Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 553–559

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Effects of bile salts on propranolol distribution into liposomes studied by capillary electrophoresis

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ARTICLE INFO

Article history:
Received 22 March 2011
Received in revised form 31 May 2011
Accepted 24 June 2011
Available online 2 July 2011

Keywords:
Affinity capillary electrophoresis
Bile salt
Distribution coefficient
Frontal analysis
Liposome

ABSTRACT

The objective of this study was to study the effect of four different bile salts, cholate (C), deoxycholate (DC), taurocholate (TC), monoketocholate (MKC), on the membrane binding of a cationic model drug, propranolol, using capillary electrophoresis. The apparent distribution coefficient of propranolol in a buffer/liposome system, in the absence and presence of various concentrations of the bile salts, was measured using capillary electrophoresis frontal analysis. At bile salt concentrations which did not disrupt the liposomes, the bile salts increased the apparent distribution coefficient of propranolol in a concentration-dependent manner, to various extents (DC > C > TC > MKC). The mechanisms for these increases were inferred from studies of ion pairing between bile salts and propranolol using mobility shift affinity capillary electrophoresis and from zeta potential measurements. The bile salts ion-paired with propranolol to different extents as indicated by the estimated complexation constants (K range: 30–58 M^{-1}). This was found to have a minor effect on the membrane distribution of propranolol only. The major effect is proposed to be due to the insertion of bile salt into the liposomal membranes leading to a more negatively charged membrane surface thereby providing stronger electrostatic interactions with the positively charged propranolol.

1. Introduction

Bile salts are endogenous surfactants which have been studied widely as permeability enhancers to increase drug transport across various biological barriers such as buccal mucosa [1], intestine, skin [2], cornea [3] and blood–brain barrier [4]. They increase paracellular permeability at relatively high submicellar concentrations [5], whereas bile salt micelles are cytotoxic solubilizing cell membranes and remove membrane components [6]. At sub-lytic concentrations, bile salt monomers insert into cell membranes, the extent being determined by their lipophilicity [7]. The accumulation of bile salts in cell membranes not only changes the membrane composition but may also alter membrane biophysical properties such as membrane fluidity [8] which may increase passive diffusion of drugs across the membranes.

At physiological pH, bile salts are negatively charged in aqueous solution so that their incorporation into lipid bilayers increases the negative surface charge density. Since the interaction of ionized drugs with membranes may involve electrostatic interactions [9], membrane incorporated bile salts have the potential to affect the partitioning of ionized drugs. Moreover, bile salts are not only able to interact with membranes but can also interact with drug molecules [10,11]. Several studies have shown that bile salts form ion pairs with cationic compounds thereby increasing the apparent lipophilicity of the parent drugs and subsequently increasing drug absorption [12–14].

An anisotropic liposome/buffer system is considered as a more bio-relevant model to predict drug absorption, compared to the isotropic octanol/water system, particularly when electrostatic interactions between ionized drugs and membranes are significant [15]. Various techniques have been developed to determine buffer/liposome distribution coefficients: equilibrium dialysis [16], potentiometry [17], NMR [18] and second-derivative spectrophotometry [19]. CE-FA has been widely explored for the investigation of plasma protein binding and polyelectrolyte complexation and the data have generally been found to be in good agreement with results obtained using other techniques, such as equilibrium dialysis and ultrafiltration [20–22]. More recently, capillary electrophoresis frontal analysis (CE-FA) methods have been developed to study drug liposome interactions and to determine liposome/buffer distribution coefficients [23–25]. The compara-

Abbreviations: ACE, affinity capillary electrophoresis; C, cholate; CE, capillary electrophoresis; DC, deoxycholate; FA, frontal analysis; TC, taurocholate; MKC, monoketocholate.

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doi:10.1016/j.jpba.2011.06.020

tive studies available have demonstrated satisfactory agreement between affinity data obtained using equilibrium dialysis and CE-FA [26] and electrokinetic chromatography and CE-FA [27], respectively. Compared with techniques such as equilibrium dialysis, CE-FA is relatively fast, less labour intensive and requires small amounts of sample [26].

The aim of this study was to investigate the effects of four bile salts (Fig. 1(a)), cholate (C), deoxycholate (DC), taurocholate (TC), monoketocholate (MKC), which have been characterized as permeability enhancers using a 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Fig. 1(c)) monolayer model [28], on the distribution of propranolol (Fig. 1(b)) (pK_a 9.5), a cationic model drug at physiological pH, into lipid bilayers. The same lipid as used in the monolayer studies, DPPC [28], was employed in the preparation of the unilamellar liposomes subject to investigation. A CE-FA method, suitable for determining the apparent liposome/buffer distribution coefficient of propranolol in the absence and presence of bile salts, was developed. The interactions between the bile salts, at submicellar concentrations, and propranolol were characterized by mobility shift affinity capillary electrophoresis (ACE) [29–33]. The results of this study should help in understanding the effects of bile salts on the distribution of cationic drugs into biological membranes and, consequently, the effect of bile salts on the passive diffusion of these drugs across biological barriers via the transcellular pathway.

2. Materials and methods

2.1. Materials

DPPC, HEPES, propranolol hydrochloride, Ringer's buffer (10 mM D-glucose; 0.5 mM $MgCl_2$; 0.45 mM KCl; 120 mM NaCl; 0.70 mM Na_2HPO_4; 1.5 mM NaH_2PO_4), C, DC and TC were purchased from Sigma–Aldrich (St. Louis, MO, USA). MKC, purity 96.5% with 3.1% cholate as the major impurity determined by HPLC and MS, was a gift from Professor Ksenija Kuhajda (University of Novi Sad, Serbia). All other chemicals and reagents were of at least analytical grade. Purified water prepared from a Milli-Q deionization unit (Millipore, Bedford, MA, USA) was used throughout.

2.2. Methods

2.2.1. Preparation and characterization of liposomes

Unilamellar liposomes were prepared in a round-bottomed flask by dissolving 20 mg DPPC in chloroform. The organic solvent was removed by rotary evaporation and dried overnight under vacuum. The lipid film was hydrated with 5.0 mL Ringer's–HEPES buffer (Ringer's buffer containing 10 mM HEPES, pH 7.4) for 1 h at 55 °C. Upon hydration, the dispersion was left at room temperature to settle for 1 h. In order to obtain unilamellar liposomes, extrusion of the liposomal dispersion was performed 10 times through two stacked polycarbonate filters (Whatman International, UK) with pore size of 100 and 50 nm at 55 °C using an extruder (LipexBiomembranes, Vancouver, Canada) under nitrogen pressure. The concentration of DPPC in the liposome preparation was determined using the Stewart assay [34]. The liposome preparations were stored at 4 °C until use.

2.2.2. Determination of particle size and zeta potential

Zetapotential (ζ-potential) values and the size distribution of the DPPC liposomes were determined in Ringer's–HEPES buffer at pH 7.4 and 25.0 °C by dynamic light scattering (DLS) analysis using a Zetasizer ZS90 (Malvern Instruments Ltd., Malvern, UK). The lipid concentration was kept constant at 2 mM and the bile salt concentration was in the range 0–3 mM. Viscosity and refractive index of the dispersion medium (water) were taken as 1.02 cP and 1.330, respectively.

2.2.3. CE-FA experiments

CE-FA was performed on a HP ^{3D}CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector (DAD). Uncoated fused silica capillaries, 50 μm id and length of 32.5 cm, with a length of 24.5 cm to the detector, were used in all experiments (Polymicro Technologies, Phoenix, AZ, USA). New capillaries were conditioned by flushing sequentially with 1 M NaOH, 0.1 M NaOH and Ringer's–HEPES buffer for 30 min each. The capillary was flushed daily with 1% SDS solution, 1 M NaOH and Ringer's–HEPES buffer for 5 min each before conducting experiments. Between runs, the capillary was flushed with 0.1 M NaOH and Ringer's–HEPES buffer for 2 min each. UV detection was performed at 214 nm. The applied voltage was +5 kV (~50 μA) and samples were introduced by hydrodynamic injection (50 mbar for 20 s) unless otherwise reported. The temperature of the capillary cassette was set to 25 °C. Ringer's–HEPES buffer was used for all CE experiments and sample preparation. All samples were mixtures of 100 μM propranolol, bile salts at various concentrations and 2 mM DPPC liposomes. Standard samples were mixtures of 100 μM propranolol and bile salts without DPPC liposomes. All samples and standards were analyzed in triplicate. Standard samples were analyzed immediately before liposome-containing samples with the same total drug concentration.

2.2.4. Calculation of distribution coefficient from CE-FA experiments

The propranolol concentration in the aqueous phase (C_{aq}) was calculated from the total drug concentration (C_{total}) and the plateau peak heights measured by CE-FA for the drug substance in the liposome-containing sample (H_{sample}) and in the standard solution (H_{std}) containing the same total drug concentration but without liposomes:

$$C_{aq} = \frac{H_{sample}}{H_{std}} C_{total} \quad (1)$$

The apparent membrane distribution coefficient was defined as:

$$D_{mem} = \frac{C_{mem}}{C_{aq}} \quad (2)$$

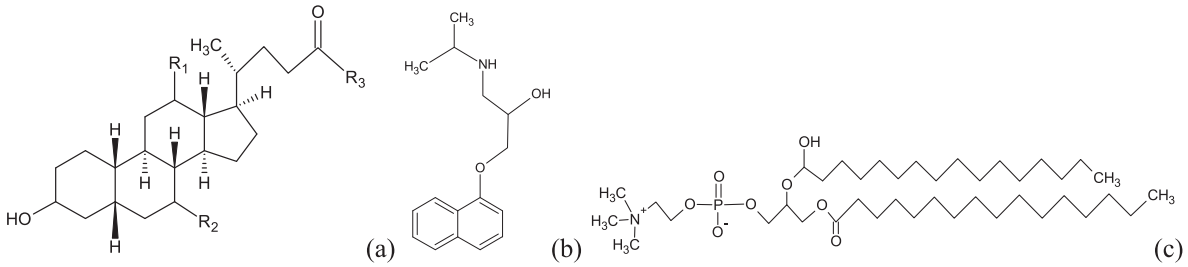
where C_{mem} is the concentration of drug in the membrane (liposomal) phase. The drug concentration in the membrane phase was calculated by mass balance:

$$C_{mem} = \frac{C_{total}V_{total} - C_{aq}V_{aq}}{V_{mem}} \quad (3)$$

where V_{total}, V_{aq} and V_{mem} are the total sample volume, volume of the aqueous phase and volume of the membrane phase, respectively. V_{mem} was calculated from the lipid concentrations determined using the Stewart assay assuming the density of the lipid membrane phase to be 1.00 g/mL [35,36]. The volume of the aqueous phase, V_{aq}, was calculated from the relationship:

$$V_{total} = V_{aq} + V_{mem} \quad (4)$$

Note that V_{mem} was defined to include only the volume of the phospholipid and, thus, does not take into account the increase in volume due to incorporation of the bile salts into the liposomes. Experiments were conducted in triplicate. Mean and variance of a ratio of peak heights of liposome-containing sample (H_{sample}) and the standard solution (H_{std}) were calculated according to Taylor expansions [37].



	Cholate	Deoxycholate	Monoketocholate	Taurocholate
R1	- OH	- OH	= O	- OH
R2	- OH		- OH	- OH
R3	- OH	- OH	- OH	- $NH(CH_2)_2SO_3H$

Fig. 1. Chemical structures of bile salts (a), propranolol (b) and DPPC (c).

2.2.5. Mobility shift affinity capillary electrophoresis

The instrumentation for the mobility shift ACE assay was identical to that used in the CE-FA study. Separation buffers consisted of Ringer's–HEPES buffer (pH 7.4) containing various concentrations of the bile salts. Sample solutions were mixtures of 50 μM propranolol and 0.05% (v/v) DMSO (electroosmotic flow (EOF) marker) in Ringer's–HEPES buffer. The samples were introduced into the capillary by applying a pressure of 50 mbar for 2 s. Measurements were performed in triplicate.

The relative viscosity of each bile salt solution (relative to Ringer's–HEPES buffer) was determined by measuring the time for a 0.1% v/v DMSO sample plug to reach the detector window in a capillary filled with the bile salt solution upon application of pressure (20 psi), using a Beckman PACE 5010 CE instrument (Fullerton, CA) with a 97(90) cm × 50 μm id uncoated fused silica capillary (Polymicro Technologies) at 25 °C. The relative viscosity (η_1/η_2) is given by:

$$\frac{\eta_1}{\eta_2} = \frac{t_1}{t_2} \tag{5}$$

where η_1 and η_2 are the viscosities and t_1 and t_2 are the peak appearance times for the solutions 1 and 2, respectively. The samples were run in triplicate.

For a charged spherical molecule, the effective electrophoretic mobility, μ, is determined by the charge-to-size ratio and the viscosity of the electrophoresis medium according to Eq. (6):

$$\mu = \frac{q_{eff}}{6\pi\eta r} \tag{6}$$

where q_{eff} and r are the effective charge and the radius of the analyte, respectively, and η is the viscosity of the electrophoresis buffer. The effective electrophoretic mobility was calculated from:

$$\mu = \frac{l_c l_d}{U}\left(\frac{1}{t} - \frac{1}{t_0}\right) \tag{7}$$

where l_c is the total length of the capillary, l_d is the length of the capillary from inlet to the detector, U is the applied voltage, t and t_0 are the peak appearance times of the model compound and EOF, respectively.

The effective electrophoretic mobility, μ, of propranolol in the buffer solutions containing bile salt is the weighted average of the mobilities of the compound in the free and complexed form:

$$\mu = \frac{[D]}{[D]+[DB]}\mu_f + \frac{[DB]}{[D]+[DB]}\mu_c \tag{8}$$

where μ_f and μ_c are the electrophoretic mobilities of free compound and compound/bile salt complex, respectively, and [D] and [DB] are the concentrations of free compound and complex, respectively.

To calculate the binding constant between propranolol and bile salt, 1:1 stoichiometry and an electrophoretic mobility of the complex, μ_c, equal to zero were assumed. The complexation constant K is given by:

$$K = \frac{[DB]}{[D]\cdot[B]} \tag{9}$$

After substituting Eq. (9) into Eq. (8) and rearranging, μ can be expressed as

$$\mu = \frac{\mu_f + \mu_c K[B]}{1 + K[B]} \tag{10}$$

where [B] is the concentration of bile salt. The concentrations of the bile salts were below their CMCs in order to avoid the presence of micelles. The assumption $\mu_c = 0$ enabled Eq. (10) to be simplified to

$$\mu = \frac{\mu_f}{1 + K[B]} \tag{11}$$

The K and μ_f were determined by nonlinear regression using GraphPad Prism 5.0 (Graphpad software, Inc., La Jolla, CA).

3. Results

3.1. Particle size and zeta potentials

Particle size of the DPPC liposomes was determined to ensure the integrity of the liposome membrane after incubation with the bile salts. The Z-average size of DPPC liposomes in the absence of bile salts was around 76 nm. Exposure to bile salts (concentration ranges investigated: 1–3 mM for C, MKC and TC and 0.2–0.6 mM for DC) produced significant increases in the Z-average size and the polydispersity index of the liposomes relative to the liposomes in the absence of bile salt (Fig. 2), presumably due to insertion of bile salts into the lipid bilayers. Liposome particle size decreased at higher concentrations of DC (>0.2 mM), C and TC (>1 mM), but additional peaks due to micelles or mixed micelles were not observed in the particle size distribution measurements (DLS).

Incorporation of anionic bile salts into DPPC liposomes decreased the zeta potential (Fig. 3). In the absence of bile salts, the liposomes were slightly positively charged with a zeta potential of around 2.3 mV. After addition of bile salts, the zeta potential decreased to different extents depending on the structure and concentration of the bile salt.

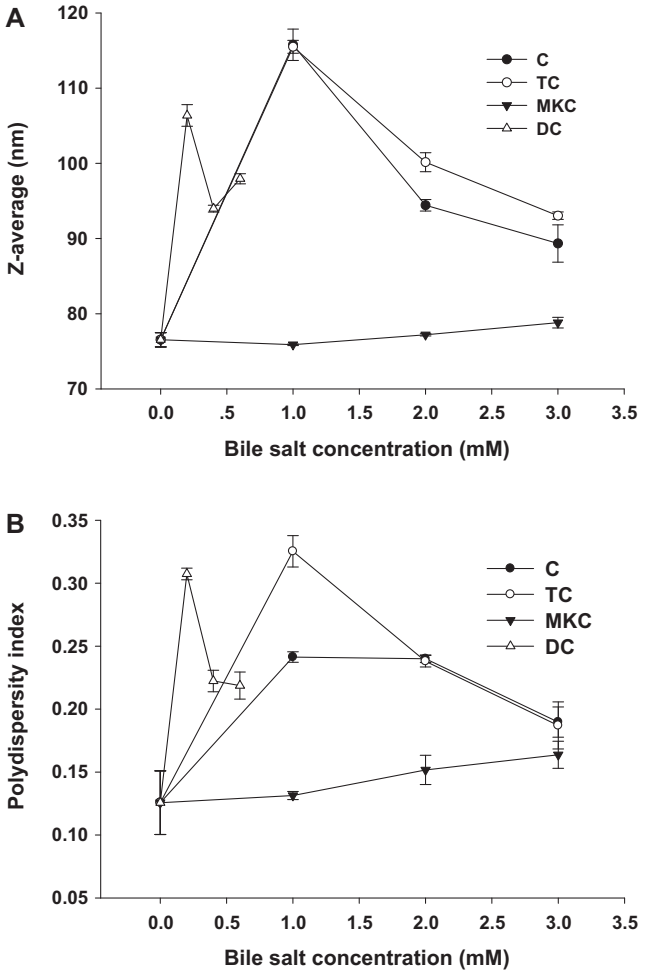


Fig. 2. Effect of bile salts on (A) Z-average size and (B) polydispersity index of DPPC liposomes (2 mM lipid) in Ringer's–HEPES buffer at pH 7.4 and 25 °C. Data are means ± SD (n = 3).

3.2. Ion pair formation between bile salts and propranolol

The apparent complexation constants between bile salts and propranolol were determined using mobility shift ACE. This involved addition of bile salt to the CE running buffer and measuring the changes in electrophoretic mobility of propranolol.

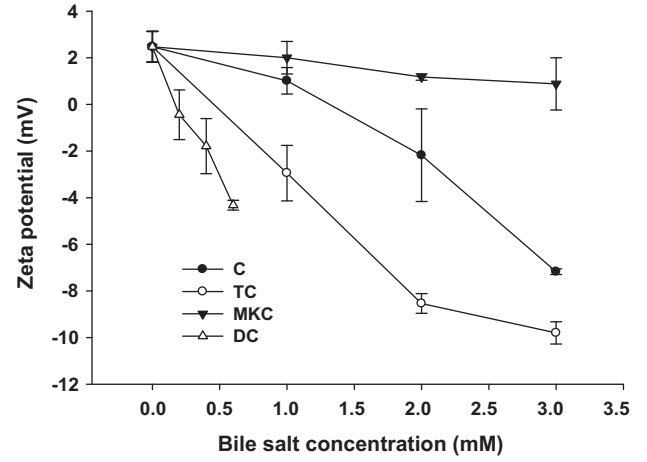


Fig. 3. Effect of bile salts on the zeta potential of DPPC liposomes (2 mM lipid) in Ringer's–HEPES buffer at pH 7.4 and 25 °C. Data are means ± SD (n = 3).

Table 1
MC values of bile salts [28] and complexation constants (K) between bile salts and propranolol in Ringer's–HEPES buffer at pH 7.4 and 25 °C. Data are means ± SD (n = 3).

	C	DC	MKC	TC
CMC (mM)	4.1	1.7	13.4	3.6
K (M^{-1})	41 ± 2.2	58 ± 7.5	30 ± 1.7	37 ± 5.4

The addition of complexation agents (bile salts in the present case) to the CE running buffer may induce changes in the analyte electrophoretic mobility which are not due to the specific analyte–ligand interaction but rather due to a medium effect. That is, that the complexation agent/additive added causes changes, for instance in the viscosity, pH and/or ionic strength of the back ground electrolyte [38–40]. Therefore, prior to determining the complexation constants, the effect of high concentrations of bile salts on viscosity, pH and ionic strength of the running buffer was investigated. ANOVA showed there were no significant (P > 0.05) differences in migration time of EOF between the running buffer with and without bile salts. There were also no differences in the pH of the running buffers. There were small increases in ionic strength (3 mM for C, MKC and TC containing buffers and 0.6 mM for DC containing buffer) upon addition of bile salts. The ionic strength of the Ringer's–HEPES buffer was calculated to 0.129 M. These changes are not expected to significantly affect the electrophoretic mobilities of propranolol. Fig. 6 shows the effective electrophoretic mobility of propranolol as a function of bile salt concentration. The presence of the four bile salts led to a decrease in the effective electrophoretic mobility of propranolol, indicating an interaction (ion-pairing) between propranolol and the bile salts (Fig. 6). One-to-one complexation constants were calculated from Eq. (11) and are listed in Table 1.

3.3. Effect of bile salts on membrane/buffer distribution coefficient of propranolol

As a part of the development of the CE-FA method various sample injection times were investigated (5, 10, 20, 30 and 60 s), for both standard and sample solutions, to make sure that frontal analysis conditions were achieved. That is, attaining electropherograms characterized by plateau peaks, and peak heights, which are independent of the injection time [41]. An injection time of 20 s was found suitable and used throughout. A linear relationship (r^2 > 0.999) between the plateau peak heights and the propranolol concentration was observed in the concentration range 20–1000 μM. For propranolol standards and liposome containing samples, the RSD on the peak heights was below 3.5% (n = 3). Fig. 4 shows representative electropherograms for a propranolol standard solution and a liposome containing sample. It is apparent that the presence of liposomes led to decreases in the peak height which is due to propranolol distributing into the liposomal membrane. The apparent membrane/buffer distribution coefficient of propranolol was determined in the absence and presence of bile salts at various concentrations using CE-FA. All bile salts significantly increased the apparent distribution coefficients of propranolol (Fig. 5) in a concentration-dependent manner. DC had the most predominant effect, followed by C and TC, whereas the semi-synthetic bile salt, MKC, showed least effect.

4. Discussion

4.1. Effect of bile salts on liposome characteristics

DPPC is a zwitterionic phospholipid thereby the zeta potential of DPPC liposomes should be around zero, theoretically. However, in this study the zeta potential of the DPPC liposomes, in the absence

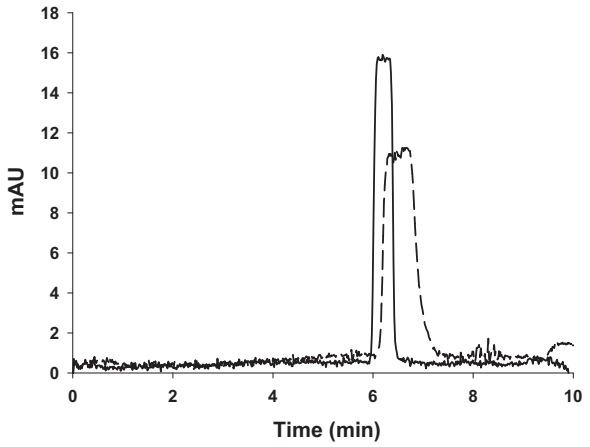


Fig. 4. Electropherograms of propranolol (100 μM) standard (solid line) and liposome containing sample (2 mM DPPC, dashed line) in Ringer's–HEPES buffer at pH 7.4 and 25 °C.

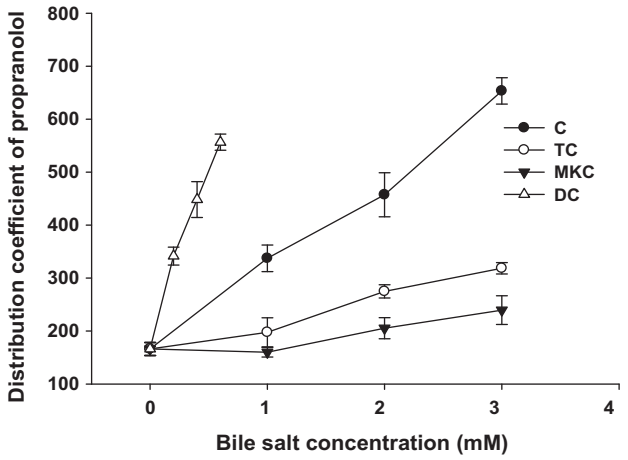


Fig. 5. Effect of bile salt concentration on the apparent distribution coefficient of propranolol in DPPC liposome (2 mM lipid)/buffer system (Ringer's–HEPES buffer) at pH 7.4. Data are means ± SD (n = 3).

of bile salts, was found to be slightly positive. This is not unusual because factors such as pH, ionic strength and the presence of counter ions could influence the zeta potential. Other studies have also determined slightly positive zeta potentials for DPPC liposomes [42,43]. The zeta potential measurements suggested that the addition of bile salts changed the membrane surface charge of the DPPC liposomes from slightly positive to negative. DC had the greatest effect on membrane surface charge, followed by TC, C and MKC (Fig. 3). The negative surface charge would provide an attractive electrostatic force for propranolol, leading to stronger membrane interactions. The changes in membrane surface charge induced by the bile salts would depend on the concentration of bile salt in the membrane and their ionization state which is a function of the bile salt ionization constant in the lipid membrane. The pK_a of the common C24 unconjugated bile salts, DC, C and MKC, in water as measured by potentiometric titration is ~4.8–5.0 [44]; conjugation of the bile acids with taurine lowers the pK_a by about 5 units [45]. Although the pK_a values of bile salts are about 2 units higher when they are located in phospholipid membranes [46], the rank order of the pK_a values would not be affected. The changes in liposome zeta potential induced by the bile salts are in accordance with the penetration of the bile salts into DPPC monolayers (DC > TC > C > MKC) as well as their CMC values (DC < TC < C < MKC) previously measured [28], but, interestingly, not aligned with the bile salt octanol/water distribution coefficients (rank order: DC > C > MKC > TC) [28]. Altogether, this points to the importance of bile salt amphiphilicity for membrane interactions, in addition to hydrophobicity as indicated by the octanol/water distribution coefficients.

The addition of bile salts to the DPPC liposome containing solutions also led to an increase in liposomal size (Fig. 2). The relative decreases in particle size at higher concentrations may be due to changes in the liposome shape. As the liposome particle size increased in the presence of bile salts at all concentrations relative to the size in the absence of bile salt, it was assumed that the bile salts did not solubilize the liposomal membranes at the investigated concentrations. The changes in liposome z-average were related to the effect of the bile salts on the zeta potential. DC and MKC which had the largest and smallest effect on the zeta potential also had the largest and smallest effect on the liposomal size, respectively. The distribution of the bile salts into the DPPC liposomes may, in addition to the potential electrostatic interactions, affect the distribution of propranolol by simply providing a larger distribution phase. It should be noted that the DPPC liposomes (phase transition temperature around 42 °C) was in the gel phase whereas cell membranes are in a liquid crystalline phase at room temperature. It has been shown that membranes in the gel phase are more resistant to interaction with bile salt than a membrane in a liquid crystalline phase [47,48]. Thereby, it may be expected that bile salts would show more significant effect on the propranolol binding to cell membranes.

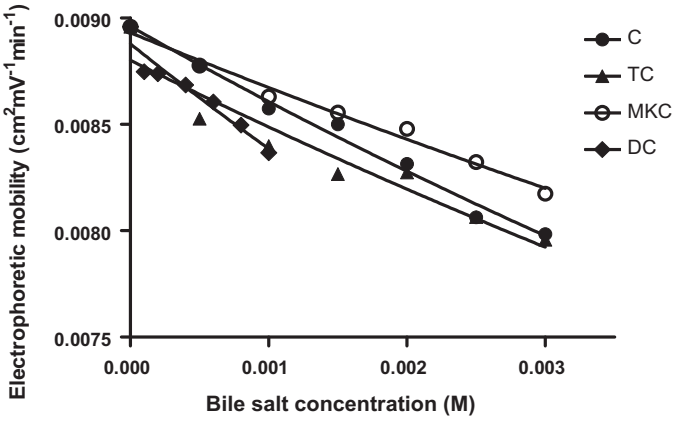


Fig. 6. Electrophoretic mobility of propranolol in the presence of bile salts in Ringer's–HEPES buffer at 25 °C.

4.2. Ion pair formation between bile salts and propranolol

The mobility shift ACE experiments revealed that the bile salts interacted with propranolol in the Ringer's–HEPES buffer. In order not to exceed the CMC values (Table 1), the highest concentration of bile salt added to the buffer was 3 mM and 0.6 mM for C, TC, and MKC and DC, respectively. In the analysis of the mobility shift data, it was assumed that only 1:1 bile salt–propranolol complexes were formed. In previous studies, bile salt–propranolol complexation stoichiometries higher than 1:1 have been reported. It has to be noted that most of bile salt concentrations in the investigated concentration range was above the bile salt CMCs [49,50]. Under such conditions, it may not be surprising that the stoichiometry is higher than 1:1 because propranolol is distributed into the bile salt micelles. However, this does not necessarily imply that the stoichiometry is larger than 1:1 at bile salt concentrations below the CMC even though bile salts may form small aggregates (dimers or trimers) stepwise even at the concentrations below their CMC. For taurodeoxycholate–tetrabutylammonium (K = 50 M^{-1}) and taurodeoxycholate–isopropamide (K = 12 M^{-1}) complexation

data were consistent with 1:1 complexation at bile salt concentrations below the CMC of taurodeoxycholate [51]. Bile salts also formed 1:1 ion pairs with long-chain alkyltrimethylammonium ions with complexation constants in the range 13–1740 M^{-1} as determined by conductometry in 0.1 mole fraction ethanol–water [52]. We suggest that the 1:1 binding model which is the simplest model is also likely to be the predominant form in the investigated system. In order to determine complexation constants with high accuracy it is important to cover a large part of the binding isotherm [53–55]. In the current investigation, this was not possible due to the limited bile salt concentration range. Consequently, it has to be emphasized that the complexation constants listed in Table 1 should be considered estimates only. The magnitudes of the complexation constants are in line with previous results [51]. Overall, mobility shift ACE showed that propranolol–bile salt ion-pairing occurs in the aqueous solution. Furthermore, Table 1 indicates that ion pair formation between propranolol and bile salts differed with DC having the highest affinity, followed by C, TC and MKC. Electrostatic attraction between bile salts and cationic compounds is a major driving force in the formation of ion pairs. Other factors including hydrophobic interaction [56], hydrogen binding [57] and steric hindrance may, however, affect the degree of ion-pairing. At a propranolol concentration of 50 μM and bile salt concentrations of 3 mM, it can be estimated that approximately 12% of the propranolol is complexed with C, MKC and TC (~3% propranolol on complexed form in the presence of 0.6 mM DC). Due to the limited concentration range covered in the mobility shift ACE experiments and the associated uncertainty of the estimated complexation constants, attempts to discern possible structure activity relationships were not made.

4.3. Effect of bile salts on membrane/buffer distribution coefficient of propranolol

A CE-FA method suitable for investigating the interaction between propranolol and DPPC liposomes was developed. The presence of an additional species (bile salt) in the pre-incubation sample represents a new development as compared to previous liposome CE-FA investigations [23–26]. Also, the previous studies [23–26] have mostly applied highly negatively charged liposome compositions in order to minimize capillary wall adsorption. However, the presence of the additional bile salt species did not affect the shape of the attained propranolol plateau peaks (Fig. 4). DPPC lipid most certainly adsorbs onto the capillary wall. Adsorption of lipid could not be detected in the electropherograms, most likely because propranolol migrates out of the liposome zone and reaches the detection window prior to the liposome. Indirect evidence of lipid adsorption was, however, observed. Attempts to extend the CE-FA method to encompass the negatively charged analyte piroxicam instead of the positively charged propranolol were not successful. This may be due to interactions between the analyte and lipid adsorbed onto the capillary wall. The CE-FA studies showed that addition of bile salts significantly enhanced the fraction of propranolol interacting with the liposomal phase. The increase in the apparent membrane/buffer distribution coefficient of the cationic propranolol induced by the bile salts was in the rank order DC > C > TC > MKC (Fig. 5).

It is suggested that the higher apparent distribution coefficients may primarily be due to electrostatic interactions increasing the distribution of propranolol into the membranes. The increase in the volume of the liposomal phase associated with the addition of bile salts and their distribution into the lipidic phase may also be a contributing factor. The calculated fractions of bile salt complexed propranolol (Section 4.2) are small and will be even smaller in the presence of the liposomal phase. Addition of MKC had a limited effect on the apparent distribution of propranolol although the propranolol–MKC complex formed to almost similar extents as for the other propranolol–bile salts ion pairs. Based on this observation, it may be suggested that the formation of propranolol–bile salt ion pairs is likely to have a relatively limited influence on the apparent membrane/buffer distribution of propranolol. However, previous studies have suggested that ion pair formation between propranolol and the bile salt taurodeoxycholate caused higher propranolol absorption *in vivo* [58]. Also, it has been reported that the structure of the bile salts influences the extraction of the quaternary ammonium *N*,*N*-dimethyl derivative of propranolol into octanol (ion-pairing occurring mainly in the organic phase) [59]. Thus, possible effects due to ion-pairing cannot be ruled out. In addition to these electrostatic interactions, membrane binding may be influenced by changes in membrane fluidity and phospholipid chain disorder caused by bile salts [60], as it has been reported that membranes with high fluidity provide a more flexible structure for drug binding [61].

5. Conclusions

A CE-FA method was successfully developed for the investigation of propranolol liposome/buffer distribution in DPPC liposomes. Incorporation of an additional species, the bile salts, into the frontal analysis methodology was successfully accomplished. Also, testifying to the versatility of affinity capillary electrophoresis, the formation of propranolol–bile salt ion pairs was detected and complexation constants estimated. Bile salts predominantly enhanced the distribution of propranolol into DPPC lipid membranes by increasing the negative surface charge, as inferred from zeta-potential measurements. The effect of adding bile salts is influenced by the lipophilicity of the bile salts, however, other effects including amphiphilicity and steric hindrance, are involved. The formation of propranolol–bile salt ion pairs in the aqueous phase is likely to have a comparatively smaller effect on the membrane distribution of propranolol. This study suggests that bile salts may facilitate the distribution of cationic compounds into cell membranes via electrostatic interactions.

Acknowledgement

Authors thank the Drug Research Academy, Faculty of Pharmaceutical Sciences, University of Copenhagen for providing financial assistance to support Lin Yang's visit.

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