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# Dimerization: First Step for Micelle Preorganization of Bile Salts

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**Abstract.** The dimerization constants of sodium cholate, sodium deoxycholate and sodium chenodeoxycholate have been determined in dilute alkaline aqueous solution. The high stabilities of the dimers cannot be explained by single hydrogen bonds but multiple interactions must be involved simultaneously.

**Key words.** Bile acids, dimerization, oligomerization, natural detergent.

## 1. Introduction

Bile salts are natural detergents having some peculiar properties. Their configuration, based on a steroid nucleus (5$\beta$-cholan-24-oic acid), developed in the course of evolution. The sodium salts of the 3$\alpha$,7$\alpha$,12$\alpha$-trihydroxy (cholate, $C^-$), 3$\alpha$,12$\alpha$-dihydroxy (deoxycholate, $DC^-$) and the 3$\alpha$,7$\alpha$-dihydroxy (chenodeoxycholate, $CDC^-$) derivatives (see Figure 1) are water soluble, but the organization of the surface active molecular aggregates depend on several factors (concentration, temperature, pH, ionic strength, kind of host, etc.) [1–4].

Although the equilibrium character of micelle formation, viz.:

$$\text{monomer} \rightleftharpoons \text{oligomer} \rightleftharpoons \text{polymer}$$

is generally accepted, few data can be found about the formation constants. First of all dimerization is emphasized, or the dimers are sometimes mentioned as the most important or single species in the concentration range before c.m.c. This concept is mentioned several times [5–9] but only two dimerization constants can be found: 18($\pm$1) for NaC and 13($\pm$1) for NaDC [7]. (The first one was subsequently corrected to 15($\pm$1) [10].) The values seem too low and their ratio is rather strange. It can be assumed that the small changes in pH due to the dilution of NaC and NaDC solutions and also the extrapolation of the measured data are reflected in these results.

More recently Bottari *et al.* have reported many data, among them a log $K$ value of 1.51 for the formation of $Na_2(DC)_2$ measured in 0.5 M $Bu_4NCl$ at 25°C [11]. The log $K$ of the $Na_2(DC)_2$ species was calculated to be 7.1 in 0.1 M $Me_4NCl$ and the values for the $NaH(DC)_2$ species vary between 11.6 and 10.2 depending on the concentration of the ionic media [12]. (The authors used a special ion sensitive electrode based on $Pb(DC)_2$ precipitate and assumed that $DC^-$ ions are monomeric in the $Me_4NDC$ solution used for calibration [13].) The above values (covering several orders of magnitude) show that the problem is far from being solved.

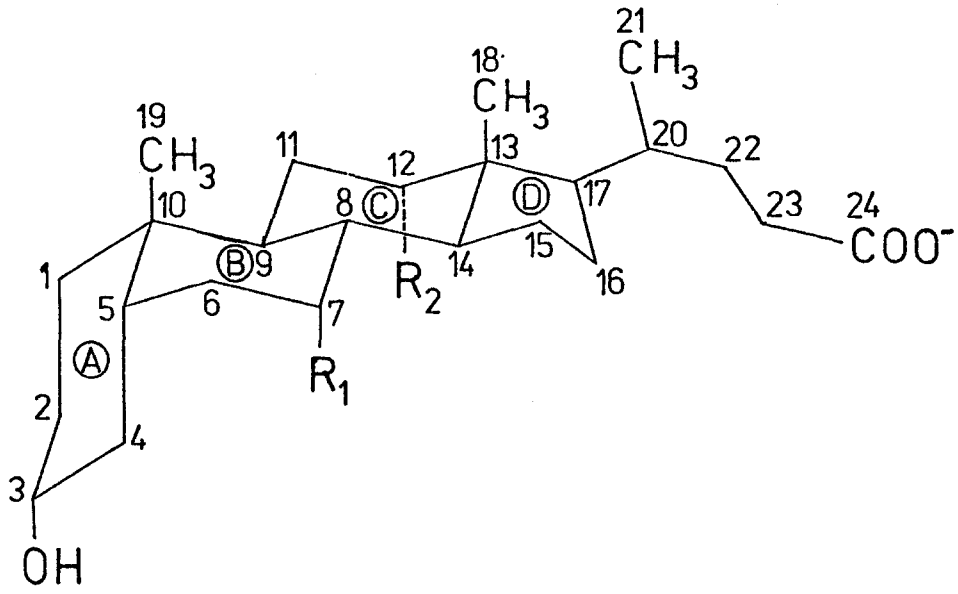


Fig. 1. Formulae of cholate ion ($C^-$; $R_1 = OH$, $R_2 = OH$), deoxycholate ion ($DC^-$; $R_1 = H$, $R_2 = OH$) and chenodeoxycholate ion ($DCD^-$; $R_1 = OH$, $R_2 = H$).

It is known that the composition of a bile salt solution is extremely sensitive to any influence, particularly to any change in medium or pH. It follows that the media have to be kept as constant as possible and a significant excess of alkali is required as for the very detailed SAXS studies [14] for more concentrated NaDC solutions. Accepting these conditions our aim was to reinvestigate the crucial question of dimerization for the three bile salts mentioned.

## 2. Experimental

For solving similar problems [15], we could satisfactorily use a special method based on conductivity measurements under very strict conditions [16]. In the case of bile salts both the temperature and the relatively low concentration of sodium ions were kept constant while the hydroxide ions were exchanged for the anions of the bile acids stepwise up to a given limit, at most to a 1:1 ratio. Under such circumstances the activity coefficients (due primarily to the constant cation concentration) remain strictly constant [16] and the conductivity measured can be characterized as:

$$K = \sum \lambda_i c_i$$

where $c_i$ is the concentration of the $i$th species and $\lambda_i$ is its special molar contribution to the conductivity. Since all of the total concentrations are known and the mass balances can easily be drawn, having a relatively high number of measured values, the characteristic constants can be computed.

The calculation and the control of (dimerization) constants are easy tasks theoretically but knowing the high probability of the polymerization, which is

characterized in the case of bile salts in the form of low c.m.c values, the requirement for using very dilute solutions is essential.

The reagents used were of analytical grade. The twice distilled water and the sodium hydroxide stock solutions were purified to eliminate any carbonate content, and the solutions were stored under nitrogen free of any traces of carbon dioxide. CA, DCA and CDCA (the appropriate acids) were recrystallized from ethanol-water and/or acetone-water mixtures, dried at 100°C and controlled by TLC and potentiometric titration.

The series of solutions with constant sodium ion concentration were prepared in parallel: equivalent amounts of sodium hydroxide stock solutions were diluted under carbon dioxide free nitrogen at 25°C in the presence or absence of the given bile acid. (Measured by weight, its maximum amount was equivalent to one half of the sodium hydroxide content.) Mixing these parallel solutions, several bile salt concentrations could be prepared with constant ionic strength and the desired excess of sodium hydroxide. Conductivity data were recorded with a Radiometer CDM-2d type conductometer having a special conductivity cell [16] at least at 56 different concentrations of every series, and the measurements were repeated. The starting concentration of sodium hydroxide (which is equal to the constant sodium ion concentration) and the highest concentration of the different bile anions are summarized in Table I.

As typical examples, three series of measurements are represented in Figure 2. Like the other cases, no breakpoints or other dramatic changes can be observed in

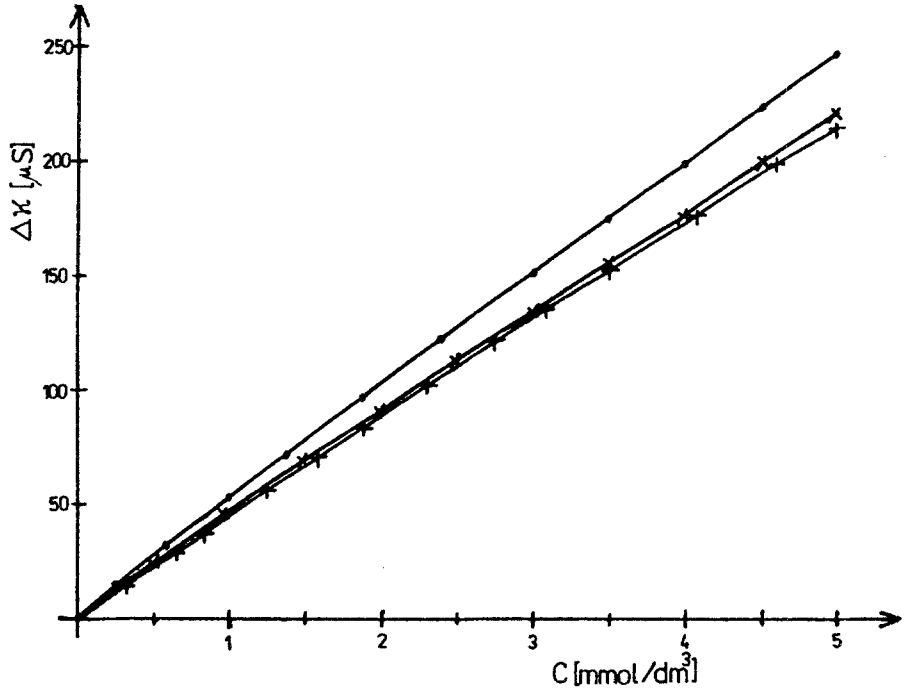


Fig. 2. Differential conductivities of bile salts in 0.02 mol $dm^{-3}$ NaOH at 25°C as a function of their concentration (NaC: ●; NaDC: ×; NaCDC: +).

Table I

| Bile salt | Const. conc. of sodium ion, mol $dm^{-3}$ | Highest conc. of bile salt, mmol $dm^{-3}$ | Dimerization constant, $dm^3\ mol^{-1}$ |
|---|---|---|---|
| Sodium cholate | 0.10 | 15 | $(1.12 \pm 0.2) \times 10^2$ |
| | 0.02 | 10 | $(9.26 \pm 0.1) \times 10^1$ |
| | 0.04 | 5 | $(1.68 \pm 0.08) \times 10^3$ |
| Sodium deoxycholate | 0.02 | 5 | $(1.59 \pm 0.05) \times 10^3$ |
| | 0.01 | 2.5 | $(1.53 \pm 0.03) \times 10^3$ |
| Sodium chenodeoxycholate | 0.02 | 5 | $(2.38 \pm 0.07) \times 10^3$ |
| | 0.01 | 2.5 | $(2.17 \pm 0.04) \times 10^3$ |

the plots of the conductivities vs. bile anion concentration. These curves prove that there is no micellization under the given conditions but the data measured fit well to the simplest assumption: to the monomer–dimer equilibrium. The dimerization constants were calculated as follows:

$$2A \rightleftharpoons A_2$$

$$K = [A_2]/[A]^2$$

where A indicates the given bile anion and the brackets denote equilibrium concentrations in mol $dm^{-3}$ units. (The dimerization constants are also collected in Table I. It must be mentioned that the fit between the measured and calculated values grow worse by including other assumed oligomers into the calculations.)

## 3. Discussion

Our results again prove dimerization before micellization and the importance of the dimeric form which can interact with single bile anions, with other dimeric species or any guest molecule. This simple conclusion agrees well with the findings of some recent papers [17, 18], first of all with the results of Kano *et al.* [9], who prepared some water-insoluble analogues of DCA and proved, unambiguously, that the analogues having at least two α-hydroxy groups attached to the C-3 and C-12 positions of the steroid nucleus are able to interact with monomeric DC anions.

Considering the structure of the compounds under discussion, Figure 1 presents both their arched shapes due to the *cis* fusion of the A and B rings as well as their two different faces: the convex nonpolar one (called the β-plane or 'back') characterized by β-methyl groups, and the other concave polar one (often called the α-plane or 'face') characterized by the secondary α-hydroxy groups. (The conformations of the D ring and the side chain having the carboxylate group are also influenced by interactions.)

When we extend the accepted picture of an ordinary detergent – consisting of a hydrophobic group (generally a long alkyl chain) and a hydrophilic head – to these natural detergents, the 'hydrophobic' steroid skeleton of the bile anions can be regarded as bifunctional. It follows that the monomeric bile salts also have several possibilities of forming dimers or a supermolecular host, and the high capacity of self-accommodation to the conditions is the result of the evolution.

Two models of dimerization are mainly discussed. The first (mostly accepted) is the 'back-to-back' model where the two monomers are held together through hydrophobic interactions between $\beta$-planes [6, 17, 18], while the second is the 'face-to-face' model, presenting the dimer as a double hydrogen bonded species [7, 9]. (Both theories can be accepted or criticized, but one very characteristic feature is that both of them have to assume the other type of interaction in further steps of oligomerization.)

Returning to the dimerization constants summarized in Table I, their order as a function of ionic strength and the individual values can be well correlated with the known critical micelle concentrations [1–4], and with the more complex, more rigid micelle structures of bile salts having two hydroxy groups [19]. These differences are demonstrated with the change in relative concentrations of monomeric and dimeric species in Figure 3, calculated with the values given in Table I for the three characteristic cases. (It should be mentioned that the data are valid for dilute

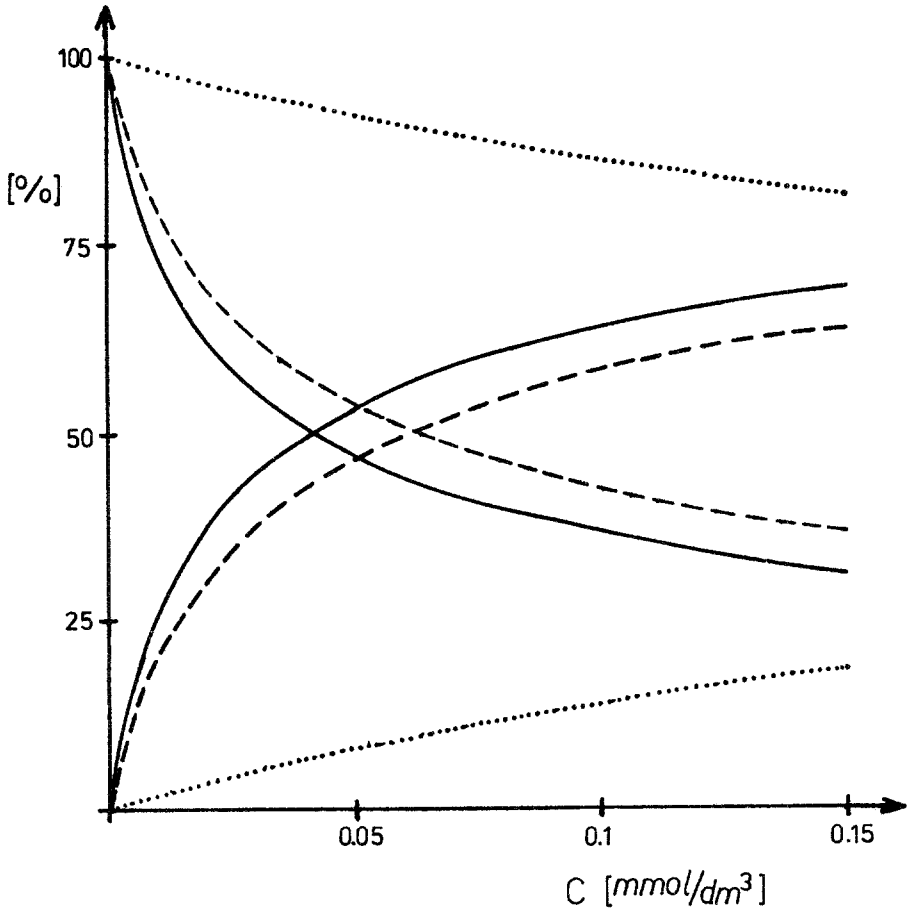


Fig. 3. Relative concentrations of monomeric (upper lines) and dimeric (lower lines) species in diluted alkaline solutions of sodium cholate (NaC: · · ·), sodium deoxycholate (NaDC: – – –) and sodium chenodeoxycholate (NaCDC: ——).

aqueous solutions having a large excess of sodium hydroxide. The constant interactions, e.g. the high sodium ion concentration and the presence of hydroxide excess are incorporated into the stability constants as a consequence of the method used for their determination.)

The order of magnitude of dimerization constants is even more remarkable. In aqueous solution, no single interaction can produce such high stability [20] and even assuming double hydrogen bonds, much lower dimerization constants (like those calculated in Ref. [7]) can only be explained. It must be assumed that very complex interactions can also exist in the concentration range below micelle formation, including hydrogen bonds, ion-dipole, hydrophobic, etc. interactions and at least three-point connections must be the result [20]. It seems that the same possibilities are somehow realized in more concentrated solutions or in the crystalline state, investigated in detail by Giglio *et al.* using X-rays, SASX, EXAFS and NMR analyses [14, 21, 22].

## Acknowledgement

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