1. Introduction

The transport properties, the rate of uptake and finally the pharmacokinetic behavior of oral applied lipophilic drugs depend to a high degree on the solubilization and emulsification of these substances by the intestinal fluids [1–3].

These fluids contain the amphiphilic bile salts, that exhibit a good solubility in water, but they also form micelles at higher concentrations. The shape, size and charge of the micelles and hence their physicochemical properties may be further modified by other constituents of the digestive fluid as lecithin and fatty acids.

To obtain some information about the interactions of drugs with these bile salt aggregates, we investigated model systems with different concentrations of bile salts and lecithin. As already pointed out in Parts

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I and II, capillary electrophoresis (CE) is a versatile tool to monitor these interactions [4,5].

Bile salts have the tendency to form mixed micelles (e.g., with phospholipids) in addition to the simple micelles.

The effect of various concentrations of phosphatidylcholine at constant bile salt concentration in the separation buffer on the net mobility of the selected drugs was used to obtain a quantitative measure of the strength of interactions between the drug and the mixed bile salt micelles. The mobility of the micellar aggregates was determined as the reference value to the calculations.

It was shown in a recent paper [4] that the partition of drugs between micelle and free solution strongly depends on the kind of bile salt (di- and trihydroxy bile salt, conjugated and unconjugated bile salts) and the pH value. The partition coefficient is related to the size and shape of the simple micelles, ionic interactions play a secondary role. The drugs are associated to the micelles mainly by hydrophobic interactions and by hydrogen bonds.

Numerous studies indicate that the solubilities of water-insoluble substances depend on the total lipid concentration and the bile salt/lecithin mixing ratio [6,7]. Pure bile salts form globular micelles of about 10–15 Å in size [8], whereas pure lecithin forms bilayers. A transformation from a globular structure (in a pure bile salt solution) to an ellipsoidal one takes place when the lecithin concentration exceeds 35 mol%. Above 70 mol% lecithin, the system changes from a micellar into a lamellar arrangement. Structural studies on micellar systems indicate, that different types of micelles exist (simple and mixed micelles) [9,10]. Mazer et al. [11] suggested the coexistence of simple and mixed micelles at low lecithin (LC)/bile salt (BS) ratios (0–0.6). At higher LC/BS ratios (>0.6) only mixed micelles are present. Beyond a ratio of 2:1 insoluble bilayers and metastable liposomes (up to 300 Å) are formed.

Higuchi et al. [12] proved the coexistence of simple and mixed micelles by equilibrium dialysis studies. It was shown that at a constant lecithin concentration bile salt molecules were built in the mixed micellar phase up to a certain limit of concentration. An excess of bile salt forms additional simple micelles.

A new aspect has appeared recently, indicating that quite different molecular arrangements in the range of the divergence limit coexist. Large phospholipid vesicles coexist with mixed micelles [13,14]. Due to the much higher water solubility of bile salt monomers, the LC/BS ratio of the vesicle is changed by dilution. At concentrations below the micellar phase limit, mixed LC/BS vesicles form spontaneously [15].

Changes of size, shape and charge of the mixed micelles understandably entail a modification of the electrokinetic properties of these aggregates. Therefore it is necessary to determine the electrophoretic mobilities of the new formed micelles over the whole range [0–20 m*M* dipalmitoylphosphatidylcholine (DPPC) at constant bile salt concentration of 20 m*M*].

## 2. Materials and methods

### 2.1. Chemicals

Propranolol·HCl, quinine·HCl, etilefrine·HCl, atenolol, chloramphenicol, ibuprofen·Na and diclofenac were purchased from COM-Pharma Handels GmbH (Hamburg, Germany). The model samples (1 m*M*) were prepared by dissolving analytical pure substances in bidistilled water. The sodium salts of taurocholic acid (NaTC), glycodeoxycholic acid (NaGDC), DL-α-phosphatidylcholine, dipalmitoyl (C16/0) (DPPC) and L-α-phosphatidylcholine (LC) from fresh egg yolk (approx. 99%) of analytical grade were obtained from Fluka (Buchs, Switzerland). 1,2-Dipalmitoyl-*sn*-glycero-3-(N-(7-nitro-2-1,3-benzooxadiazol-4-yl))phosphoethanolamine (N-BDPE) was obtained from Avanti Polar Lipids (Alabaster, AL, USA). Mixed micellar solutions were prepared by adding the phosphatidylcholine to the bile salt solution. All systems were stirred continuously at 50°C until the solutions became clear. All samples were measured three-fold. From this an average standard deviation of $\Delta\mu = 4.03 \cdot 10^{-6}$ resulted.

### 2.2. Capillary electrophoresis

A Hewlett-Packard (Waldbronn, Germany) HP$^{3D}$CE system fitted with a 600 (515)×0.05 mm

(extend lightpath) fused-silica capillary and an on-column diode array detector (190–600 nm) were used for MACE. 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 subsequent run. The separation conditions were: −30 kV voltage (inlet), 200 (mbar/s) pressure injection, 25°C capillary temperature. The detection was done on the cathodic side at 200 and 220 nm. All micellar solutions and samples were filtered through a membrane filter of 0.2 μm pore size and degassed by ultrasound before use.

### 2.3. CE–MS

CE–MS results were obtained with the above-mentioned CE system coupled to a LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA). The ionization was done by electrospray (ESI) at a voltage of 4.7 kV mainly in the positive mode. The effective separation voltage is calculated from the difference between nominal CE voltage applied to the capillary inlet and electrospray voltage applied to the outlet instead of the ground potential in normal CE experiments. Sheath liquid was added by a syringe pump (Unimicro Corp.) via PTFE tubing.

For experiments with additional UV detection a 800 (215)×0.05 mm fused-silica capillary was used. Without UV detection, a capillary of 500 mm length was sufficient.

## 3. Theory

In principle, a change of the mobility ($\mu$) of drugs at an increasing phospholipid and constant bile salt concentration can have different reasons:

Case I: formation of one micellar phase – only simple bile salt micelles or bile salt/phospholipid mixed micelles exist. (1) The electrophoretic mobility of the micellar phase is changing. (2) The partition coefficient is modified.

An interpretation of the interactions is more complicated in the presence of different micellar phases.

Case II: formation of two micellar phases – simple bile salt micelles coexist with bile salt/phospholipid mixed micelles. (1) The electrophoretic properties of the simple micelles are influenced by the concentration of phospholipids (e.g., changes in the aggregation number of the micelle). (2) $\mu_{mmc}$ of a mixed micelle is not constant with dependence on the lipid concentration. (3) $k_{total}$ derives from the single capacity factors $k_{smc}$ and $k_{mmc}$.

The basis on which the capacity factor $k=n_{mc}/n_{aq}$ is calculated is Nernst's law, which describes the partition between micellar and aqueous phase (equation 1). The partition coefficient $K$ and the partial molar volume of the micelle $\bar{v}$ can be estimated provided that only one micellar phase exists (case I).

$$k = K\frac{V_{mc}}{V_{aq}} = K\frac{\bar{v}([b_0] - \mathrm{cmc})}{1 - \bar{v}([b_0] - \mathrm{cmc})} = \frac{\mu_D - \mu}{\mu - \mu_{mc}} \qquad (1)$$

The capacity factor $k$ represents the ratio of bound and free dissolved drug, $b_0$ the bile salt concentration, cmc the critical micelle concentration, $\mu_D$ the mobility of the used drug in aqueous solution, $\mu_{mc}$ the mobility of the micelle and $\mu$ the measured mobility.

With the presence of different micellar phases, equation (1) no longer holds for the calculation of the capacity factor $k$. Since the change of the mobility $\mu$ of the drug depends on two different micellar phases (simple and mixed micellar phase), another equation has to be formulated.

The net mobility $\mu$ of the drug results from its mobilities in aqueous solution and micellar phases corresponding to its partition between the three phases.

$$\mu = x\mu_D + y\mu_{smc} + z\mu_{mmc} \qquad (2)$$

$$\Delta\mu = \mu - \mu_D = y(\mu_{smc} - \mu_D) + z(\mu_{mmc} - \mu_D) \qquad (3)$$

$\mu_D$ represents the electrophoretic mobility of the free dissolved drug, $\mu_{smc}$ the mobility of the simple bile salt micelle, $\mu_{mmc}$ the mobility of the mixed micelle, $\mu$ the measured mobility of the drug depending on the phosphocholine concentrations in the run buffer and $x$, $y$, $z$ the corresponding molar ratio of the drug ($n_{smc,mmc}/n_{total}$).

Thus the following equation results from Eq. 3:

$$y = \frac{k_{smc}}{1 + k_{smc} + k_{mmc}} \quad (4)$$

and

$$z = \frac{k_{mmc}}{1 + k_{smc} + k_{mmc}} \quad (5)$$

With the capacity factors of the different partition equilibria, $k_{smc} = n_{smc}/n_{aq}$, $k_{mmc} = n_{mmc}/n_{aq}$ and $k_{total} = k_{smc} + k_{mmc}$ obtained. $k_{total}$ corresponds to the capacity factor $k$ for a one phase system.

Since the relation $k_{smc}/k_{mmc}$ is unknown, the calculation of the capacity factor $k_{mmc}$ is only possible if one of the following conditions is met: (1) if the drug shows only slight or no interactions with the bile salt micelles, $y = 0$ (e.g., diclofenac), (2) if only one micellar phase exists in a certain concentration limit (e.g., only mixed micelles) and (3) if the drug is completely dissolved in the micellar phase, $x = 0$. Otherwise, only estimations of the interactions can be made.

## 4. Results and discussion

### 4.1. Mobility of the drugs

#### 4.1.1. Bile salt/DPPC

In this investigation the interactions between bile salts (NaGDC, NaTC) mixed with phosphatidylcholine and the drugs propranolol, quinine, atenolol, etilefrine, chloramphenicol, diclofenac and ibuprofen were studied. In the system BS/PC/drug, the hydrophobic interactions play a major role in terms of influencing the electrokinetic migration behaviour.

Fig. 1 shows typical electropherograms where propranolol and etilefrine were run at different concentrations of DPPC at constant bile salt concentration in the buffer. Not only the peak areas and shapes but the mobilities undergo a striking change. At 20 m*M* DPPC propranolol shows a maximum shift.

Fig. 2a and Fig. 2b show the changes in the mobilities of the used drugs as influenced by the

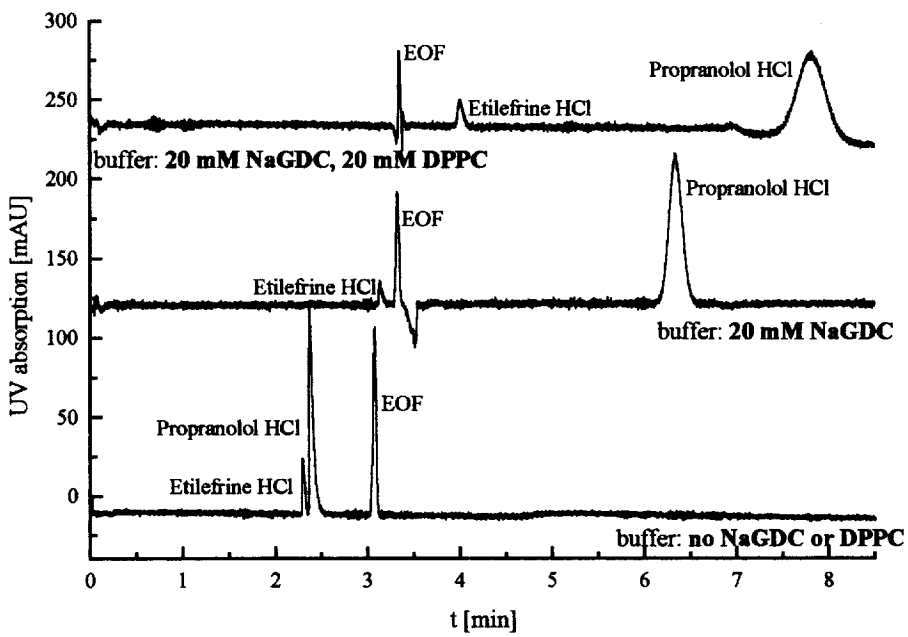


Fig. 1. Electropherograms of the cationic drugs propranolol and etilefrine in the presence of NaGDC and various concentrations of DPPC in the run buffer (buffer: different concentrations of NaGDC and DPPC, 20 m*M* phosphate, pH 7.4, detection: 220 nm).

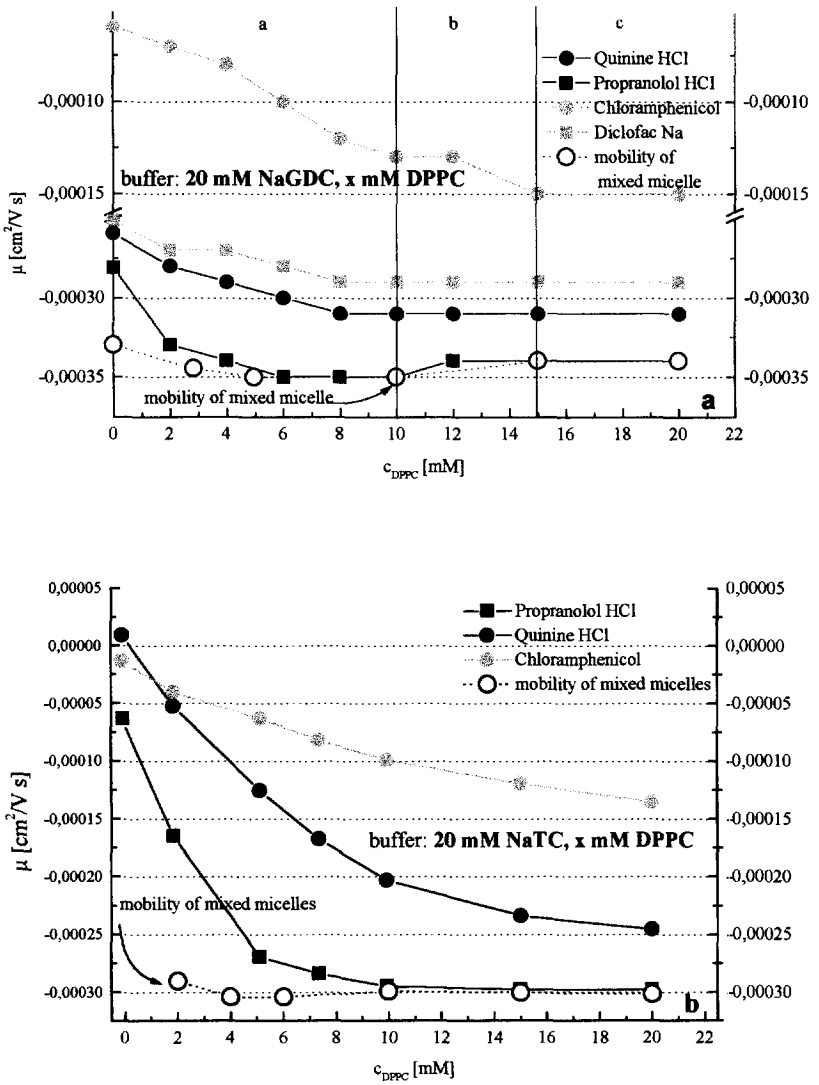


Fig. 2. Electrophoretic ionic mobilities $\mu$ ($cm^2/V\,s$) of different drugs as influenced by DPPC concentration – a comparison between NaGDC (a) and NaTC (b) (buffer: 20 m*M* bile salt, *x* m*M* DPPC, 20 m*M* phosphate, pH 7.4, detection: 220 nm).

DPPC concentration at a constant bile salt concentration (NaGDC or NaTC). Within this concentration range (2–20 m*M* DPPC) the coexistence of simple micelles and mixed micelles (range a) and the exclusive existence of only mixed micelles (range b) (in Fig. 2a) is supposed. It is not possible to specify whether bilayer vesicles are formed over the range c.

For all drugs the ionic mobility is independent of higher DPPC concentrations starting at a concentration of 15 m*M* DPPC and 20 m*M* NaGDC (Fig. 2a). The maximum shift for chloramphenicol is $-0.00015\ cm^2/V\,s$. The change in the mobilities of the basic propranolol and quinine is not as drastic as in the nonionic drug chloramphenicol. The assumption that propranolol is completely solubilized over the whole range in the mixed micelles (NaGDC/

DPPC) could be confirmed, because a rapid increase of mobility between 0 and 2 m*M* DPPC was observed and the mobility corresponds to $\mu_{mmc}$ which will be discussed in Section 4.2.

In the presence of NaTC (Fig. 2b) the mobility shift for propanolol is significantly higher. Due to the fact that the interactions between propranolol and NaTC micelles are weaker than for propranolol and NaGDC micelles even low concentrations of DPPC have a strong effect on the mobility shift.

As a matter of principle the net mobilities of propranolol, quinine and diclofenac are dependent on the used bile salt in the ranges b and c, but this is not applied to the strength of interactions. Only with the knowledge concerning the difference between the mobility of micellar phase and the mobility of drug an estimation of the strength became possible. The higher the concentration of DPPC becomes the lesser the influence of the bile salt. For a concentration of 20 m*M* DPPC only a small difference for the values $\mu - \mu_{mmc}$ for the various drugs is observed. The estimation of the ionic mobilities is described in Section 4.2.

#### *4.1.2. Bile salt/lecithin*

Fig. 3a and Fig. 3b demonstrate the dependence of the mobilities of various drugs on the lecithin concentration under presence of NaGDC and NaTC. Since natural egg lecithin is a mixture of different zwitterionic phosphocholines (mainly OPPC, SPPC and LPPC; O oleic acid, P palmitic acid, S stearic acid, L linoleic acid, PC phosphocholine), the molar mixing ratio of which is not exactly fixed, only the mass ratios have been indicated. The curves show an increase in mobilities except in the case of ibuprofen. A drastic change is observed both for the nonionic drug chloramphenicol and the anionic diclofenac. The intensity of the interactions might be explained by the lipophilic character of the mixed micelles and by the presence of positively charged quaternary amino groups. Propranolol is again nearly completely solubilized in the mixed micelles over the whole concentration range (Fig. 3a). The mobilities of the more hydrophilic, basic drugs atenolol and etilefrine are not as strongly influenced compared to propranolol.

### *4.2. Characterization of the micellar phase*

An important foundation for the quantification of interactions is the determination of the electrophoretic properties of the mixed micellar phase itself.

The formation of mixed micelles bile salt/phosphatidylcholine is a very complex process, because the limit of the coexistence range (i.e., below it simple micelles coexist with simple ones, above only mixed micelles exist) and the divergence limit (change from mixed micelles to bilayers and liposomes) depend on the type of bile salt molecule (di- or trihydroxy bile salts), the total lipid concentration, the ionic strength and the pH value.

It was supposed that the formation of mixed micelles leads to a decrease in ionic mobility due to an increase in size and a reduction of the relative charge of the micelles.

Beside the poor UV absorption of the mixed and simple micelles it remains difficult to trace them as two distinguished species. This is since the bile salt molecules are exchanged between the two phases with a high rate. Substances like caempferol and bilirubin which were used as markers of pure bile salt micelles turned out to be unsuitable for these systems, because they spread over the different phases in an unknown way.

The mobilities $\mu$ of the mixed micelles were estimated according to two different methods described below, which allow an estimation of the electrophoretic velocities of the mixed micelles. Both methods have to be coherently considered.

#### *4.2.1. Method I*

Since the mixed micelles (in contrast to pure bile salt micelles) remain stable during dilution, it is possible to use a phosphate buffer without bile salts. This is since the bile salt molecules are exchanged between the two phases with a high rate. For that purpose a mixture containing NaGDC and DPPC was applied as a sample on the capillary, while the CE run buffer only contains phosphate.

The first peak (peak 1′ in Fig. 4) corresponds to the mobility of the bile salt monomer ($\mu = -0.00019$ cm$^2$/V s for NaGDC), because simple bile salt micelles are not stable under these conditions. A second peak (2′) appears with a mobility of

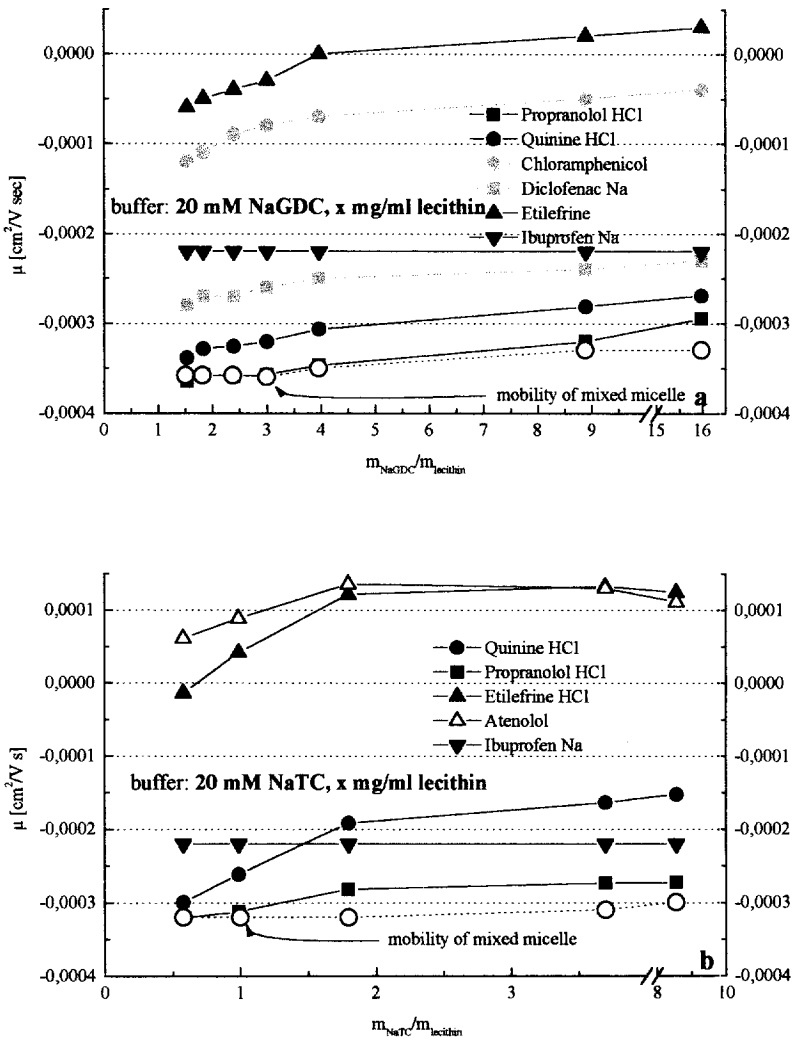


Fig. 3. Electrophoretic ionic mobilities $\mu$ ($cm^2/V\,s$) of different drugs as influenced by egg lecithin concentration – a comparison between NaGDC (a) and NaTC (b) (buffer: 20 m*M* bile salt, *x* m*M* lecithin, 20 m*M* phosphate, pH 7.4, detection: 220 nm).

$-0.00026$ $cm^2/V\,s$ at a constant NaGDC concentration and is not influenced in its area by the DPPC concentration. A possible interpretation could be the formation of vesicles caused under these conditions.

At a constant NaGDC concentration of 20 m*M* another peak is formed (peak 3′) if the concentration of DPPC exceeds 6 m*M*, whose area grows remarkably with a further increase of DPPC concentration until it remains constant at 15 m*M*. At a molecular mass ratio NaGDC/DPPC of 1:1 the mobility is $-0.00034$ $cm^2/V\,s$. The reason as to why the UV absorption increased so much is not clear at the moment. It was shown by means of MS detection (positive and negative ionization mode) that DPPC is the main constituent of this aggregate. A list of the found adducts is given in Section 4.3. These adducts are most probably formed at the ESI nozzle during the electrospray ionization process. So the structure of possible adducts or aggregates formed under CE conditions, which might explain the extraordinary high mobility, remain undetermined.

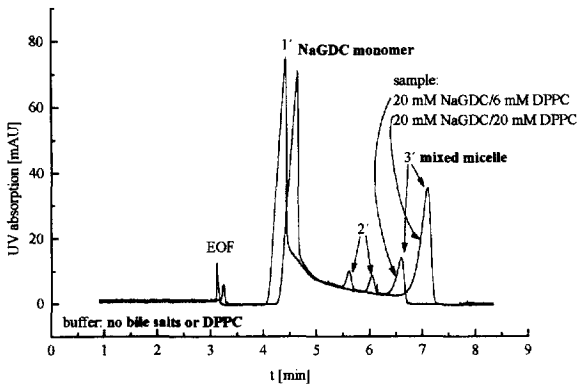


Fig. 4. Electropherograms of two samples containing 6 or 20 m*M* DPPC respectively, solubilized in 20 m*M* NaGDC (buffer: 20 m*M* phosphate, pH 7.4, detection 200 nm, peaks 1′ and 2′ are explained in Section 4.2.1).

### *4.2.2. Method II*

This method is characterized by the presence of bile salt in the CE run buffer and the sample as well, typically at a concentration of 20 m*M* DPPC was solubilized in the bile salt solution, so equilibrium conditions were reached provided that the formation of mixed micelles occurs reversibly and not kinetically controlled (see Section 4.2.1). Two different negative peaks appear by using UV detection (200 nm). The first one (peak 1 in Fig. 5a) corresponds to the simple bile salt micelles (i.e., a lower bile salt concentration in the sample through formation of mixed micelles), the second corresponds to the mixed micelles (peak 2 in Fig. 5a). These conclusions are explained by the results described as follows.

In MS experiments it could be shown that this second peak consists of DPPC and NaGDC. Its mobility in ammonium acetate buffer was $3.0 \cdot 10^{4}$ $cm^2/V$ s. Because of the high bile salt concentration in the CE run buffer (20 m*M*) it was not possible to determine an exact concentration profile of NaGDC. Another peak at masses related to DPPC was not found, which means that only one phospholipid containing phase exists.

Another independent way for the determination of the micellar mobilities was the introduction of NBDPE with only a slight perturbation of the equilibria. Sample and running buffer had the same composition, but the sample contained the additional marker. The results in Fig. 5b are comparable with Fig. 5a.

The slight shift of the migration time of peak 2 in presence of the marker (10 mol%) results from the influence of NBDPE on the general mobility of the micelle. Fig. 5b also shows the net mobility of NBDPE under the same conditions. At 220 nm a positive peak appears instead of the second negative one. A more elegant method by far is the use of laser induced fluorescence [16]. It was shown by MS detection, that NBDPE is completely incorporated in the DPPC phase (detection at $m/z$ 855 for $[M+H]^+$ and $m/z$ 877 for $[M+Na]^+$).

Since obviously the mobility – and therefore shape, size and charge of the mixed micelle is too dependent on the strength of the interactions Fig. 2a and the results of methods I and II are compared.

The various sections of Fig. 6a and Fig. 6b are described as follows.

Range a: for the direct determination of the new appearing BS/DPPC peak 3′ (Fig. 4) an increase in the mobility with increased DPPC concentration is observed (Fig. 6a). When the phospholipid concentration increases, bile salts are built in the mixed micelles in order to increase the stability. The same phenomenon is observed for peak 2 of the mixed micelle under equilibrium conditions and the presence of NaGDC ($\mu_2$). In this range a drastic change of the mobilities and $k$ values of chloramphenicol, quinine and propranolol occurs.

Range b: when a ratio of 1:0.5 for NaGDC/DPPC is reached, $\mu_2$ remains constant, but the area $A_2$ increases enormously. Obviously, only DPPC is built in the micelles in order to keep a certain bile salt concentration outside the micelles. This assumption is supported by the fact, that above this concentration ratio, the area of the bile salt monomer peak $A_1$, remains constant, after a significant decrease (Fig. 6b). Above a DPPC concentration of 10 m*M* $\mu_3$, and $\mu_2$ are identical. In this range an estimation of the mobilities of the mixed micelles is possible under using method I. The mobilities of all drugs change only slightly.

Range c: the areas $A_2$ and $A_3$, and the mobilities $\mu_2$ and $\mu_3$, remain nearly unchanged above a concentration ratio of 1.33:1. Whether mixed micelles and pure DPPC vesicles coexist or whether more

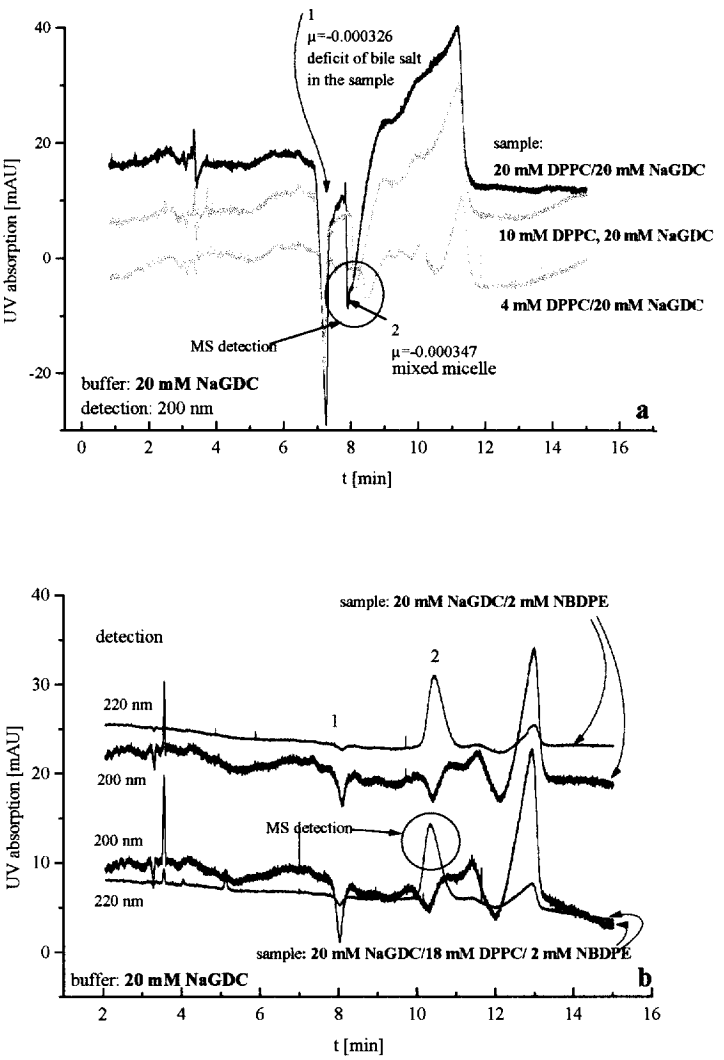


Fig. 5. Electropherograms of DPPC/NaGDC (a) and NBDPE/NaGDC and DPPC/NBDPE/NaGDC mixtures (b), $\mu$ ($cm^2V\ s$) (buffer: 20 m*M* bile salt, 20 m*M* phosphate, pH 7.4, detection: 220 nm).

DPPC is built in the mixed micelles has not yet been answered. The assumption that peak 2′ (Fig. 4) derives from pure DPPC could not be confirmed. Interestingly, this peak area seems to be unaffected by the lipid concentration. CE–MS runs did not clarify the problem.

We assume that in the range between 10 and 15 m*M* DPPC mixed micelles exist nearly exclusively. Vesicles could not be traced. The fact that propranolol has the same mobility as $\mu_2$ (NaGDC/DPPC) confirm that it is completely incorporated in the mixed micelles. The systems NaGDC/DPPC do not show a further change of the drug mobilities.

### 4.3. MS and CE–MS

For MS experiments without CE sample solutions can be supplied via the sheath liquid line by the same syringe pump without further alteration.

The nature of phospholipid headgroups and the

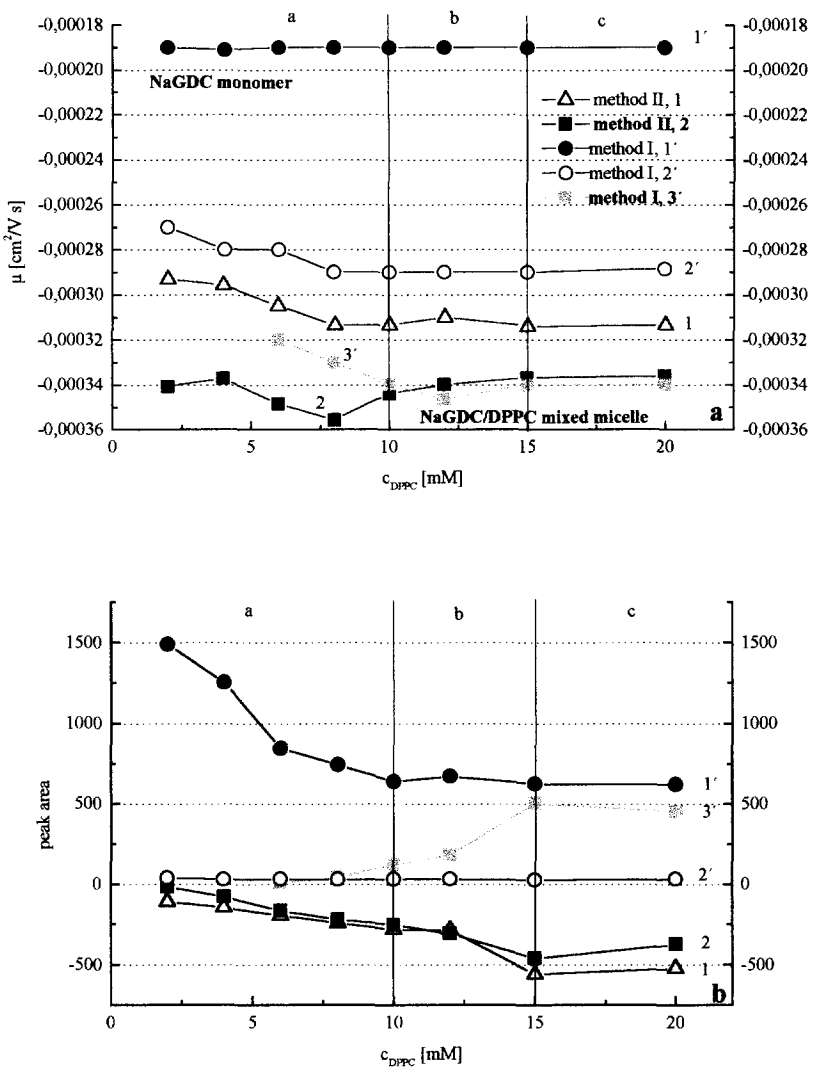


Fig. 6. Curves of Fig. 4 and Fig. 5a received mobilities of micellar phases as influenced by the DPPC concentration (a); peak areas of peaks 1', 3', 2', 2, 1 (b).(conditions are described in Section 4.3).

fatty acid composition can be determined by MS–MS or $MS^n$, respectively, which is an advantage of the ion trap technique.

In CE–MS experiments a stable electrospray ionization has to be guaranteed. Since the flow-rate coming from CE capillary is too low for it (for the given conditions 40 nl/min at an EOF of 6 min), it was necessary to add sheath liquid at a rate of at least 1.5 μl/min, resulting in sample dilution. The composition of sheath liquid is important: a mixture of methanol–water (50:50) allowed much lower flow-rates than pure methanol.

The MS detection can be done in the full scan or in the selected ion monitoring (SIM) mode. The advantage of full scan is that even unexpected adducts are found by taking mass spectra at peaks in the total ion current (TIC) or other known mass ranges. Every $m/z$ can be filtered out later. The SIM

mode was preferred for routine determinations of up to ten $m/z$ ranges to reduce the amount of data. Dimethylsulfoxide was used as electroosmotic flow (EOF) marker (detected at $m/z$ 79). The mass ranges ($m/z$ ratios) of highly concentrated CE buffer constituents like bile salt should be excluded, otherwise the sensitivity decreases drastically. For CE–MS experiments a volatile buffer substance, ammonium acetate, was used instead of phosphate at a concentration of 10–20 m*M*.

According to method I the bile salt (NaGDC) peak could be measured in the form of $[M+H]^+$ at $m/z$ 450, $[2M+2H]^+$ at $m/z$ 900, $[2M+Na+H]^+$ at $m/z$ 922. The DPPC peak could be detected as $[M+H]^+$ at $m/z$ 735, $[M+Na]^+$ at $m/z$ 757, $[M+acetate+2Na]^+$ at $m/z$ 839, $[M+GDCA+Na]^+$ at $m/z$ 1206, $[M+GDCA+2Na-H]^+$ at $m/z$ 1228 and sometimes as $[2M+2H]^+$ at $m/z$ 1470. In experiments according to method II only one large DPPC peak could be detected at the $m/z$ values mentioned above.

In the negative ionization mode the following adducts were found: $[GDCA-H]^-$ at $m/z$ 449, $[2GDCA+Na-H]^-$ at $m/z$ 921, [DPPC + acetate] at $m/z$ 794 and $[DPPC+GDCA-H]^-$ at $m/z$ 1183. All these adducts were generated during the ESI process. Although ESI is not completely understood up to now, it is known that amphiphilic substances tend to form adducts because of their enrichment at the surface of the formed droplets.

GDCA itself as a continuous buffer constituent could not be measured.

### 4.4. Capacity factor

According to equation 3 the calculation of the capacity factor is only possible if only one micellar phase exists or a partial capacity factor is known. For example, chloramphenicol as well as diclofenac and ibuprofen show very weak interactions with the simple micellar bile salt phases of NaTC and NaGDC, so that $k_{smc}$ is about 0 and $k_{total}=k_{mmc}$. Therefore a calculation of $k$ is also possible for the range a.

$k_{mmc}$ was calculated in the concentration range from 10 to 15 m*M* DPPC, above this range it is not sure if only mixed micelles exist or not.

Furthermore, because of the stability of the mixed micelles in the whole concentration range only a small part of the total bile salt amount is outside of the mixed micellar phase. Higuchi et al. [12] showed that at a total concentration of about 20 m*M* BS/20 m*M* LC less than 5 mol% (i.e., 1 m*M*) BS are outside of the mixed micelle (coexistence limit for 20 m*M* NaTC was determined at 10 m*M* lecithin). In the range from 1–3 m*M* BS even propranolol and quinine only show very slight interactions to the micellar phase [4].

In principle, in the presence of phosphocholines an increase in the partition coefficient is observed. For instance $k_{total}$ of diclofenac increases more than ten-fold, $k_{total}$ of chloramphenicol increases five-fold.

The influence of the bile salt type on $k_{total}$ is not significant. Only for drugs that show a strong affinity to the bile salt phase itself the kind of bile salt plays a major role for the formation of mixed micelles when the concentration of DPPC falls below 10 m*M*. Up to a concentration of DPPC of 10 m*M* a smaller capacity factor was obtained when NaTC is present.

The phospholipids play a small role regarding the interactions, while bile salts are just stabilizing the system. Furthermore, conjugated bile salts additionally form ion pair bonds with the phosphocholines and are therefore unable to interact with the lipophilic cationic drugs (e.g. propranolol, quinine; see Table 1). A direct comparison between DPPC and lecithin was not made, but it can be assumed that an influence caused by unsaturated acyl groups exists.

Hydrophilic drugs like atenolol and etilefrine only show slight interactions compared to quinine and propranolol. Both drugs have quite strong affinity to the micellar phase [4]. Ibuprofen has no interactions even though it is a quite lipophilic substance. Its chemical structure could be the reason for its behaviour since the absence of an amino or amide group or any other hydrogen acceptor basic- or hydrogen donor acidic groups prevents it from undergoing stronger molecular interaction.

Naylor et al. [17] found that the more hydrophilic steroidal drugs hydrocortisone and betamethasone in comparison to danazol have no remarkable interactions to NaTC/LC (4:1). However, the solubility of danazol increased when lecithin was added to the bile salt dissolution medium. It is evident, that the chemical structure has an important influence on the

Table 1
Calculated capacity factors $k_{total}$ of various drugs (conditions are described in Section 4.1)

| $k$ / $k_{smc}$ | Propranolol·HCl | Quinine·HCl | Chloramphenicol | Diclofenac·Na |
|---|---|---|---|---|
| 20 m*M* NaGDC | 7.4 | 5.4 | 0.18 | 0.17 |
| 20 m*M* NaTC | 1.2 | 0.8 | 0.08 | 0.14 |
| 20 m*M* BS | 10 m*M* DPPC<br>15 m*M* DPPC | 10 m*M* DPPC<br>15 m*M* DPPC | 10 m*M* DPPC<br>15 m*M* DPPC | 10 m*M* DPPC<br>15 m*M* DPPC |
| $k_{mmc}=k_{total}$ | 76.8 | 11.5 | 0.5 | 0.9 |
| NaGDC/DPPC | ∞ | 17.5 | 0.7 | 2.2 |
| $k_{mmc}=k_{total}$ | 59.7 | 3.8 | 0.4 | 0.9 |
| NaTC/DPPC | ∞ | 5.9 | 0.8 | 2.1 |
| 20 m*M* BS | $m_{NaGDC}/m_{LC}$<br>1.5:1 | $m_{NaGDC}/m_{LC}$<br>1.5:1 | $m_{NaGDC}/m_{LC}$<br>1.5:1 | $m_{NaGDC}/m_{LC}$<br>1.5:1 |
| $k_{mmc}=k_{total}$ NaGDC/LC | ∞ | 16.5 | 0.5 | 0.6 |
| | Basic drugs $m_{BS}/m_{LC}=1.5:1$<br>Propranolol·HCl | Quinine·HCl | Atenolol | Etilefrine·HCl |
| $k_{smc}$ 20 m*M* NaTC | 1.2 | 0.8 | 0.002 | 0.2 |
| $k_{mmc}=k_{total}$ NaTC/LC | ∞ | 4.3 | 0.2 | 0.3 |

affinity behaviour too. Danazol contains an amino group in the form of an isooxazol.

## 5. Conclusions

The present investigation has shown that the content of phosphocholines has an influence on the partition behaviour of the used drugs. It is shown that basic as well as acidic drugs are influenced in a different degree. Lipophilicity, structure and size have stronger influence on the behaviour than the charge of the molecule.

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## References

[1] R. Neubert, B. Fritsch, G. Dongowski, Pharmazie 48 (1993) 723–728.
[2] S. Muranishi, N. Muranushi, H. Sezaki, Int. J. Pharm. 2 (1979) 101–111.
[3] S. Muranushi, M. Kinugama, Y. Nakajima, S. Muranushi, H. Sezaki, Int. J. Pharm. 4 (1980) 271–279.
[4] M.A. Schwarz, R.H.H. Neubert, H.H. Rüttinger, J. Chromatogr. A 745 (1996) 135–143.
[5] M.A. Schwarz, R.H.H. Neubert, G. Dongowski, Pharm. Res. 13 (1996) 1174–1180.
[6] M.C. Carey, D.M. Small, J. Clin. Invest. 61 (1978) 998–1026.
[7] St. Bader, M. Guarneri, Cosmet. Toileteries 108 (1993) 63–68.
[8] N.A. Mazer, M.C. Carey, R.F. Kwasnick, G.B. Benedek, Biochemistry 18 (1979) 3064–3075.
[9] K. Müller, Hepatology 4 (1984) 134S–137S.
[10] W.I. Higuchi, M. Arakawa, P.H. Lee, S. Noro, J. Coll. Interface Sci. 119 (1987) 30–37.
[11] N.A. Mazer, G.B. Benedek, M.C. Carey, Biochemistry 19 (1980) 601–615.
[12] W.I. Higuchi, M. Arakawa, P.H. Lee, S. Noro, J. Coll. Interface Sci. 119 (1987) 30–37.
[13] G.Y. Somjen, T. Gila, FEBS 156 (1983) 265–268.

[14] J.S. Pedersen, S.U. Egelhaaf, P. Schurtenberger, J. Phys. Chem. 99 (1995) 1299–1305.
[15] S. Egelhaaf, M. Müller, P. Schurtenberger, Prog. Colloid Polym. Sci. 97 (1994) 267–270.
[16] M.A. Schwarz, K. Raith, G. Donkowski, R.H.H. Neubert, in preparation.
[17] L. Naylor, V. Bakatselou, N. Rodriguez-Hornedo, N. Weiner and J. Dressman, personal communication.