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# Mixed micelles of 7,12-dioxolithocholic acid and selected hydrophobic bile acids: Interaction parameter, partition coefficient of nitrazepam and mixed micelles haemolytic potential

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ABSTRACT

The formation of mixed micelles built of 7,12-dioxolithocholic and the following hydrophobic bile acids was examined by conductometric method: cholic (C), deoxycholic (D), chenodeoxycholic (CD), 12-oxolithocholic (12-oxoL), 7-oxolithocholic (7-oxoL), ursodeoxycholic (UD) and hiodeoxycholic (HD). Interaction parameter ($\beta$) in the studied binary mixed micelles had negative value, suggesting synergism between micelle building units. Based on $\beta$ value, the hydrophobic bile acids formed two groups: group I (C, D and CD) and group II (12-oxoL, 7-oxoL, UD and HD). Bile acids from group II had more negative $\beta$ values than bile acids from group I. Also, bile acids from group II formed intermolecular hydrogen bonds in aggregates with both smaller (2) and higher (4) aggregation numbers, according to the analysis of their stereochemical (conformational) structures and possible structures of mixed micelles built of these bile acids and 7,12-dioxolithocholic acid.

Haemolytic potential and partition coefficient of nitrazepam were higher in mixed micelles built of the more hydrophobic bile acids (C, D, CD) and 7,12-dioxolithocholic acid than in micelles built only of 7,12-dioxolithocholic acid. On the other hand, these mixed micelles still had lower values of haemolytic potential than micelles built of C, D or CD. The mixed micelles that included bile acids: 12-oxoL, 7-oxoL, UD or HD did not significantly differ from the micelles of 7,12-dioxolithocholic acid, observing the values of their haemolytic potential.



## 1. Introduction

Bile acids are steroid amphiphilic molecules, which form micelles (aggregates) above the critical micellar concentration [1–5]. The formation of micelles determines the physiological role of bile acids in digestion (absorption) of lipids [5,6]. Micelles of bile acids are used in the pharmaceutical formulations (alone or in combination with lecithin) where they form mixed micelles with poorly soluble molecules (drugs) [7,8]. It is known that bile acid micelles interact with phospholipids in cell membranes altering the permeability of membrane [9,10]. Certain bile acids form hydrogen bonded aggregates with some drugs (lidocain, verapamil), which may lead to changes in drug bioavailability [11,12]. Ursocholic and chenodeoxycholic acids form mixed micelles with cholesterol and are applied in dissolution of cholesterol gallstone [6,13].

The more hydrophobic bile acid is, the lower critical micellar concentration it has and accordingly tends more to form micellar aggregates [1,5,14–16]. This means that the micellar interior is more hydrophobic and accepts guest molecule more efficiently [8]. However, membranolytic potential of bile acids arises together with the increase of their hydrophobicity [9,17,18]. Substitution of steroid OH groups with oxo groups in bile acids decreases their hydrophobicity (increasing at the same time their cmc) and membranolytic potential (toxicity) of bile acids, as well as the tendency to accept hydrophobic guest molecule [14–16,18]. Apart from the chemical modification, the properties of surface-active agents can be modified by means of forming their binary mixtures (mixed micelles), using surfactants with different individual aggregation abilities (cmc values) [19–25].

Mixed micelles built of bile acids and fatty acids, cholesterol or various phospholipids are extensively studied, as well as mixed micelles built of different OH derivatives of bile acids. However, according to the authors' knowledge, mixed micelles consisting of different bile acids' oxo derivatives or bile acids' oxo and OH derivatives have not been described in literature.

In this study, mixed micelles consisting of 7,12-dioxolithocholic acid (cmc = 95 mM, present in each mixed micelle) and more hydrophobic bile acids (oxo and hydroxyl derivatives) (Fig. 1), in their binary mixtures, are examined. Namely, 7,12-dioxolithocholic acid is not membranotoxic [18], but the inner side of its micelle is

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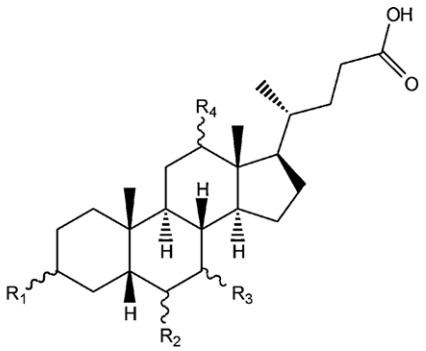


| | |
|---|---|
| $R_1 = OH; R_3 = R_4 = =O; R_2 = H$ | 3α-hydroxy-7,12-dioxo-5β-cholanoic acid (7,12-dioxolithocholic acid); **7,12-dioxoL**; **(1)** |
| $R_1 = R_3 = R_4 = OH; R_2 = H$ | 3α,7α,12α-trihydroxy-5β-cholanoic acid (cholic acid); **C**; **(2)** |
| $R_1 = R_4 = OH; R_2 = R_3 = H$ | 3α,12α-dihydroxy-5β-cholanoic acid (deoxycholic acid); **D**; **(3)** |
| $R_1 = R_3 = OH; R_2 = R_4 = H$ | 3α,7α-dihydroxy-5β-cholanoic acid (chenodeoxycholic acid); **CD**; **(4)** |
| $R_1 = OH; R_4 = =O; R_2 = R_3 = H$ | 3α-hydroxy-12-oxo-5β-cholanoic acid (12-oxolithocholic acid); **12-oxoL**; **(5)** |
| $R_1 = OH; R_3 = =O; R_2 = R_4 = H$ | 3α-hydroxy-7-oxo-5β-cholanoic acid (7-oxolithocholic acid); **7-oxoL**; **(6)** |
| $R_1 = R_3 = OH; R_2 = R_4 = H$ | 3α,7β-dihydroxy-5β-cholanoic acid (ursodeoxycholic acid); **UD**; **(7)** |
| $R_1 = R_2 = OH; R_3 = R_4 = H$ | 3α,6α-dihydroxy-5β-cholanoic acid (hiodeoxycholic acid); **HD**; **(8)** |

**Fig. 1.** Structures of tested bile acids.

poorly hydrophobic. Therefore, one of the objectives of this study is the derivation of mixed micelles whose haemolytic potential would remain low whereas the hydrophobicity of their interior and the availability to accept hydrophobic guest would increase. The physicochemical parameters (Table 1) of the mixed micelles are determined and mutual interactions of bile acids in their binary systems (mixed micelles) are analyzed. Furthermore, the ability of the mixed micelles to accept nitrazepam (model for hydrophobic guest-drug) is evaluated, as well as their haemolytic potential.

Comparison of stability between the mixed micelles built of different oxo derivatives and the mixed micelles built of oxo and OH derivatives, based on the values of their interaction parameter ($\beta$) (Table 1), is innovative in the area of mixed micelles. In this study it is questioned, whether mixed micelles which have greater hydrophobic interactions between the building units are more stable in comparison to mixed micelles in which hydrophobic interactions are smaller, but the presence of hydrogen bond is possible.

It is well known that micelles of cholic and deoxycholic acids, as well as mixed micelles of these bile acids with lecithin, show the affinity towards nitrazepam (probe molecule) [8]. The analysis of whether the stability of mixed micelle influences the partition of nitrazepam into mixed micelle and whether more stable mixed micelles build more stable systems with nitrazepam has not been previously reported, according to the authors' knowledge.

## 2. Materials and methods

### 2.1. Materials

The synthesis of 7-oxolithocholic acid was realized by regioselective oxidation of the C7 OH group of chenodeoxycholic acid, following the procedure of Tullar [30], while 12-oxolithocholic acid was synthesized according to Miljković et al. [31]. The starting compound for the synthesis of 7,12-dioxocholic acid was cholic acid from which 12-oxodeoxycholic acid was derived [31], followed by the oxidation of C7 OH group [31]. The bile acids cholic, deoxycholic, chenodeoxycholic, ursodeoxycholic and lithodeoxycholic were purchased from Sigma, New Zealand (98%). Each analyzed bile acid was previously two times recrystallized from methanol. The analyzed bile acids were transformed to sodium salts according to the procedure of Roda et al. [1]. In the experiments were used: methanol (Sigma, HPLC grade), $KH_2PO_4$ (Lachner, analytical reagent grade), $Na_2PO_4$ (Lachner, analytical reagent grade), nitrazepam (Sigma, 99.9%), defibrinated rat blood and double distilled water.

**Table 1**
Physical–chemical parameters of mixed micelles.

| Physical–chemical parameters | | Equations |
|---|---|---|
| The ideal critical micellar concentration for the mixed micelle according to Clint [26] | $cmc^{id}$ | $\frac{1}{cmc^{id}} = \sum_i \frac{\alpha_i}{cmc_i}$, (1) |
| | | $\alpha_i$ and $cmc_i$ molar ratio in the solution and cmc component $i$ |
| Molar ratio of the more hydrophobic bile acids in the real mixed micelle according to Rubinghu [27] | $x_1$ | $\frac{x_1^2 \ln(cmc^{ex}\alpha/cmc_1 x_1)}{(1-x_1)^2 \ln[cmc^{ex}(1-\alpha)/cmc_2(1-x_1)]} = 1$, (2) |
| | | $cmc^{ex}$ experimentally determined cmc in the mixed micelle, $cmc_1$ = cmc for more hydrophobic bile acids, $cmc_2$ = cmc for 7,12-dioxolithocholic acid |
| Molar ratio of the more hydrophobic bile acids in the real mixed micelle according to Motomura [28] | $x^{id}$ | $x^{id} = \frac{cmc_2\alpha}{cmc_2\alpha + cmc_1(1-\alpha)}$ (3) |
| The interaction parametar according to Rubinghu [27] | $\beta$ | $\beta = \frac{\ln(cmc^{ex}\alpha/cmc_1 x_1)}{(1-x_1)^2}$ (4) |
| Activity coefficient of the more hydrophobic bile acid in the mixed micelle [29] | $f_1$ | $f_1 = \exp[\beta(1-x_1)^2]$ (5) |
| Activity coefficient of 7,12-dioxolithochic acid in the mixed micelle [29] | $f_2$ | $f_2 = \exp[\beta x_1^2]$ (6) |
| Free energy of mixed micelle formation | $\Delta G_{ex}$ | $\Delta G_{ex} = RT[x_1 \ln f_1 + (1-x_1)\ln f_2]$ (7) |