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Solubility and the Solubility Product of Some Calcium(II) Salts of Bile Acids

Emilio Bottari, Maria Rosa Festa,* and Lorella Gentile

Dipartimento di Chimica, S. Cannizzaro, Università Sapienza, Piazzale A. Moro 5, 00185 Roma, Italy

ABSTRACT: The solubility of calcium(II) salts of some salts of bile acids was investigated. Among the considered compounds, the slightly soluble salts were prepared by adding a moderate excess of calcium(II) chloride solution to each solution of the selected sodium bile salts. The obtained solids were analyzed to check their composition and the crystallization water content. Each solid compound was brought in contact at 25 °C with an aqueous solution (0.15 or 0.50) mol·dm^{-3} NaCl or 0.50 mol·dm^{-3} $N(CH_3)_4Cl$, as the ionic medium. The obtained suspension was stirred until reaching equilibrium. The equilibrated mixture was analyzed for the free hydrogen ion concentration and for the calcium(II) concentration. Solubility and solubility products were determined for the calcium(II) salts of the following bile salts: cholate, deoxycholate, glycodeoxycholate, ursodeoxycholate, chenodeoxycholate, lithocholate, and dehydrocholate. Calcium(II) glycocholate, taurocholate, and taurodeoxycholate were soluble compounds. The solubility of the investigated salts followed the order: cholate > dehydrocholate > glycodeoxycholate > ursodeoxycholate > chenodeoxycholate > deoxycholate > lithocholate. The solubility in 0.50 mol·dm^{-3} NaCl was higher than in 0.15 mol·dm^{-3} NaCl. Concerning the contained −OH groups the solubility order was the following: trihydroxycholate > dihydroxycholate > monohydroxycholate.

1. INTRODUCTION

The sodium salts of cholic and deoxycholic acids and their conjugates with glycine and taurine are present in human bile[1] and play a fundamental role in many physiologic and biological systems. The bile salts are natural steroids with surface-active and detergent-like properties capable of forming micellar aggregates and solubilizing in water many compounds, such as cholesterol and lecithin.[1−4] Natural bile acids are C_{24}-saturated and belong to the cholanic acid series. Those studied in our laboratory are among the most important bile salts and contain two (dihydroxycholanic) or three (trihydroxycholanic) OH groups in the positions 3α and 12α (deoxycholic acid = HDC and its conjugates) or 3α, 7α, and 12α (cholic acid = HCol and its conjugates). The structure, size, and shape of bile salt micelles, their aggregation numbers, and their critical micellar concentrations (cmc's) were studied by many researchers.[4−22]

Structure investigations on sodium taurocholate (NaTC)[14,20] and glycocholate (NaGC)[16,21] were performed, showing that dimers and octamers could be considered the building blocks of their micellar aggregates. The investigation about aqueous solutions of trihydroxycholanic salts was improved studying the behavior of the sodium cholate.[22]

To explain the results obtained for aqueous sodium salt solution of dihydroxycholanic acids (NaDC,[17] NaGDC,[18] and NaTDC[19]), the presence of several species with different odd high aggregation numbers and also with uptake of protons was assumed. In all cases the presence of a trimer, which seems to constitute the micellar aggregates building unit, is observed for all of the ionic medium concentrations.

The influence of the ionic composition of the solution on the behavior of sodium glycocholate and cholate,[23] gel formation, and protonation constants of taurocholate and taurodeoxycholate[24] were investigated.

The composition of aqueous solutions containing contemporary sodium glycocholate and glycodeoxycholate in gas and solution phases with formation of mixed aggregates was also studied.[24,25]

Recently, reactions occurring between calcium(II) and salts of bile acids were the subject of research of some authors.

De Castro et al.,[26] by investigating the formation of compounds between calcium(II) and magnesium(II) with some bile acids, ascertaining the great disparity among the values reported for the solubility and the acidity constants of HBil, decided to determine again the acidity constants of these acids. However, they proposed values of acid constants for deoxycholic, dehydrocholic, chenodeoxycholic, and their conjugates with glycine obtained in conditions of nonequilibrium and in the presence of precipitate.

Reliable, accurate, and obtained in real equilibrium conditions solubility and acid constants for several bile acids were determined.[27] Such values are directly applicable not only under the analytical point of view, but also for biology and medicine, in particular the values proposed at 0.15 mol·dm^{-3} NaCl. This knowledge is necessary also to evaluate the solubility products of calcium(II) bile salts in physiological conditions.

Jones et al.[28] and Gu et al.[29] studied the enterohepatic circulation, where dehydroxylation and deconjugation of bile acids, as occurs in the large intestine, were considered to promote precipitation of bile acids, and indicated that a factor limiting the bile acid solubility, next to pH, could be the presence of calcium(II), since the solubility products of calcium(II) salts of many anionic detergents are quite low. For this reason, they reported measurements of the solubility of the calcium(II) salts of 10 unconjugated bile acids as well as of three taurine conjugates of these bile acids and proposed approximate solubility products of the relative calcium(II) bile salts. Gu et al.[29]

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explained that their proposed values represent "approximate" solubility products because concentrated solutions of calcium chloride, sodium bile salt, and sodium chloride were used. Often high concentrations of calcium(II) and bile salt had to be used. The activity coefficient of calcium(II) was calculated, and arginine was used to keep constant the hydrogen ion concentration.

The aim of the developed research here is to obtain the solubility and solubility product of calcium(II) salts of some bile acid, in diluted solutions to avoid micellar aggregate formation, in solutions where the reagent activity coefficient should be kept constant by applying the constant ionic medium method, proposed by Biedermann and Sillèn[30] and in the absence of other reagents which can be able to complex calcium(II).

In this paper the solubility of calcium(II) salts of cholate (Col), deoxycholate (DC), glycocholate (GC), glycodeoxycholate (GDC), chenodeoxycholate (CDC), ursodeoxycholate (UDC), lithocholate (Lit), dehydrocholate (DHC), was investigated at 25 °C and in 0.150 mol·dm^{-3} and 0.500 mol·dm^{-3} NaCl. The solubility of calcium(II) deoxycholate was studied also in 0.500 mol·dm^{-3} $N(CH_3)_4Cl$.

The formation of micellar aggregates of calcium(II), taurocholate (TC) and taurodeoxycholate (TDC), was investigated in previous papers.[31,32] It was found that both these salts are soluble and the composition of premicellar and micellar aggregates was found.

2. EXPERIMENTAL SECTION

2.1. Method of Investigation. To investigate the behavior of the considered calcium(II) bile salts, it is necessary to prepare the solid salts of calcium of exactly known composition and to study the resulting equilibria in solutions where the solid is in contact with solutions with different compositions.

The investigation starts from the preparation of the solid compounds obtained according to the main criteria of gravimetric analysis, which means from diluted and warm solutions. It was necessary to have precipitates easily filtered, in a stable form and with known stoichiometry. Each solid is equilibrated with aqueous solutions of different composition to study equilibria taking place between solid and solutions.

By assuming calcium(II) and bile anions as independent reagents, in aqueous solution, the following general equilibria can be expressed:

$$qCa + pH + r\text{Bil} \leftrightarrow Ca_qH_p\text{Bil}_r \quad (1)$$

with constant $\beta_{q,p,r} = [Ca_qH_p\text{Bil}_r]\,([Ca]^q[H]^p[\text{Bil}]^r)^{-1}$ and the solubility product

$$k_s = [Ca]^{q'}[H]^{p'}[\text{Bil}]^{r'} \quad (2)$$

In eqs 1 and 2 and in the following, charges are omitted, and [X] is the free concentration of X, while c_X will indicate the total concentration of X.

The analysis of the solutions is focused on the knowledge of q, q', p, p', r, and r' and the constants k_s and $\beta_{q,p,r}$.

In the above expressions, if q or $q' \geq 1$ (if they are equal to 1, polynuclear species are not formed) and p and $p' \neq 0$, species with the participation of protons are in place; in particular if p and $p' > 0$, species with the assumption of protons are formed, while if p and $p' < 0$, OH are assumed. The symbol $r \geq 1$ depends on the number of bile ions that are bound to calcium(II), and Bil indicates one of the investigated bile salts.

Also, the sodium bile salts not found in commerce, prepared as below described, were analyzed by thermoanalysis to verify their correct composition.

To study the solubility and determine the solubility product of each investigated calcium(II) salt, a slight excess of each solid was brought in contact with solutions with the following general composition:

- Solution S1: c_{OH} M in OH^-; μM in Na^+; $(\mu - c_{OH})$ M in Cl^-;
- Solution S2: c_{OH} M in OH^-; c_{Ca} M in Ca^{2+}; $(\mu - 2c_{Ca})$M in Na^+; $(\mu - c_{OH})$ M in Cl^-;
- Solution S3: c_{OH} M in OH^-; c_{Bil} M in Bil^-; μM in Na^+; $(\mu - c_{Bil} - c_{OH})$ M in Cl^-.

In the above expressions M stands for mol·dm^{-3}, and μ indicates the concentration of the ionic medium, which was (0.150 or 0.500) mol·dm^{-3}.

Several series of solutions, S1, S2, and S3, were added with the solids and, after reaching equilibrium, analyzed. For each series c_{OH}, c_{Ca}, and c_{Bil} were varied to investigate if species with the participation of protons or complexes with a different ratio between calcium(II) and bile salt could be formed.

In all cases solutions containing the suspended solid were shaken until equilibrium was reached. After repeated checks, it could be concluded that 20 h were enough to obtain equilibrium between solid and solutions.

For each solution the free hydrogen ion concentration, [H], was obtained by measuring the electromotive force (emf) of a galvanic cell containing a glass electrode (GE).

Before each measurement, at least three buffer solutions at known hydrogen ion concentrations were introduced in the measurement vessel. Generally, as the range $9 \leq -\log[H] \leq 11$ was investigated in this case, the liquid junction potential, depending on the hydrogen ion concentration, was negligible.

After the determination of $-\log[H]$, the solutions were filtered or centrifuged so that their content in calcium(II) could be determined by atomic absorption (AA) spectrometry and titration with EDTA. The solubility and free hydrogen ion concentration were the basis to obtain the solubility product, K_s, of the investigated calcium(II) bile salts.

2.2. Materials and Analysis. Sodium bile salts were generally Sigma or Calbiochem products used without further purification. When sodium bile salts were not found, the corresponding acids were purchased from Sigma or Calbiochem. To prepare the sodium salts, weighted amounts of the bile acid were added with the equivalent quantity of NaOH to have a moderately alkaline solution ($-\log[H] \approx 8$). To the clear solution, a little excess of slight soluble bile acid was added so that a precipitate was formed. The solution after filtration was limpid. When evaporated until dryness it provided the solid sodium salt. The absence of excess of water was checked because the solid was dried in an oven at 105 °C until constant weight. The prepared sodium salts were analyzed by thermogravimetry.

To prepare the solids with suitable physical properties, a calcium(II) chloride solution was added under stirring to warm and diluted solutions of each selected sodium bile salt until precipitation was obtained. A moderate excess of $CaCl_2$ solution was further added. The mixture was stirred and held under heating water bath overnight, so that the precipitate could reach the best composition.

The obtained precipitate was filtered or centrifuged and washed with twice-distilled water until the absence of excess of

calcium(II) was checked. It was then dried in oven at 70 °C until a constant weight was obtained. The obtained solid was analyzed both for water content and for the ratio of calcium(II)/bile salt. The analysis was performed by applying three methods. At first a known sample of the solid was ignited in a muffle furnace at 550 °C to obtain the corresponding carbonate, which was analyzed by titration with standard HCl. The second method was the dosage of calcium(II) in a weighted sample by titration against standard EDTA. Finally thermoanalysis was employed to check both water content and composition.

Particular care was dedicated to the preparation of calcium(II) lithocholate. As expected, lithocholic acid (HLit) is not soluble in water, but also its sodium salt (NaLit) is slightly soluble in water. As both compounds (HLit and NaLit) are soluble in ethanol, adding some drops of calcium(II) chloride solution to an ethanolic solution of sodium lithocholate (NaLit), the expected precipitate of calcium(II) lithocholate is obtained.

The obtained composition corresponded to a 1:2 ratio between calcium(II) and bile anion, while a content of water generally corresponded to $2H_2O$. In Figure 1 the thermogram obtained for $Ca(UDC)_2$ is represented, as an example.

Hydrochloric acid, sodium chloride, sodium hydroxide, tetramethylammonium chloride, and tetramethylammonium hydroxide were prepared and analyzed as previously[21,24] described. Calcium(II) chloride (RP C. Erba) was used to prepare the calcium(II) bile salts and to prepare standards for AA spectrometry. It was analyzed as described previously.[32] A solution of EDTA (as disodium salt) was prepared from a C. Erba RP reagent and analyzed using a $CaCO_3$ standard. The final point of the titration was obtained by using a magnesium(II) EDTA complex, ammonia buffer at $-\log[H] = 10$, and Eriochrom black T as an indicator.

2.3. Details on Experimental Apparatus. All of the measurements were carried out in a thermostat room at 25.0 ± 0.5 °C. Solutions containing the excess of solid calcium(II) bile salts were transferred in suitable glass bottles with an emeried plug and sealed with parafilm. The bottles were shaken mechanically in a thermostat at 25.00 ± 0.05 °C for 20 h, time necessary to reach equilibrium.

Emf measurements of the galvanic cell were performed with a Metrohm mod. 654 pH meter equipped with a glass electrode no. 6.0102.000 from the same firm. The reference electrode (RE) was prepared according to Brown[33] (RE = Ag, AgCl/μ NaCl, saturated with AgCl/μ NaCl). Constant values of emf were obtained within few minutes. The values were reproducible within ± 0.2 mV, and μ indicates the ionic medium. The glass electrode response agreed with that of a hydrogen electrode until $-\log[H] \leq 10$. Beyond this limit, the emf values obtained from GE were adjusted assuming the response provided by the hydrogen electrode as correct.

The emf measurements were carried out in solutions at real equilibrium, because data obtained from solutions prepared with different procedures agreed well.

After the emf measurements, solutions were filtered and centrifuged for about one hour at 4000 rpm (round per minute). Clear solutions were analyzed for the calcium(II) content with an AA spectrometer.

A thermoelectron AA spectrometer (model Solaar) equipped with lamps of the same firm was used to determinate calcium(II) in the test solutions in flame obtained from C_2H_2 and N_2O. A preheating device was necessary for the use of nitrogen protoxide and to improve sensitivity. Acetylene and nitrogen protoxide from cylinders fuelled the flame.

Nitrogen (99.995 %) from a cylinder, further purified by passing through 10 % NaOH, 10 % H_2SO_4, and the selected ionic medium and bubbling through the solutions, was used to eliminate oxygen and CO_2.

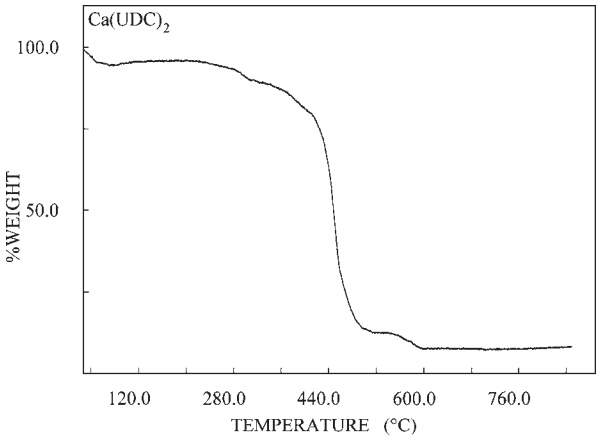

Figure 1. Thermogram of $Ca(UDC)_2$, as an example. The percentage of water corresponds to two water molecules.

3. RESULTS AND DISCUSSION

The determination of the solubility product of the calcium(II) bile salts requires the preparation of solid compounds of defined composition of the selected bile salts of calcium(II).

For each salt the ratio between calcium(II) and the selected bile salt was checked as described above. For all of the salts analyzed, the ratio of 1:2 in the solid was found, and it was proven by means of thermogravimetric analysis that all of the salts contained two water molecules. This means that in the expression of the solubility product (eq 2) $q' = 1$, $p' = 0$, and $r' = 2$, so that for each calcium(II) bile salt the formula $Ca(Bil)_2 \cdot 2H_2O$ could be deduced.

Previous papers[17−22,27] showed that it was convenient to operate for this investigation in moderate alkaline solutions, to avoid the problem of slight solubility of the corresponding cholanic acids. However, to check the possibility of formation chemical species with the participation of protons, solutions in the range $9.5 \leq -\log[H] \leq 11.5$ were prepared and analyzed. The upper limit of the value of $-\log[H]$ was selected to avoid the possibility of precipitation of calcium(II) hydroxide. Solutions at different OH^- in the range $0.1 \leq [OH] \leq 5 \cdot 10^{-3}$ mol·dm^{-3} were prepared.

As solutions at different $-\log[H]$ provided the same results, it could be concluded that species with the participation of hydrogen ions are not present in appreciable concentrations and in eq 1, $p = 0$.

Solutions with the suspended solid were added with an excess of calcium(II) ion, alternatively with an excess of sodium salt of the selected bile salt. The excess was in any case very limited so that the formation of micellar aggregates was avoided. Few sodium salts of the studied bile acid have been studied,[17−23] and some species explained the experimental data satisfactorily. For such bile salts a greater excess of sodium bile salt could be added because, as explained below, the knowledge of the sodium−bile salt system was very useful.

Table 1. $-\log K'_s$ Values of Calcium(II) CDC, UDC, DHC, and Lit, at 25 °C and in 0.15 M NaCl

	$Ca(CDC)_2$	$Ca(UDC)_2$	$Ca(DHC)_2$	$Ca(Lit)_2$
1.	8.72	8.40	6.74	15.50
2.	8.78	8.35	6.76	15.40
3.	8.70	8.32	6.72	15.35
4.	8.74	8.26	6.65	15.54
5.	8.73	8.39	6.68	15.50
6.	8.66	8.36	6.69	15.40
7.	8.71	8.37	6.71	15.60
8.	8.74	8.35	6.71	15.35
9.	8.73	8.38	6.74	15.45
10.	8.73	8.35	6.72	15.50
average	8.72 ± 0.08	8.35 ± 0.09	6.71 ± 0.06	15.45 ± 0.15

The solubility S, corresponding to the total concentration of calcium(II) for each bile salt, by taking in consideration the mass action law, can be expressed, without preliminary hypothesis, as the material balance of calcium(II), as follows:

$$S = c_{Ca} = [Ca] + \sum\sum q\beta_{q,r}[Ca]^q[Bil]^r$$
$$= [Ca](1 + \sum\sum q\beta_{q,r}[Ca]^{q-1}[Bil]^r) \quad (3)$$

In eq 3, symbols have the same meaning as indicated above, and as proven, the presence of species formed with the participation of protons is neglected.

Equation 3, on the basis of the results of the above indicated analysis, can be arranged as follows:

$$\log K'_s = \log k_s + \log(1 + \sum\sum q\beta_{q,r}[Ca]^{q-1}[Bil]^r) \quad (4)$$

To obtain the solubility product k_s from eq 4, knowledge of the free concentration of [Bil] is necessary. It can be obtained from the material balance of the correspondent bile salt, as follows:

$$C_{Bil} = [Bil] + k_1[H][Bil] + \sum\sum\sum r''\beta_{q'',p'',r''}[Na]^{q''}[H]^{p''}[Bil]^{r''}$$
$$+ \sum\sum r\beta_{q,r}[Ca]^q[Bil]^r \quad (5)$$

The free concentration of the bile anion can be calculated in the first approximation if the contribute of the last term of eq 3 is negligible and the contribution of the species formed between sodium and bile anion is known or negligible. The protonation constants, k_1, of the bile anions investigated here was previously determined in the same experimental conditions.[27]

Previous[31,32] studies carried out on solutions containing calcium(II) and taurocholate and taurodeoxycholate showed that no precipitate is formed in a wide range of concentration, but only aggregates with different compositions.

In this research it was observed that by adding different amounts of calcium(II) to solutions of sodium glycocholate in a wide range of concentration no precipitation occurred. It could be concluded that the calcium(II) salts of glycocholate, taurocholate, and taurodeoxycholate are soluble.

As the composition of solutions containing sodium ions and CDC, UDC, DHC, and Lit anions are not known, but their critical micellar concentration (cmc) is known even in different experimental conditions, it was possible to perform experiments mixing the reagents in concentration range under cmc. The investigation was carried out by employing very low concentrations of calcium(II) and bile salts. Generally solutions where calcium(II) and bile salts concentrations in excess as regards the solid were less than $1 \cdot 10^{-3}$ mol·dm^{-3} were studied. In these concentration ranges, the formation of aggregates was neglected, and in the first approximation [Bil] was calculated from eq 5. The first approximation value could be affined successively.

Table 2. $-\log K'_s$ Values of Calcium(II) CDC, UDC, DHC, and Lit, at 25 °C and in 0.50 M NaCl

	$Ca(CDC)_2$	$Ca(UDC)_2$	$Ca(DHC)_2$	$Ca(Lit)_2$
1.	8.52	8.20	6.24	15.00
2.	8.58	8.25	6.23	15.09
3.	8.50	8.32	6.30	15.15
4.	8.54	8.26	6.35	15.03
5.	8.53	8.19	6.25	15.08
6.	8.46	8.26	6.25	15.05
7.	8.49	8.26	6.28	14.98
8.	8.55	8.25	6.32	15.12
9.	8.54	8.22	6.22	15.15
10.	8.56	8.25	6.32	15.09
average	8.53 ± 0.07	8.27 ± 0.09	6.28 ± 0.08	15.07 ± 0.12

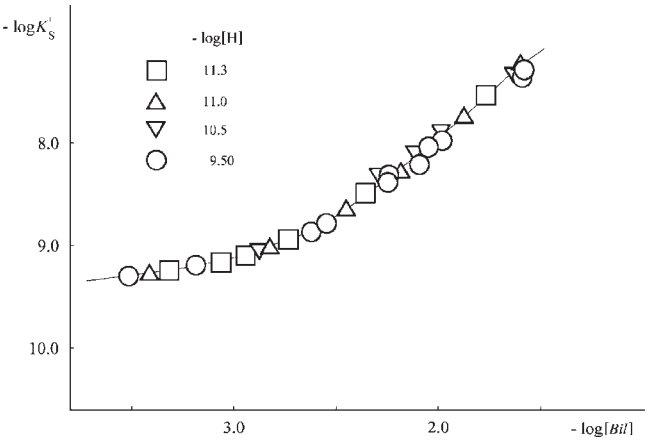

Figure 2. Dependence of the apparent solubility product ($-\log K'_s$) of $Ca(DC)_2$ on the concentration of bile anion at 25 °C and 0.15 mol·dm^{-3} NaCl. Points obtained at different hydrogen ion concentrations fall on the same curve.

For the above considered bile salts, it can be observed that the logarithm of the products of calcium(II) concentration and [Bil], obtained from eq 5 with the explained approximations, remains constant within about ± 0.1. As constant values were obtained for $-\log K'_s$ without any trend depending on the concentration of calcium(II) or bile salts, it seems reasonable to assume the average of the obtained values as solubility product, $-\log k_s$, both in (0.15 and 0.50) M NaCl.

In Table 1 (for 0.15 mol·dm^{-3} NaCl ionic medium) and Table 2 (for 0.50 mol·dm^{-3} NaCl ionic medium) as examples, the obtained values of $\log K'_s$ for the above listed bile salts are collected. The constancy of the obtained values can be observed.

Since the formation of aggregates between sodium and deoxycholate, cholate, or glycodeoxycholate was previously[17,18,22]

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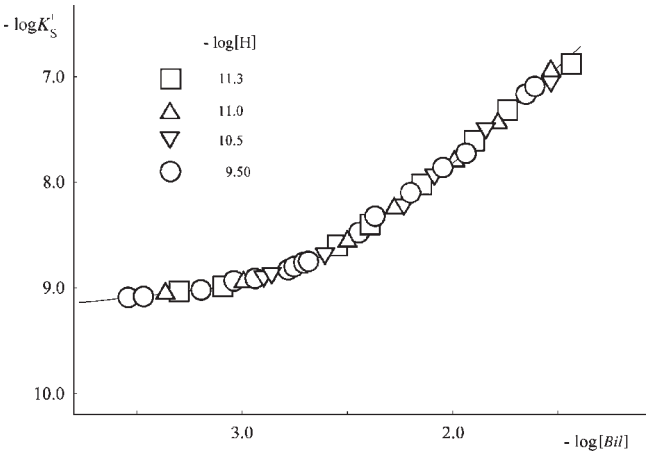

Figure 3. Dependence of apparent solubility product ($-\log K'_s$) of $Ca(DC)_2$ on the concentration of bile anion at 25 °C and 0.50 mol·dm^{-3} $N(CH_3)_4Cl$. Points obtained at different hydrogen ion concentrations fall on the same curve.

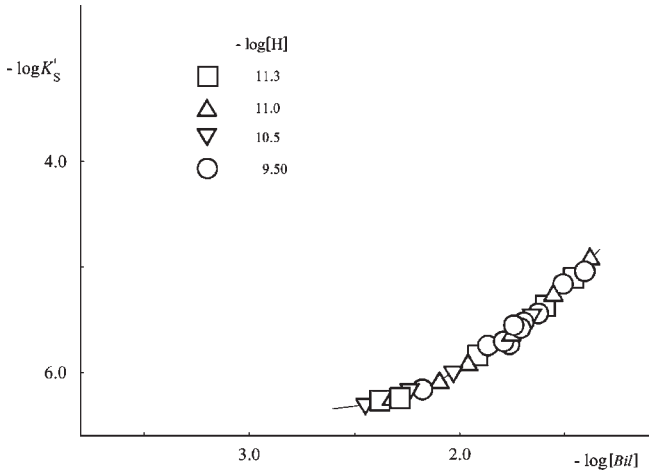

Figure 4. Dependence of apparent solubility product ($-\log K'_s$) of $Ca(Col)_2$ on the concentration of bile anion at 25 °C and 0.50 mol·dm^{-3} NaCl. Points obtained at different hydrogen ion concentrations fall on the same curve.

studied, the dependence of solubility of $Ca(Bil)_2 \cdot 2H_2O$ on the concentrations both calcium(II) and the selected bile anion could be studied. The previous investigation offered the opportunity to evaluate the contribution of the sodium bile salt aggregates so that the free bile anion concentration can be calculated from eq 5 with more accuracy.

The solubility of the calcium(II) salts (DC, Col, GDC) was investigated both in (0.50 and 0.15) mol·dm^{-3} NaCl, in a wide range of calcium(II) and bile anion concentration. Furthermore, the system calcium(II)−deoxycholate was studied also in 0.50 mol·dm^{-3} $N(CH_3)_4Cl$, so that the influence of the composition of the ionic medium could be evaluated.

The experimental data of calcium(II) deoxycholate plotted in Figure 2 show the dependence of $-\log K'_s$ on $-\log[DC]$ for experiments carried out in 0.15 M NaCl. As all of the points fall on the same trend independently of the hydrogen ion concentration, it is confirmed that species formed with the participation of protons are not present in appreciable concentration and the hypothesis formulated with eq 3 is valid. Furthermore, all points independently of the calcium(II) fall on the same curve, and no appreciable presence of polynuclear species in solutions can be observed (i.e., $q = 1$). As the slope of the trend of the points is more than 1, r can assume the values 1 and 2, so that eq 4 can be expressed in the form:

$$-\log K'_s = -\log k_s + \log(1 + \beta_{1,1}[\mathrm{Bil}] + \beta_{1,2}[\mathrm{Bil}]^2) \quad (6)$$

By applying in the first approximation the graphic method proposed by Sillèn,[34] eq 6 can be normalized in the form:

$$y = \log(1 + \alpha u + u^2) \quad (7)$$

where $-\log K'_s - y = -\log k_s$; $\log u = \log[\mathrm{Bil}] + 1/2 \log \beta_{1,2}$ and $\log \beta_{1,1} = 1/2 \log \beta_{1,2} + \log \alpha$.

The graphic of eq 7 was superimposed to the experimental points, and the two plots were moved parallel to both axes until the best fit was obtained. In this position and on the basis of the mathematical position, the values of $\log k_s$, $\log \beta_{1,2}$, and $\log \beta_{1,1}$ could be obtained.

The same procedure was applied to the points obtained for the investigation on calcium(II)−deoxycholate in 0.50 mol·dm^{-3} $N(CH_3)_4Cl$, for which the dependence of $-\log K'_s$ on $-\log[DC]$ is shown in Figure 3.

Also in this case the slope of the trend of the points was more than 1, and the experimental data were explained by assuming the formation of two species with the ratios 1:1 and 1:2, between calcium(II) and deoxycholate.

The experimental data obtained for the system calcium(II)−cholate are plotted in Figure 4, in the form $-\log K'_s$ as a function of $-\log[Col]$. Although also in this case all of the points fall on the same trend ($q = 1$), the trend is independent of $-\log[H]$, and the slope is more than 1, it can be observed that the form of the curve is different with respect to those of Figures 2 and 3. In this case the trend of the points cannot be normalized by an equation similar to eq 7. The hypothesis was formulated that the experimental data could be explained by assuming next to the precipitate, the existence only of the species $Ca(Col)_2$ of composition similar to that of the precipitate. On this basis, the dependence of $\log K'_s$ on $-\log[Col]$ could be expressed as follows:

$$-\log K'_s = -\log k_s + \log(1 + \beta_{1,2}[\mathrm{Bil}]^2) \quad (8)$$

which was normalized by the equation: $y' = \log(1 + u^2)$, where:

$$-\log K'_s - y' = -\log k_s;$$

$$\log u = \log[\mathrm{Bil}] + 1/2 \log \beta_{1,2}$$

By applying a procedure similar to that above explained the k_s and $\beta_{1,2}$ values could be obtained.

The experimental data obtained studying the solubility of calcium(II) glycodeoxycholate are plotted in Figure 5, and they can be explained by assuming the existence of CaGDC and $Ca(GDC)_2$ next to the formation of the solid.

In Table 3 the species assumed to explain the experimental data obtained for deoxycholate, cholate, and glycodeoxycholate with the relative constants are collected.

The solubility product ($\log k_s$) of the salts calcium(II) deoxycholate, calcium(II) cholate, and calcium(II) glycodeoxycholate,

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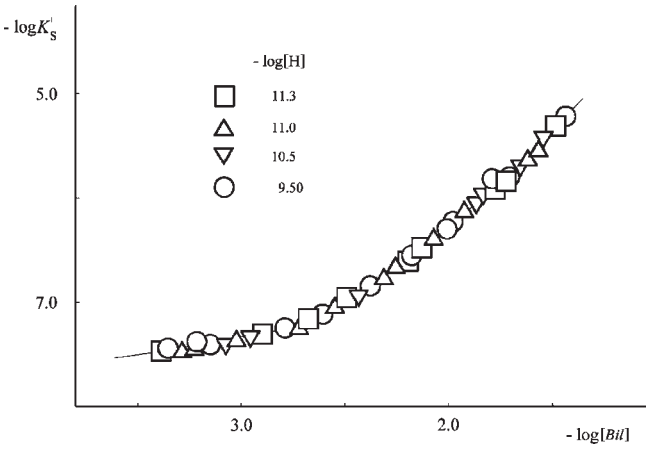

Figure 5. Dependence of apparent solubility product ($-\log K'_s$) of $Ca(GDC)_2$ on the concentration of bile anion at 25 °C and 0.15 mol·dm^{-3} NaCl. Points obtained at different hydrogen ion concentrations fall on the same curve.

Table 3. Soluble Species and Relative Constants Assumed to Explain the Data Obtained for the Systems Calcium(II) and Deoxycholate, Cholate, and Glycodeoxycholate, Respectively, in 0.15 and 0.50 M NaCl[a]

species		0.15 M NaCl	0.50 M NaCl	$N(CH_3)_4Cl$
Ca(DC)	$\log \beta_1$	2.82 ± 0.10	2.75 ± 0.10	2.80 ± 0.10
$Ca(DC)_2$	$\log \beta_2$	5.28 ± 0.08	5.25 ± 0.08	5.24 ± 0.07
$Ca(Col)_2$	$\log \beta_2$	4.46 ± 0.05	4.24 ± 0.05	
Ca(GDC)	$\log \beta_1$	2.80 ± 0.10	2.72 ± 0.10	
$Ca(GDC)_2$	$\log \beta_2$	5.20 ± 0.10	5.12 ± 0.10	

[a] For deoxycholate also the species observed in 0.50 M $N(CH_3)_4Cl$ are collected.

obtained by the above explained procedure, together with the other studied bile salts log k_s is collected in Table 4. As it can be observed the value of log k_s for calcium(II) deoxycholate was determined also in the ionic medium 0.50 mol·dm^{-3} $N(CH_3)_4Cl$.

From an inspection of Table 4, it can be observed that the solubility product values in both ionic medium follow the order: lithocholate < deoxycholate < chenodeoxycholate < ursodeoxycholate < glycodeoxycholate < dehydrocholate < cholate.

It is evident that the solubility increases by increasing the OH groups present in each studied compound. Monohydroxy compounds (calcium(II)−lithocholate) have the smallest solubility; dihydroxycholanic (like calcium(II) deoxycholate or conjugates) are about 10^6 times more soluble, and trihydroxycholanic (calcium(II)−cholate) are about 10^3 times more soluble than the deoxycholate or its conjugates. Generally simple calcium(II) bile salts are less soluble than conjugates. Glycoconjugates are more soluble than deoxycholate and cholate. The calcium(II) glycocholate is soluble in both ionic media. Calcium(II) tauroconjugates both for deoxycholate and taurodeoxycholate are soluble.

The log k_s values are different in the different ionic media. It can be observed an increasing of solubility by increasing the concentration of the NaCl of the ionic medium. This effect can be attributed both to the change of the activity coefficient and to the increasing of solubility due to the interaction between the bile anion with the sodium ions. This second hypothesis is supported

Table 4. Solubility of Calcium(II) Bile Salts at 25 °C and in (0.15 and 0.50) M, Respectively[a]

	$-\log k_s$ (0.15 M)	$-\log k_s$ (0.50 M)	$-\log k_s$ (0.50 M)
bile anion	NaCl	NaCl	$N(CH_3)_4Cl$
(1) deoxycholate	9.40 ± 0.10	9.03 ± 0.10	9.20 ± 0.10
(2) cholate	6.50 ± 0.10	6.40 ± 0.10	
(3) glycodeoxycholate	7.58 ± 0.10	6.75 ± 0.10	
(4) chenodeoxycholate	8.72 ± 0.10	8.53 ± 0.10	
(5) ursodeoxycholate	8.35 ± 0.10	8.27 ± 0.10	
(6) dehydrocholate	6.71 ± 0.10	6.28 ± 0.10	
(7) lithocholate	15.45 ± 0.15	15.07 ± 0.15	

[a] For the slightly soluble salts, solubility products ($-\log k_s$) are collected.

by the increasing of the solubility by increasing of sodium ions in the ionic medium (from (0.15 to 0.50) mol·dm^{-3}).

Data of Tables 3 and 4 show that the change of ionic medium from NaCl to $N(CH_3)_4Cl$ has a slight influence. However, the presence of NaCl seems to play a higher role than $N(CH_3)_4Cl$ on the solubility of $Ca(DC)_2$, even if the k_s are not far, by considering the limits of error. By comparing all three k_s values (0.15, 0.50 NaCl, and 0.50 mol·dm^{-3} $N(CH_3)_4Cl$), it can be deduced that the concentration of the ionic medium plays the main role.

The observed behavior can be compared with that previously found by studying the composition of solutions containing sodium and the here studied bile salts.[17−21] It was stressed many times that aggregates formed between sodium and dihydroxycholanic are bigger than those formed by trihydroxycholanic and the former are more stable than the latter. Furthermore, from an inspection of Table 3, it can be observed that simple or conjugate dihydroxycholanics are able to form with calcium(II) compounds with the ratio 1:1 and 1:2, while the trihydroxycholanic compounds (for instance cholate) is able to form only 1:2 species. By comparing this evidence with the composition of species assumed to explain the experimental data obtained for solutions containing sodium ions and di- and trihydroxycholanic bile salts an agreement can be observed, because aggregates formed for the former have very frequently an odd number of bile anions, while the latter (trihydroxy) quite always form smaller aggregates and with even number of bile anions. It was found that for small aggregates a dimer was proposed as the building unit of the micellar aggregates.[21] Similarly, it can be observed in Table 3 that only cholate forms a soluble compound with the ratio of 1:2 and no appreciable quantity of compounds with an odd number is present.

As explained above, the solubility of some of the bile anions here studied were investigated also by Gu et al.[29] Our results agree with the conclusions presented by these authors with respect the solubility of tauroconjugates and of calcium(II) glycocholate, because in both investigations these compounds are defined as soluble.

In all other cases (DC, GDC, Col, UDC, CDC, Lit), the values proposed by Gu et al. defined as approximate solubility products are higher than those proposed in this paper. In ref 29, the procedure to obtain the results was very different with respect to that followed in our research. The solid was prepared from concentrated solutions, where the formation of aggregates was not accurately avoided and the activity coefficients were not kept constants, but they were calculated. Finally, these authors to keep constant the hydrogen ion concentration added arginine to each

Table 5. $-\log k_s$ Values at 25 °C and in 0.50 M $N(CH_3)_4Cl$ for Lead(II) Bile

bile ion	$-\log k_s$	$\log \beta_2$	$\log \beta_3$
DC	11.40		7.50
GDC	10.58	6.00	7.50
Col	9.08		4.60
GC	8.33	4.55	

solutions. It was found that arginine is a ligand of calcium(II)[35] forming two complexes with ratios 1:1 and 1:2. All of these considerations are explained very well because by Gu et al. found solubility values higher than those proposed in this paper.

However, the solubility of the systems calcium(II)−bile anions found in that research follows the same order as proposed above.

It seems of interest to compare the behavior of the calcium(II) with that of lead(II) toward the same bile salts, previously studied for deoxycholate, glycodeoxycholate, cholate, and glycocholate and the results found for the system barium(II) taurodeoxycholate.[36−40]

Barium(II) is alone among the investigated cations able to form a slight soluble compound with taurodeoxycholate, even if the solubility product is higher than that of deoxycholate (6.42 to compare to 9.20, both determined in 0.50 M $N(CH_3)_4Cl$).

In Table 5 the solubility products of precipitate formed by lead(II) with some bile salts are collected.

As expected, lead(II) forms salts less soluble than calcium(II), so that even lead(II) glycocholate is a slight soluble compound. Furthermore, some difference can be observed about the composition of the soluble species relative to the cholate. Table 5 shows that lead(II) forms with cholate a soluble species with the ratio 1:3, while generally cholate, being a dihydroxycholanic, also forms species with an odd number with calcium(II).

4. CONCLUSION

This paper presents several important characteristics of bile acids, their sodium salts, and the soluble or slight soluble calcium(II) bile compounds.

The solubility and acid constants of the bile acids were previously investigated and accurately determined.[27] Sodium bile salts both purchased and prepared were in this research analyzed to find the composition and the stoichiometric quantity of crystallization water. Each compound was analyzed by means of thermogravimetric analysis to check the percentage purity and crystallization water. Each calcium(II) salt of the selected bile anion was prepared from diluted solutions and after the suitable digestion was analyzed to obtain its stoichiometric composition and the number of coordinated water molecules.

Solid calcium(II) bile salts were added in moderate excess to ionic medium solution, (0.50 and 0.15) mol·dm^{-3} NaCl (physiological solution), and stirred until equilibrium reaching. As solutions with ionic medium were prepared at different hydrogen ion concentrations and the data did not show any dependence on pH, it could be deduced that species with the participation of protons are not present in appreciable concentration.

The resulting experimental data were explained by assuming that for all of the studied compounds the ratio between calcium(II) and the selected bile anion was 1:2, and the solid contained two water molecules. The solubility in 0.15 mol·dm^{-3} was in any case less than in 0.5 mol·dm^{-3} of the same salt. The solubility of trihydroxyl cholanic calcium(II) salt is more than that of dihydroxyl, which is more than that of monohydroxycholanic. Generally the conjugates are more soluble than the simple ones.

A comparison with the alone literature data proposed by Gu et al.[29] shows some elements in agreement, but mainly the $-\log k_s$ data here obtained are lower than those proposed by Gu.

For few cases (DC, GDC, Col), to explain the obtained experimental data obtained here, it was necessary to also assume the presence of soluble species with the ratio 1:1 or 1:2 between calcium(II) and bile anion.

The conclusion of this research seems important in foreseeing the probability of precipitation of calcium(II) bile salts in human bile for the compound present in the human body.

The good results obtained here suggest how the solubility of the studied compounds can be increased, for example, by adding a suitable ligand of calcium(II).

AUTHOR INFORMATION

Corresponding Author

*Tel.: +390649913643. Fax: +3906490420. E-mail: mariarosa.festa@uniroma1.it.

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