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Protolytic Equilibria in Aqueous Sodium Deoxycholate Solutions

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Protolytic equilibria taking place in aqueous solutions of sodium deoxycholate (*DC*Na) have been studied at 25 °C using 0.5 *M* NaCl as ionic medium. Electromotive force measurements of a galvanic cell were carried out by means of a glass electrode.

The reagent necessary to change the acidity of the solutions was produced "*in situ*" by supplying a constant small current.

Solubility and acid constant of deoxycholic acid (H*DC*) have been determined for the chosen experimental conditions. Experimental data obtained in less acid solutions have been explained by assuming the presence of the species $H(DC)_2$. The relative stability constant has been determined. At higher deoxycholate concentration the presence of a polymeric micellar species has been assumed.

(Keywords: Micelles; Polymeric species; Solubility)

Protolytische Gleichgewichte in wäßrigen Natriumdesoxycholat-Lösungen

Die Protonierung von Natrium-Desoxycholat (*DC*Na) in wäßrigen Lösungen mit 0.5 *M* NaCl wurde bei 25 °C mit Hilfe von E.M.K. Messungen mit einer Glaselektrode untersucht.

Das notwendige Reagens für die Umwandlung der Säure in den untersuchten Lösungen wurde „*in situ*" durch einen konstanten schwachen Strom erzeugt.

Löslichkeit und Dissoziationkonstante von Desoxycholsäure (H*DC*) wurden unter den gewählten experimentellen Bedingungen bestimmt. Die experimentellen Daten in schwach sauren Lösungen konnten mit der Annahme der Existenz von $H(DC)_2$ erklärt werden. Die entsprechende Konstante wurde bestimmt. Zur Erklärung der Daten in stärker konzentrierten Lösungen von Desoxycholat ist die Annahme einer polynuklearen Spezies nötig.

Symbols: H = analytical excess of hydrogen ions, if negative it corresponds to OH^-; h = free concentration of hydrogen ions; A = total concentration of deoxycholate; a = free concentration of deoxycholate; K_a = acid constant of deoxycholic acid (H*DC*) defined as follows: $[HDC]\ K_a = h\,a$; $\beta_{q,p}$ stability constant of a species $H_p(DC)_q$ defined as follows: $[H_p(DC)_q] = \beta_{q,p} h^p a^q$; C^0 = solubility of H*DC*; $\bar{n}$ = formation function representing the average number of H^+ bonded to deoxycholate.

Introduction

Ekwall et al.[1,2] have studied solutions of sodium deoxycholate (*DC*Na) extensively. They derived the solubility and the acid constant of H*DC* from electromotive force (e.m.f.) measurements. Their measurements were carried out at 20 °C, and at variable ionic strength (μ); results were corrected for μ. The same workers reported the only reliable references.

Josephson[3] calculated a value of K_a from e.m.f. measurements performed in solutions containing *DC*Na and variable amounts of HCl.

Kumler and *Halverstadt*[4] calculated approximate values of K_a in water from their measurements carried out in water—ethanol. The existing values are inconsistent and are affected by remarkable approximations since the workers mentioned above neglect both the change of activity coefficients and the liquid junction potential. No data have been collected in a definite ionic medium and nothing is known about the eventual presence of polymeric species. Moreover a localised precipitation takes place by adding HCl to *DC*Na and the precipitate redissolves very slowly.

The aim of our work is to study the equilibria occurring in aqueous solutions of *DC*Na in a wide range of concentration of reagents.

The investigation was performed at 25 °C using 0.5 *M* NaCl as constant ionic medium in order to minimise the variation of the activity coefficients. To avoid local precipitation or supersaturation, *H* was changed by producing the reagent *"in situ" via* coulometry under stirring. A wide range of $-\log h$ was studied. In the range $-\log h \leqslant 6$, the value of K_a and the solubility of H*DC* could be determined, whereas more complicated equilibria take place in the range $6 \leqslant -\log h \leqslant 9$.

Method

All measurements were performed at 25 °C. The e.m.f. of the cell

$$(-)\quad \text{R.E.} \mid \text{Solution } S \mid \text{G.E.}\ (+) \qquad \text{(I)}$$

[where G.E. = glass electrode; R.E. = Ag, AgCl/0.5 *M* NaCl (sat. with AgCl)/0.5 *M* NaCl] was measured. Solution *S* contained an excess of NaCl with respect to the reagents, in order to have the following general composition:

$$H\,M \text{ in } \mathrm{H}^+;\ (0.5-H)\,M \text{ in } \mathrm{Na}^+;\ A\,M \text{ in } DC^-;\ (0.5-A)\,M \text{ in } \mathrm{Cl}^-$$

According to *Biedermann* and *Sillén*[5], by employing a constant ionic medium, it was possible to neglect the variations of the activity coefficients in spite of variations of the concentration of the reagents. Concentrations can thus replace activities in all the expressions. The e.m.f. of the cell (I) at 25 °C and in mV units, can be expressed as:

$$E_I = E_I^\circ + 59.16 \log h + E_j$$

where E_I° is a constant and E_j is the liquid junction potential. Both terms were determined in the first part of each measurement, when $H = h$, i.e. in the absence of deoxycholate. E_j is a function only of h according to Ref.[5]. We found $E_j = -100\, h\, \mathrm{mV}\, M^{-1}$. The terms E_I° and E_j were also determined after each measurement.

The second part of the measurements was carried out in a different way, depending on the $-\log h$ range investigated.

Determination of K_a and of the Solubility of H*DC* ($-\log h \leqslant 6$)

After the determination of E_I° and E_j, a slight excess of solid H*DC* was introduced into solution *S*. All measurements were carried out in the presence of the solid, by gradually decreasing H and measuring E_I. The alcalinisation of solution *S* was performed by means of coulometry, by producing the reagent ($-H$) *"in situ"* in the stirred solution. In such a way, the volume was kept constant, no impurities were introduced, and a slow neutralisation was achieved.

From the material balance, one can write:

$$a = h + i\,t\,(F v)^{-1}$$

where i is the current, t the time, F the *Faraday* i. e. 96 487 Coulombs and v the volume of solution *S*. All these quantities are known. Since h was measured, a couple of values (h, a) was obtained for each experimental point. As the measurements were performed in the presence of solid H*DC*, the concentration of H*DC* is constant as is the product $h\,a = [\mathrm{H}DC]\, K_a = K$.

In order to acquire the value of K_a, it was necessary to determine the solubility, C^0, of H*DC* under the same experimental conditions. The solubility of H*DC* was estimated coulometrically, titrating saturated and filtered solutions. The equivalence point was determined by *Gran*'s method[6]. Since C^0 is $[\mathrm{H}DC]$, the calculation of the value of K_a is straigth forward.

Equilibria in the Range $6 \leqslant -\log h \leqslant 9$

After the determination of E_I^0 and E_j, a current was applied to obtain an alkaline solution with $-\log h \geqslant 9$. Afterwards a solution of *DC*Na was added until a concentration $A\,M$ was reached. In the second part of the measurements, A was kept constant while H was gradually increased. Titrations were interrupted when a little amount of precipitate could be detected.

Also in this $-\log h$ range, known quantities of H or $-H$ were produced coulometrically and h was measured for every point. From measured h and analytical A and H data, the formation function $\bar{n} = (H - h) A^{-1}$ could be calculated. The values $\bar{n}(\log h)_A$ represent the basis for further elaborations.

Experimental

Material and Analysis

H*DC* Calbiochem (A grade) was used without further purification.

*DC*Na Calbiochem (A grade) was recrystallised from acetone—water.

Traces of acetone in *DC*Na were removed in a desiccator under vacuum and by keeping the salt at 60 °C overnight. In order to have a known quantity of crystallisation water, *DC*Na was left for a week at constant humidity. Analysis of the purified product sometimes showed the presence of little amounts of acid or alkali. Analysis was then performed for each titration during the e.m.f. measurements between the first and the second part, after the addition of the *DC*Na solution. The quantity of impurity was determined with good accuracy according to *Gran*[6]. Corrections were often necessary.

Hydrochloric acid and sodium chloride were prepared and analysed as described previously[7].

Commercial ultrapure nitrogen was bubbled through all solutions during the measurements. Water (freshly bidistilled from a solution of $KMnO_4$) was used to prepare all solutions.

Details on the Equipment

All measurements were carried out at 25.00 ± 0.05 °C. The cell arrangement was similar to that described by *Forsling, Hietanen,* and *Sillén*[8]. The reference electrode (Ag, AgCl) was prepared according to *Brown*[9]. E_I was measured by means of a mod. *pHM* 64 Radiometer potentiometer and a Beckman glass electrode No. 40498. Coulometric production of H or $-H$ was achieved in the following cell:

$$(\pm)\ \mathrm{Ag,AgCl}\ |\ 0.5\,M\,\mathrm{NaCl}\ |\ \mathrm{Solution}\ S\ |\ \mathrm{Pt\ net}\ (\pm) \qquad \text{(II)}$$

A mod. 6186 C Hewlett Packard DC source current was used to obtain a constant current. Time was measured automatically by means of a digital chronometer with an accuracy of 0.01 s. During flow of current, solution S was magnetically stirred. The current was checked by measuring the voltage at the terminals of a standard resistor placed in series with (II). The current remained constant within ± 0.03%. Generally values between $2 \cdot 10^{-4}$ and $2 \cdot 10^{-3}$ A were selected. E_I became constant in a few minutes after each current addition in the absence of *DC*Na. In solutions $A\,M$ in *DC*Na it was often necessary to wait 4–5 h to reach the constancy of E_I within ± 0.2 mV. Equilibria were reached very slowly. When E_I was constant, it remained constant for several hours. It was assumed that measurements were carried out at equilibrium because duplicate measurements as well as direct and back titrations agreed.

Results

According to the description of the methods, the results are also reported in two sections.

Determination of K_a and of the Solubility of H*DC* ($-\log h \leqslant 6$)

Five series of measurements were performed to evaluate the best value for K. The average value was $-\log K = 9.57 \pm 0.02$. K remained constant up to $-\log h \simeq 6$. At high A and $-\log h$, K varied systematically. This trend can be explained by assuming that a constant value of K indicates the presence of the monomeric species, the amount of polymeric or associated species being neglegible. On increasing A and $-\log h$, appreciable quantities of polymeric species formed, thus affecting the value of K. In order to calculate K_a from K, the knowledge of the solubility of H*DC* was needed. Several determinations were carried out to determine C^0 under the selected experimental conditions. The average value was $-\log C^0 = 4.68 \pm 0.04$. By combining C^0 and K, $-\log K_a$ was found to be 4.89 ± 0.04.

Solubility measurements could be used to verify the mononuclearity of H*DC* within this $-\log h$ range. The titration of H*DC* solutions in the absence of solid could be used to calculate approximate K_a values for every point. The average value was very close to the value reported above (within experimental error) but, as expected, values were not precise because the solutions S were not buffered in the absence of solid.

Equilibria in the Range $6 \leqslant -\log h \leqslant 9$

Measurements at several A values were performed in the range $5 \cdot 10^{-4} \leqslant A \leqslant 0.05\,M$. First, data at $A = (0.5;\ 1.0;\ 2.0;\ 5.0) \cdot 10^{-3}\,M$ were considered. Data were plotted in the form $\bar{n}$ vs. $-\log h$ in Fig. 1. It can be seen that the experimental points fall on different curves for each A value. $\bar{n}$ is an increasing function of A. This can be explained by assuming the presence of polynuclear species in *DC*Na. From the K_a value the concentration of H*DC* can be considered negligible with respect to A in this range of $-\log h$. The interval between two successive curves of Fig. 1 can be measured. In every case it was found that: $(\Delta \log A)/(\Delta \log h) = 1$. According to *Sillén*[9], one can assume that a dimeric species is prevailing in such solutions. In order to demonstrate the correctness of the above assumption, $\bar{n}$ was plotted vs. $\log(hA)$. Fig. 2 shows that the points fall on the same curve, independently of A values. $\bar{n}$ is well represented by:

$$\bar{n} = \beta_{2,1}\,h\,a/(1 + 2\,\beta_{2,1}\,h\,a)$$

From the material balance (taking into account the mass action law) it can be written:

$$A = a + 2\,\beta_{2,1}\,h\,a^2$$

According to *Sillén*[10]—in order to find out the value of $\beta_{2,1}$—the experimental points were compared with a curve of equation:

$$\bar{n} = u/(1 + 2\,u) \quad \text{where} \quad u = \beta_{2,1}\,h\,a$$

Since the experimental points were plotted as $\bar{n}$ vs. $-\log(hA)$, it was necessary to plot the normalised curve as $\bar{n}$ vs. $[\log u + \log(1+2u)]$. Experimental and normalised curves were superimposed and moved parallel along the abscissa for a best fit. In this position, the value $\log \beta_{2,1} = 9.3 \pm 0.1$ was obtained. The uncertainty of $\beta_{2,1}$ corresponds to the maximum shift that is possible with an acceptable agreement. The best fit between the normalised curve and the experimental points is reported in Fig. 2.

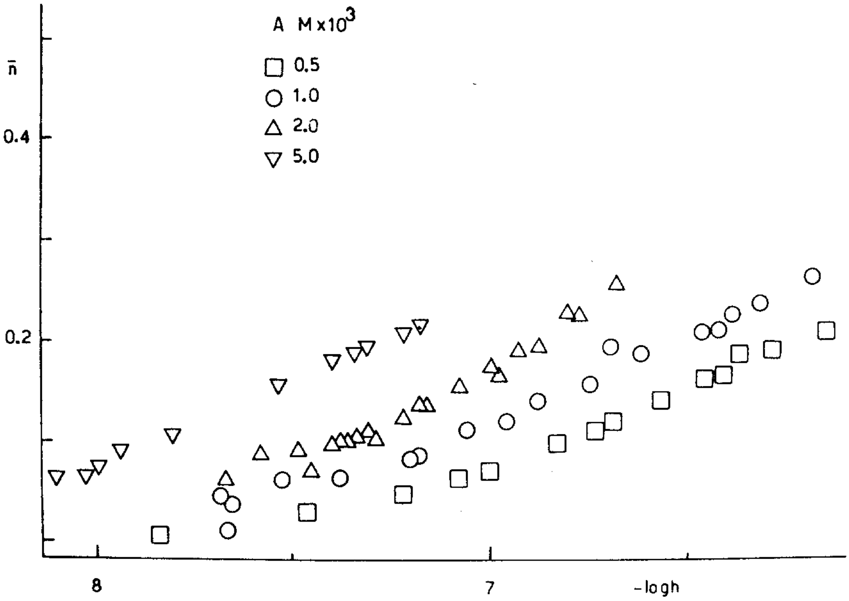

Fig. 1. Experimental data $\bar{n}$ vs. $-\log h$ at different A

Data relative to the range $5 \cdot 10^{-3} \leqslant A \leqslant 0.05\,M$ could not be explained with the assumption of the species $H(DC)_2$ with $\log \beta_{2,1} = 9.3$. As the concentration of $H(DC)_2$ was negligible with respect to A, other species were prevailing.

Several measurements at $A = 0.01$; 0.015; 0.020; 0.030; 0.035; 0.050 M were performed and in every case $\bar{n}$ was an increasing function of A. Polymeric species were prevailing when $q > 2$. Since the E_I values became constant more slowly than before and the results were less reproducible, it is not possible to make a quantitative treatment, but a few points can be made. For every A value $\bar{n}$ tends to 0.3 and the interval among the curves leads one to assume that polymeric species (with an average ratio 1 : 3 between p and q) prevail. This is confirmed by the fact that all the experimental points lie approximatively on the same curve in a plot $\bar{n}$ vs. ($-\log h^{1/3} A$). It is possible to explain the experimental data by assuming the presence of a prevailing species of composition $[H\,(DC)_3]_r$.

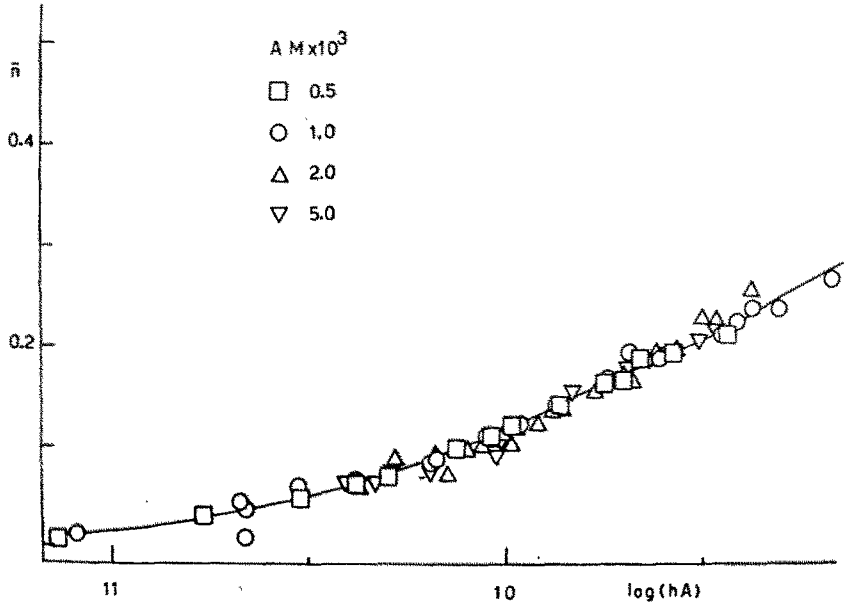

Fig. 2. $\bar{n}$ vs. $-\log(h\,A)$; the normalised best-fitting curve is shown

By comparing the plot $\bar{n}$ vs. $\log(h^{1/3} A)$ with a family of normalised curves[11] of equation $\bar{n} = p\,u^q/(1 + q\,u^q)$, it is possible to find an agreement between the two plots for $20 < r < 50$. The most probable value is $r = 30$. This means that the prevailing species seems to have an average composition $H_{30}\,(DC)_{90}$ with high stability.

Conclusions

A comparison of the value of K_a proposed in the present work with literature data is presented in Table 1.

Our value of K_a is lower than the value in Ref.[1]. Different experimental conditions could play an important role; temperature and ionic strength were different.

Also the solubility of H*DC* is seriously influenced by experimental conditions. Our value is much lower than that given by *Gillert*[12], i.e. $-\log C^0 = 3.21$ (at 15 °C and in water). The value proposed by *Gillert* was already considered too high by *Ekwall* et al.[2]. The latter authors reported $-\log C^0 = 3.98$ for the solubility of H*DC* in solutions of *DC*Na at 20 °C.

Table 1. *Comparison of reported values for K_a*

	K_a
This work	4.89 ± 0.04 (25 °C and 0.5 M NaCl)
Ref.[1]	5.17 ± 0.04 (20 °C, extrapolated for ionic strength)
Ref.[3]	6.42 (variable ionic strength)
Ref.[4]	4.97 (calculated from measurements in water—acetone)

It seems of interest to compare our results about nuclearity of *DC*Na with those of *Ekwall* et al.[1]. We found that in very diluted solutions ($A < 5 \cdot 10^{-4} M$) a monomeric species prevails. It is the only species existing in appreciable amounts when the concentration of H*DC* is lower than $10^{-4} M$. The data of this work suggest that at $A > 5 \cdot 10^{-4} M$ only the dimeric form occurs and the monomeric species is negligible. The species $\mathrm{H}(DC)_2$ with $\log \beta_{2,1} = 9.3$ exists within the range $5 \cdot 10^{-4} \leqslant A \leqslant 5 \cdot 10^{-3} M$. Beyond this limit, the concentration of $\mathrm{H}(DC)_2$ is negligible, while species with a higher polimerisation degree appear. The most probable average composition seems to be $\mathrm{H}_{30}(DC)_{90}$, which is a very stable aggregate.

Ekwall et al. explained their experimental data with similar assumptions but they propose different limits without quantitative results. They say that the monomeric form is prevailing up to $A \simeq (4.5—5.5) \cdot 10^{-3} M$. A transition occurs in the range $5 \cdot 10^{-3} \leqslant A \leqslant 0.01 M$. At higher A there are remarkable deviations and a polymeric micellar species is probably formed. The three ranges are also present in our work, but our limits of polymerisation are lower because of the greater ionic strength due to 0.5 M NaCl.

In conclusion, at 25 °C and in 0.5 M NaCl when $A \ll 5 \cdot 10^{-4} M$ the species H*DC* prevails. $\mathrm{H}(DC)_2$ predominates in the range $5 \cdot 10^{-4} \leqslant A \leqslant 5 \cdot 10^{-3} M$. At $A \geqslant 5 \cdot 10^{-3} M$ the formation of a micellar species of probable average composition $\mathrm{H}_{30}(DC)_{90}$ with high stability takes place.

Both the presence and the concentration of the ionic medium seem to play a very important role.

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