Coulometry: A Fine Procedure to Determinate Acidity Constants of Slightly Soluble Acids

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The flowing of constant direct current allows planning a coulometric method to determine solubility and acidity constants of slightly soluble acids. Coulometry combined with electromotive force (emf) measurements is applied to obtain such parameters for eight bile acids, simple and conjugated with glycine in solutions in real equilibrium and without formation of micellar aggregates. Results, obtained in different ionic media, (1.00, 0.50, and 0.15) mol·dm^{-3} NaCl (i.e., in physiologic conditions), are compared with previous data, extrapolated from mixed solvents or obtained in oversaturated solutions. The proposed method is not difficult to perform, and it can be applied to other slightly soluble acids or in very low concentrations, without a complicated elaboration of the experimental data.

Introduction

The determination of the dissociation constants of acids generally is not a difficult problem from the analytical point of view. Accurate electromotive force (emf) measurements of the free hydrogen ion concentration in solution of known analytical concentrations of the acid allow the dissociation constant of the tested acid to be obtained.

Problems can arise even for a monoprotic acid if it is slightly soluble, and further difficulties could occur for the formation of polymeric aggregates, like micelles. These difficulties are often observed for organic acids with a particular structure and among them are bile acids (HBil). In Figure 1 some examples of the structure of bile acids are reported.

Cholic and deoxycholic acids and their conjugated compounds with glycine are present in human bile and belong to cholanic acids. They can be either trihydroxycholanic (OH groups in the positions 3α, 7α, and 12α) or dihydroxycholanic (OH groups in the positions 3α and 12α) acid. Both are able to form micellar aggregates of different size, structure, and shape depending on their concentration, the hydrogenionic concentration, and in general the ionic composition of their aqueous solutions.

Knowledge of their acid dissociation constants is of interest in analytical chemistry and extremely useful in chemical, clinical, and pharmaceutical research. These values, together with the solubility, are of fundamental importance to foresee the relation existing between solubility and acidity and aggregation with formation of micelles, including also hydrogen ions and other ions eventually present in their solutions together with ions of the bile salt aggregates. Solubility and dissociation constants influence the bioavailability of the bile acid or of its salts.[1−5]

The values reported in the literature for the acidity constants of the bile acids and for their solubility are not in agreement for the very low solubility of the acid (in the protonated form HBil) and for the property of their dissociated form (Bil^-) to form aggregates, often even at low concentrations. The participation of HBil and/or Bil^- in aggregation equilibria prevents accurate knowledge of the solubility and the dissociation constants.

In 1957 Ekwall et al.,[6] in a potentiometric approach to determine the acid constants of some cholanic acids, obtained values strongly dependent on the anionic species concentration and attributed this effect to aggregation phenomena of the studied species. The same research group observed that the same micelles formed of anionic aggregates were even able to solubilize undissociated molecules of acid. They[7] concluded that the obtained values had no thermodynamic value.

Other authors[8,9] supposed that the difficulty for the determination of accurate constant values was due to their slight solubility in water. They then proved to settle the problem by determining the pK_a of some cholanic acids (simple and conjugated with glycine) performing potentiometric measurements in a mixed solvent formed by water and methanol (50 %).

The same research group[10] studied potentiometrically a series of bile acids conjugated with glycine in mixed solvents of water−methanol and water−dimethyl sulfoxide in different percentages. The values obtained with this method were extrapolated to zero percentage of organic solvent to obtain the values in aqueous solutions.

By investigating the formation of compounds between calcium(II) and magnesium(II) with some bile acids and ascertaining the great disparity among the values reported for the acidity constants of HBil, de Castro et al.[11] decided to determine again the acidity constants of these acids. These researchers supposed that the difficulties for the determination were the small solubility values and the formation of micellar aggregates. They proposed values of acid constants for deoxycholic (HDC), dehydrocholic (HDHC), and chenodeoxycholic (HCDC) acids and their conjugated compounds with glycine, determined in physiological conditions obtained from potentiometric and NMR measurements. However, the proposed values, although determined in 0.15 mol·dm^{-3} NaCl and under the critical micellar concentration (cmc), were obtained in excess of alkali and in oversaturated solution, so they cannot be considered equilibrium constants and do not have thermodynamic value.

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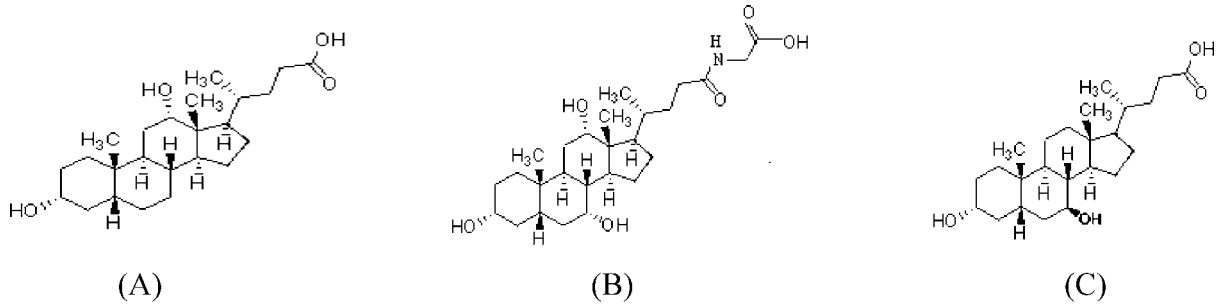

Figure 1. (A) Structure of deoxycholic acid (HDC) as an example of dihydroxycholanic acid. (B) Structure of glycocholic acid (HGC) as an example of trihydroxycholanic acid conjugated with glycine. (C) Structure of ursodeoxycholic acid (HUDC) as an example of dihydroxycholanic acid.

More recently, Fini et al.[12] published a review about "acidity in bile acid systems", where the results obtained in the last 30 years are considered to find a relationship between the values obtained in different conditions and the "true" pK_a value. In the same paper, they collected in a table the cmc and the solubility of several bile acids. In the same paper a further table reports different pK_a values for the cholic acid, that in the opinion of the authors depend on the experimental conditions.

It seems necessary to know reliable values of acid constants and the solubility of the bile acids, determined in aqueous solutions in real equilibrium and in 0.15 mol·dm^{-3} NaCl, under cmc so that aggregation formation is avoided.

The aim of this paper is to propose a method suitable for this purpose and to apply it to cholic (HCol, (3α,7α,12α-trihydroxycholanic), glycocholic (HGC), HDC (3α,12α-dihydroxycholanic), glycodeoxycholic (HGDC), HCDC (3α-7α-dihydroxycholanic), ursodeoxycholic (HUDC, 3α,7β-dihydroxycholanic), lythocholic (HLC, 3α-hydroxycholanic), and HDHC (3α,7α,12α-trihyketholanic) acids.

In this paper, solubility and acid constants are determined in a constant ionic medium constituted of (1.00, 0.50, or 0.15) mol·dm^{-3} NaCl. The values determined in 0.15 mol·dm^{-3} NaCl are directly applicable, but also the others determined in constant ionic medium have thermodynamic values, because it was proved that the adoption of the ionic medium method[13] allows minimization of the variation of the activity coefficients of the reagents in spite of the change of their concentration. In this way it is possible to substitute activities with concentrations.

Symbols are as follows: HA, a generic bile acid; C_{HA}, total concentration of bile acid (HA); C_H, analytical excess of hydrogen ion; c_{HA}, c_H, and c_A, free concentration of bile acid, hydrogen ion, and bile acid anion, respectively; and *s*, solubility of bile acid. Concentrations are expressed on the mol·dm^{-3} scale, represented by M.

Method of Investigation. The nondissociated form of bile acids or of a generic slightly soluble acid can be indicated as HA. The dissociation equilibrium written, as follows: HA $\Leftrightarrow$ $H^+ + A^-$, which is characterized by the constant:

$$K_a = c_H c_A / c_{HA} \quad (1)$$

With the adoption of the constant ionic medium[12] (for instance, 0.15 M NaCl), in the presence of an excess of HA solid, the activity of c_{HA} is constant and, like in the solubility product expression, represents the solubility of HA. As a consequence, c_{HA} can be included in a constant, so that eq 1 can be written as follows:

$$c_{HA} K_a = K = c_H c_A \quad (2)$$

where *K* is a constant in the above-described conditions.

The investigation is divided in two parts. The former is focused to determine the *K* values for each HBil in every ionic medium. The latter is performed to determine the solubility (*s*) of each bile acid in all of the selected ionic media.

In the first part, to obtain *K* values, emf measurements of the galvanic cell R.E./solution S/G.E. (I) were performed. The solution S had the following general composition: C_H M in H^+, ($\mu - C_H$) Na^+ e μ M in Cl^- saturated with HA, that is, in the presence of solid HA and in equilibrium.

G.E. and R.E. are the glass electrode and the reference electrode, respectively, M = mol·dm^{-3}, and μ represents the ionic medium.

At 25 °C and in mV units, the emf of the cell (I) can be expressed by the following relation:

$$E_I = E^\circ_I + 59.16 \log c_H + Ej \quad (3)$$

In eq 3 E°_I, a constant, and E_j, the liquid junction potential[13] due to c_H, were determined in the first part of each measurement before the addition of the solid, that is, when $C_H = c_H$. E_j was found to be a function of c_H only, that is, $E_j = -j \cdot c_H$, where *j* was dependent on the adopted ionic medium.

In the second part of the measurement, a moderate excess of solid HA was added under stirring. It was assumed that, when E_I remained constant within ± 0.1 mV during one hour, the solution could be a considered in real equilibrium with the solid. At this point, by keeping the presence of HBil solid, the solution was gradually alkalinized, generating a constant current by coulometry.

The direct current of intensity of 1 mA, exactly measured, flowed during a time exactly measured. The E_I value at equilibrium could be used to obtain c_H, for each addition of current.

The scheme of the device used to generate direct constant current is shown in Figure 2. The measurement of the potential difference between the ends of a standard resistance allowed the knowledge of exactly the intensity of the flowing current and to check its constancy. The selected intensity of current remained constant within ± 0.01 % or better.

As in the device shown in Figure 2, a stopwatch, able to measure until 0.01 s, is inserted in series, making it possible to obtain the quantity of delivered coulombs and to calculate the OH^- concentration with accuracy.

The emf measurements and the electricity quantities allow the calculation of *K* in eq 2 for each addition of current. The *K* values obtained depend on the solubility, *s*, of each acid HA.

In the second part, to determine *s*, saturated solutions of HBil were prepared by bringing into contact an excess of each tested acid with an aqueous solution of the selected ionic medium. The mixture was stirred at constant temperature until equilibrium

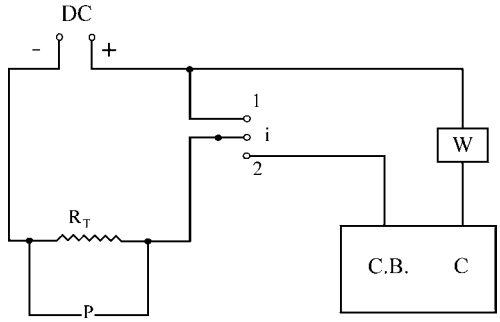

Figure 2. Device delivering direct current of constant intensity. DC = direct current generator; R_T = standard resistance; P = potentiometric circuit to check current intensity; C.B. = coulometry bridge; C = solution S vessel; i = switch; W = stopwatch.

was reached. Reaching equilibrium was verified by drawing samples at time-defined intervals and analyzing them.

The saturated solutions were filtered many times to eliminate the solid completely (solution S′) and potentiometrically titrated by means of the following galvanic cell: R.E./Solution S′/G.E. (II). The titration of solution S′ was carried out coulometrically, that is, flowing constant direct current through the solution for selected times and measuring the emf of cell (II) after each addition. The equivalence point was appreciated applying a modification to the method proposed by Gran.[14] Each equivalence point was used to obtain the corresponding solubility.

By combining the previously determined K values with solubility $s = c_{HA}$, it was possible to obtain accurate values of log K_a.

Experimental Section

Material and Analysis. The bile acids HCol, HCDC, and HUDC were Sigma products, whereas HDC, HLC, and HDHC were Fluka. The other acids, HGC and HGDC, were a gift of the firm Prodotti Chimici ed Alimentari, S.p.a., Basaluzzo Alessandria, Italy. The purity of the reagents was checked by TLC (thin layer chromatography) and polarography.

All of the reagents were pure and were used without further purification.

NaCl RP (C. Erba) was recrystallized by bubbling a current of HCl, produced from the reaction between NaCl and H_2SO_4, through a saturated solution of NaCl.

Apparatus. Emf measurements were performed with a model 654 pH meter Metrohm equipped with a glass electrode of the same firm.

In Figure 2 the device used to generate constant current is described. Two salt bridges were used. The first one connected G.E. with R.E. so that emf measurements could be carried out. The second (corresponding to a coulometry bridge, C.B., in Figure 2) was necessary to separate the Ag electrode operating as an anode, from the Belasio electrode working as a cathode present in the vessel (indicated as C in Figure 2) containing solution S or S′. Both bridges were similar to that described by Forsling et al.[15]

This arrangement allowed the following electrodic reactions to be performed separately:

$$\text{cathode: } 2H_2O + 2e = H_2 + 2OH^-$$

$$\text{anode: } Ag + C1^- = AgC1 + e$$

R.E. = Ag, AgCl/Cl^- was prepared according to Brown.[16] G.E., (15 to 30) min after each current addition, acquired a potential constant within ± 0.1 mV, which remained constant for several hours.

The constant current generator was a model 6186 C Hewlett and Packard, and the standard resistance of 100.00 Ω was model 80 Norma No. 1702676 calibrated within ± 0.01 %. The resistance, connected in parallel with a Keithley model 2000 multimeter, allowed checking the intensity of flowing current.

Nitrogen, 99.995 % from a cylinder, was further purified by passing through 20 % NaOH and 10 % H_2SO_4, and the selected ionic medium μ, bubbling through the test solutions, was used to eliminate oxygen and CO_2 from the test solutions. The solutions S or S′ were stirred during the measurements.

The correspondence between the delivered coulombs and the production of OH^- was verified several times on test solutions of known composition. The agreement between direct and back measurements proves that, in the investigated solutions, the equilibria were real.

Results and Discussion

Solutions of the above-listed bile acids, equilibrated with the corresponding solid HBil, were investigated in three different ionic media, gradually generating OH^- by coulometry and by measuring the emf for each addition of current.

To obtain the acid constants, K_a, knowledge also of the solubility of each studied acid, in the same experimental conditions, was necessary. For this reason, the obtained results are presented in two different sections. The first one deals with the determination of solubility, and the second treats the determination of the constants.

Solubility. A solution of the selected ionic medium was equilibrated until saturation with each studied acid. When equilibrium was reached, a measured volume of solution, filtered twice, was introduced in the titration vessel. The absence of cloudiness was checked by means of the Tyndall effect.

The concentration of the acid (= solubility $s = c_{HA}$) was determined by coulometric titration. For each coulometric generation of current, equilibrium was reached in about 15 min, and in correspondence the emf was measured.

The emf for each point could be expressed at 25 °C and in mV units as a function of the hydrogen ion concentration, as follows:

$$E_{II} = E_I^\circ + 59.16 \log c_H \tag{4}$$

In eq 4 E_j is neglected, as c_H was relatively low. As the bile acids are weak acids, the following relation can express c_H for each addition:

$$c_H = K_a[c_{HA} - (itF^{-1}V^{-1} - c_H)]/[(itF^{-1}V^{-1} + c_H)] \approx c_H$$
$$= K_a[c_{HA} - (itF^{-1}V^{-1})]/[itF^{-1}V^{-1}] \tag{5}$$

Equation 5 can be assumed by neglecting c_H in the numerator and denominator in proximity of the equivalence point. In eq 5 i represents the current intensity, t is the delivering time in seconds, F is the faraday = 96487 coulombs, and V is the volume of the test solution introduced in the vessel.

By introducing into eq 4 the c_H value obtained from eq 5, a function similar to that proposed by Gran is obtained:

$$\phi = K_a^{-1}it(FV)^{-1}(10 \exp(E_{II} - E_{II}^\circ)/59.16$$

$$= c_{HA} - itF^{-1}V^{-1} \tag{6}$$

The function ϕ can be obtained from the experimental values, and it decreases linearly by increasing the delivering time (t), because i, V, and F are constants.

By extrapolation, when $\phi = 0$, eq 6 becomes: $c_{HA} = itF^{-1}V^{-1}$.

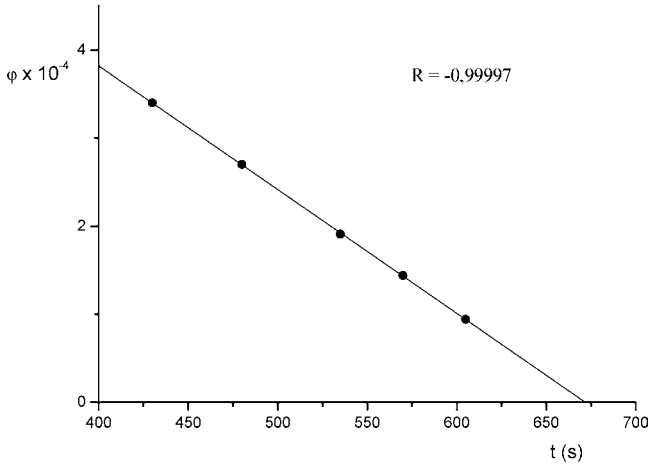

Figure 3. Determination of the HGDC solubility by means of coulometry shown as an example. The equivalent point is obtained by applying a modification of the Gran method.[14] The expression $\phi \cdot 10^{-4}$ indicates that the plotted values are multiplied for 10^{-4}.

Table 1. Solubility (*s*) Proposed Values as log *s*, in the Adopted Ionic Media for the Following Bile Acids: HCol, HDC, HLC, HCDC, HUDC, HDHC, HGC, and HGDC[a]

	μ = 1.00 M	μ = 0.50 M	μ = 0.15 M
bile acid	−log *s*	−log *s*	−log *s*
HCol	3.68 ± 0.01	3.63 ± 0.01	3.61 ± 0.01
HDC	4.09 ± 0.01	3.90 ± 0.01	3.99 ± 0.01
HLC	3.99 ± 0.01	3.94 ± 0.01	3.96 ± 0.01
HCDC	3.85 ± 0.01	3.88 ± 0.01	3.99 ± 0.01
HUDC	4.00 ± 0.01	3.89 ± 0.01	3.95 ± 0.01
HDHC	3.68 ± 0.01	3.65 ± 0.01	3.90 ± 0.01
HGC	3.22 ± 0.01	2.98 ± 0.01	2.87 ± 0.01
HGDC	3.79 ± 0.01	3.92 ± 0.01	3.86 ± 0.01

[a] M = mol·dm^{-3}.

As an example, Figure 3 shows the function ϕ (values·10^{-4}) plotted versus the delivering time *t*. The trend of the points can be well-approximated with a straight line. The value of c_{HA} corresponds to *s* for the investigated acid.

By applying the same procedure, the values of solubility for all of the studied bile acids were obtained. Table 1 collects the solubility of the studied acids obtained in the three selected ionic media, that is, (1.00, 0.50, and 0.15) mol·dm^{-3} NaCl.

Acidity Constants. From the emf measurements of cell (I) and from the delivered coulombs, the values of log *K* can be calculated for each point.

As an example, in Table 2, the obtained log *K* values, constant within ± 0.01, obtained from two different series of measurements are collected.

The constancy of log *K* supports the absence of aggregation, as expected, and the assumption that all data are obtained in a condition of real equilibrium.

The average values of log *K* obtained for each studied bile acid in each selected ionic medium are reported in Table 3.

By introducing the obtained −log *K* and −log *s* for each acid in the eq 2, the −log K_a for each investigated acid can be obtained.

Table 4 reports the −log K_a values obtained for all investigated bile acids, for each selected ionic medium.

Discussion

The main result of this investigation is represented by direct and accurate determination of solubility and acid constants of the selected bile acids in three different ionic media, without extrapolation.

From the comparison of the obtained values both for solubility and for acid constants, it can be observed that often remarkable differences exist among the solubility of the studied acids. Table 1 shows that the solubility depends also on the composition of the ionic medium.

The order of magnitude of solubility is generally about 10^{-4} mol·dm^{-3}, except for glycolic acid, which has the highest solubility.

Dependence of solubility on an ionic medium is less evident for HCol, HUDC, and HLC than for the others. Generally, dihydroxycholanic acids are less soluble than the corresponding trihydroxycholanic acid. HCol is twice as soluble with respect to HDC, which is the least soluble.

Table 2. Example of −log *K* Values, Obtained from Two Different Series of emf Measurements for HGDC in 1.00 mol·dm^{-3} NaCl as an Ionic Medium[a]

coulomb	*E* (mV)	c_H (M)	c_{GDC} (M)	−log *K*
Series a: Volume = 50.00 cm^3; $E°$ = 243.5 mV (corresponding to $C_H = c_H = 1.00 \cdot 10^{-3}$ mol·dm^{-3}); *i* = 0.9940 mA; *M* = mol·dm^{-3}				
0.03007	145.0	$2.163 \cdot 10^{-5}$	$2.786 \cdot 10^{-5}$	9.22
0.1408	134.2	$1.421 \cdot 10^{-5}$	$4.339 \cdot 10^{-5}$	9.21
0.2857	123.1	$9.220 \cdot 10^{-6}$	$6.843 \cdot 10^{-5}$	9.20
0.4501	114.0	$6.472 \cdot 10^{-6}$	$9.976 \cdot 10^{-5}$	9.19
0.8092	99.5	$3.681 \cdot 10^{-6}$	$1.714 \cdot 10^{-4}$	9.20
Series b: Volume =50.00 cm^3; $E°$ = 242.6 mV (corresponding to $C_H = c_H = 1.00 \cdot 10^{-3}$ mol·dm^{-3}); *i* = 0.9945 mA; *M* = mol·dm^{-3}				
0.04162	143.9	$2.146 \cdot 10^{-5}$	$3.009 \cdot 10^{-5}$	9.19
0.1463	132.8	$1.393 \cdot 10^{-5}$	$4.426 \cdot 10^{-5}$	9.21
0.2740	123.0	$9.514 \cdot 10^{-6}$	$6.632 \cdot 10^{-5}$	9.20
0.3978	115.3	$7.054 \cdot 10^{-6}$	$8.950 \cdot 10^{-5}$	9.20
0.7277	101.2	$4.073 \cdot 10^{-6}$	$1.549 \cdot 10^{-4}$	9.20

[a] Average value −log K = 9.20 ± 0.01.

Table 3. Obtained Values for −log *K*, in the Adopted Ionic Media, for the Following Bile Acids: HCol, HDC, HLC, HCDC, HUDC, HDHC, HGC, and HGDC[a]

	μ = 1.00 M	μ = 0.50 M	μ = 0.15 M
bile acid	−log K	−log K	−log K
HCol	8.51	8.36	8.29
HDC	10.02	9.60	9.53
HLC	12.32	12.29	12.24
HCDC	9.71	9.51	9.46
HUDC	10.24	10.04	10.03
HDHC	8.85	8.72	8.65
HGC	7.07	6.94	6.66
HGDC	9.20	9.00	8.95

[a] M = mol·dm^{-3}. The error associated to the above reported −log K can be estimated as ± 0.01.

Table 4. Obtained Values for −log K_a, in the Adopted Ionic Media, for the Following Bile Acids: HCol, HDC, HLC, HCDC, HUDC, HDHC, HGC, and HGDC[a]

	μ = 1.00 M	μ = 0.50 M	μ = 0.15 M
bile acid	−log K_a	−log K_a	−log K_a
HCol	4.83	4.73	4.68
HDC	5.93	5.70	5.54
HLC	8.33	8.35	8.28
HCDC	5.85	5.63	5.47
HUDC	6.24	6.15	6.08
HDHC	5.17	5.07	4.75
HGC	3.85	3.96	3.79
HGDC	5.41	5.08	5.09

[a] M = mol·dm^{-3}. The error associated to the above reported −log K_a can be estimated as ± 0.02.

Often the dependence on the ionic medium is not very evident. This consideration makes the extrapolation to zero ionic strength hard, as values are obtained in different conditions.

Vice versa this trend can be explained by assuming, as shown previously,[17−21] that sodium ions can associate, more or less strongly, with some bile salts.

Other remarkable differences between conjugated and nonconjugated bile acids can be observed. HGC and HGDC are more soluble and more dissociated than the corresponding HCol and HDC.

The acid HLC has a solubility comparable to the others, but it is the weakest, followed in the order by HUDC and HCDC.

HDHC has a solubility comparable to that of HCol but is weaker than HCol.

The values of the constants determined in 0.15 mol·dm^{-3} are directly applicable, except for the temperature, to the physiological conditions.

The values proposed in Table 4 cannot be compared to those obtained in different solvents or in mixed solvents,[8−10] or with values extrapolated from mixed solvents,[10] because the properties of water as a solvent are completely different to other solvents. Furthermore, the change of ionic composition of the aqueous solutions involves a variation of the activity coefficients that cannot correspond to constants determined directly in 0.15 mol·dm^{-3}.

The paper of de Castro et al.[11] reports two tables of results. In one table the authors present their obtained values, and in the other literature data are shown. These authors claim that their potentiometric titrations were performed at concentrations of bile acid lower than the relative cmc. However, from the reported pK_a and solubility, it can be calculated that precipitate of HCol takes place at pH = 3.5 and $C_{HCol} = 1 \cdot 10^{-3}$ mol·dm^{-3}.

The values of de Castro et al.[11] do not agree with the others because they were obtained in nonequilibrium conditions, in oversaturated solutions, or with unsuitable methods. Other values are also not in agreement.

Fini et al.[10] for the acidity constants of HGC and HGDC proposed 3.88 and 3.84, respectively, while in a table[11] for both acids the value 3.88 is reported. Probably the contradiction is due to a misprint, because it is very hard to assume that a conjugate dihydroxycholanic acid, HGDC, is an acid stronger than the corresponding trihydroxycholanic acid.

Moiri et al.[22] propose solubility and $-\log K_a$ values for HCol, HDC, and HCDC, determined in 0.01 mol·dm^{-3} HCl. As expected, they find different values from the others, because the presence of HCl decreases solubility and increases the possibility to form aggregates. Also from Moiri et al. data, HCol is the more soluble than HDC and HCDC, but they find a lower value than ours.

On the other hand, it is surprising that HDC appears stronger than HCol, because it is known that dihydroxycholanic acids are weaker than the corresponding trihydroxycholanic acids. Under this point of view, the values proposed by Ekwall et al.[6] are more acceptable because they show that HCol is an acid stronger than HDC.

It seems interesting to comment on the review of Fini et al.[11] They analyze the problem under different points of view, but arrive at the conclusion that "different pK_a values can be found for the same compound according the nature of the medium". It seems that these authors attribute the variation of pK_a to physical properties. We do not agree, because, in our opinion, the different values found by several authors are not in agreement because they refer to solutions not in real equilibrium or in the presence of not considered coexisting equilibria (for example, incipient precipitation, formation of dimeric species, or greater aggregation products). Also, the change of the activity coefficients plays a role. But, as Fini et al. also suggest: "experimental conditions for the study of isolated monomers of bile salts must be carefully controlled since the association cannot simply be ignored, even in a dilute solution of bile salts".

Conclusion

The method proposed in this paper is of general application for the determination of solubility and dissociation constants of slightly soluble acids, in real equilibrium. The method is applicable with success also in case of the possibility of aggregation or micelle formation.

By delivering a small, exactly measurable quantity of electricity, the possibility of addition of a very pure reagent (the current), completely free of CO_2, with a very low and exactly known concentration, allows avoidance of interfering phenomena and the gathering of reliable data in conditions selected for different purposes.

The application of the proposed method allows the solubility and acid constant values for some bile acids in three different ionic media, (1.00, 0.50, and 0.15) mol·dm^{-3} NaCl, to be obtained.

The results obtained in this work are different with respect to the literature data, both for solubility and for dissociation constants. Literature data were obtained in mixed solvents (water mixed with different organic solvents in different percentages) or in conditions of no real equilibrium.

As a general comment, it seems reasonable to consider low accuracy emf measurements with a very low concentration of HBil to obtain equilibrium constants because the system is not buffered enough to obtain stable and reliable responses. Furthermore, we are not able to find in the mentioned research any confirmation of the reproducibility of the emf measurements performed in solutions at real equilibrium. No mention of agreement between direct and back measurements is found.

We have proved that the values proposed here are reliable, accurate, and obtained in solutions at real equilibrium, where coexisting equilibria were not present in an appreciable amount. Our values of solubility and pK_a 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. For example, these values are of fundamental importance to evaluate the solubility products of calcium(II) and magnesium(II) bile salts in physiological conditions.

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