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The Conductance of Conjugated and Unconjugated Bile Acid Salts in Aqueous Solutions

Bile Acids and Steroids 80

ARNE NORMAN*

Department of Physiological Chemistry, University of Lund, Lund, Sweden

The equivalent conductances of aqueous solutions of the sodium salts of cholic, taurocholic, glycocholic, deoxycholic, taurodeoxycholic, glycodeoxycholic and dehydrocholic acids in the concentration range from 0.002 to 0.5 M were measured at 40°C. All these bile salts were found to differ from a normal uni-univalent electrolyte in their conductance behaviour. The beginning association leads to a greater than normal increase in the conductance in cholate and deoxycholate solutions. The advancement of the association in more concentrated solutions causes the slope of the curve plotting equivalent conductance against root concentration to decrease. With deoxycholate this results in a maximum which is followed by a minimum, while with cholate only a change in the slope of the curve occurs. The variations in the courses of the conductance curves are less pronounced for bile salts than for paraffin chain salts and therefore an evaluation of the critical concentration from the conductance data is not possible for bile acid salts. The conductance curves for the conjugated bile acid salts do not differ essentially from those for the unconjugated bile acid salts, which may be taken to indicate that the progress of association is similar.

Numerous studies have shown that salts of bile acids are association colloids. From the values of the osmotic coefficients of bile acid solutions Roepke and Mason [2] concluded that an association takes place in solutions of both conjugated and unconjugated bile acid salts. McBain *et al.* [3] studied the solubilization of water-insoluble lipophilic dyes by cholate and deoxycholate solutions and deduced that the hydrotropic properties of bile acid salt solutions are due to the formation of micelles. Micelle formation is not so clearly revealed by conductance data because of the weak influence of association on conductance in bile salt solutions [2]. Mellander and Stenhagen's [4] measurements showed

* Present address: Department of Chemistry, Karolinska Institutet, Stockholm.

that the conductance of sodium taurocholate solutions was normal below 0.01 M but above this concentration the conductance curve suddenly changed its slope. Ekwall and Fontell [5] studied the conductances of sodium cholate solutions over a wide concentration range (0.005 M to 0.4 M) and found that the curve plotting the equivalent conductance against the square root of the concentration resembled the curves obtained for soaps, but was less affected by the association.

Extensive studies of the association in sodium cholate and deoxycholate solutions have been carried out by Ekwall and coworkers [6,7]. A stepwise association was found to take place and three different concentration limits (limits 1, 2 and 3) were shown to exist in the solutions of the bile acid salts studied. Below the first limit, there is no association. Between the limits 1 and 2 the association is of limited scope and leads to the formation of very small aggregates, but already above the limit 2 all the added substance associates to form somewhat larger aggregates. Above the limit 3, the degree of association increases further. Aggregates of colloidal dimensions could be demonstrated to exist down to the limit 3. The concentration limits in deoxycholate solutions were 0.004—0.006 M (limit 1), 0.009—0.010 M (limit 2) and 0.04—0.05 M (limit 3), while those in cholate solutions were 0.013—

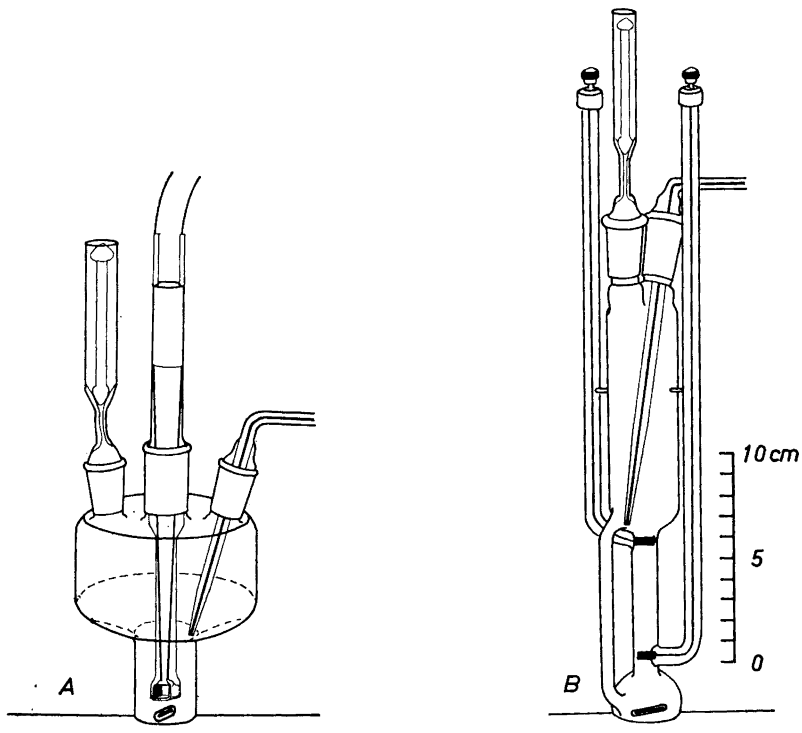

Fig. 1. Conductance cells.

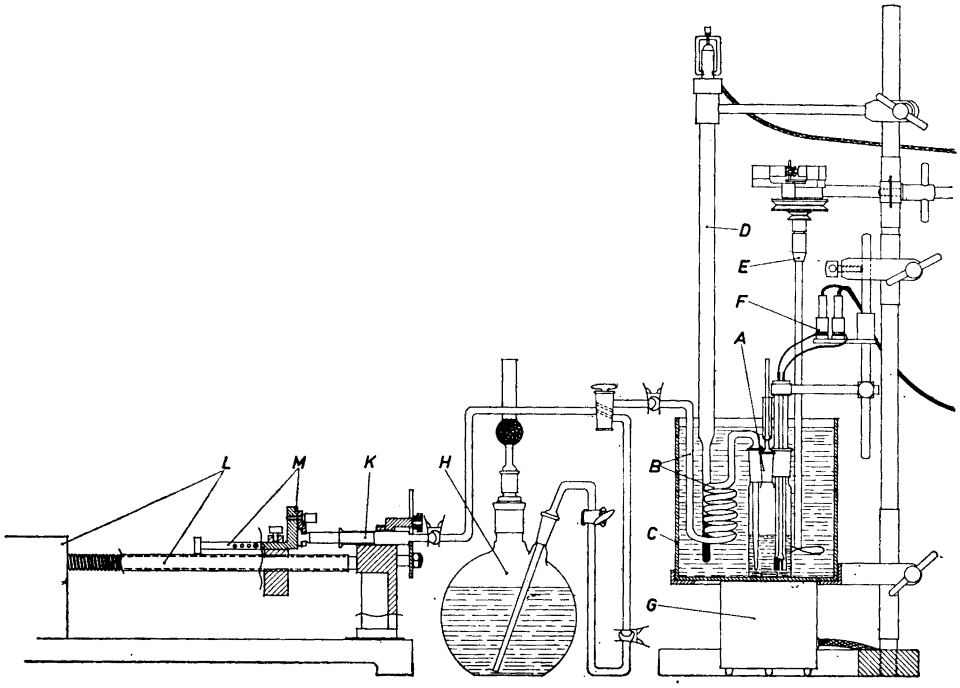

Fig. 2. Apparatus for the continuous introduction of water or solution into a conductance cell at a constant temperature. A: Conductance cell (type C). B: Glass tube through which water or solution enters the cell. C: Thermostat vessel of transparent Lucophlex. D: Contact thermometer. E: Air-driven mixer. F: Leads from platinum electrode to measuring bridge. G: Magnetic mixer. H: Storage vessel. K: All-glass syringe. L: Drive mechanism. M: Bracket moving plunger of syringe.

0.015 M, 0.045—0.050 M and 0.09—0.11 M. If one wishes to define the critical concentration for micelle formation in these solutions of bile acids salts, it lies according to Ekwall at the concentration limit 2 where the association is almost complete.

The aim of the present investigation was to study the influence of association on the conductance of aqueous solutions of sodium cholate, deoxycholate and dehydrocholate. The conductances of aqueous solutions of the glycine and taurine conjugates of cholate and deoxycholate were also measured to permit a comparison of the processes of association in solutions of conjugated and unconjugated bile salts. Automatic recording of resistance according to Andersson, Stenhagen and Mellander [1] was used to determine the resistances of bile acid salt solutions on progressive dilution.

EXPERIMENTAL

Materials. The sodium salts of the conjugated bile acids were synthesized as described previously [8]. All the salts were dried *in vacuo* at 100° for 12 h before use. The water employed had been distilled twice and had a specific conductance not greater than 0.5 $\times 10^{-6}$ ohm^{-1} cm^{-1} at 25°C.

Table 1.

Cell type	Cell constant	Minimum volume (ml)	Total volume (ml)
A	0.87	22	210
B	5.78	29	80
C	0.57	40	120

Conductance cell. The conductance cells were of the types shown is Figs. 1 and 2. It was found that the solutions in these cells could be very effectively mixed with rotating magnetic bars. The cells A and C were made of Pyrex glass and the double electrodes used in them of Jena glass. Cell B was made of Jena glass. Each cell was equipped with a valve that allowed air to escape when liquid was added to the cell. The conductance of the solution determined which cell could be used. The electric field between the electrodes varies with the level of the surface of the solution above the electrodes and the effect of this level on the resistance of the cell was studied. The resistances of the cells were measured after the cells had been filled with 0.01 N potassium chloride so that the electrodes were just covered and then continually as more potassium chloride solution was added. Fig. 3 shows the variation of the cell constant with the height of the electrolyte within the cells. It will be seen that the value of the cell constant no longer changes after a certain level has been exceeded. Characteristic values for the three cells are collected in Table 1. The values of the cell constants were checked from time to time using fresh potassium chloride standard solutions.

Thermostat. To avoid condensation of solvent, the whole conductance cell (A) and the spiral tube (B) through which water was introduced into the cell were immersed in the water of the thermostat to the level indicated in Fig. 2. The thermostat (C) was made of transparent Lucophlex, 4 mm thick, and was fastened to a stand. The heat source was a 250 W infrared lamp which was focussed on the wall of the thermostat. With the lamp connected to the electric mains through an electronic relay operated by a contact thermometer (D), the temperature of the water within the thermostat could be kept constant at 40° ± 0.005°C.

Introduction of solution into the conductance cell. The apparatus employed to effect progressive dilution of the electrolyte solution with water is shown in Fig. 2. An all-glass syringe (K) with a spherical ground surface is fastened to a drive mechanism (L) which moves the plunger at a constant velocity. The syringe is connected by a two-way stopcock and capillary tubes to the inlet tube (B) of the conductance cell and to a storage vessel (H). In this way access of carbon dioxide from the air to the water being introduced into the cell is avoided during an experiment. The rate of flow of water was varied between 1 and 2 ml/min. This rate was determined by the time required for the water to warm up to a constant temperature within the spiral tube.

Procedure. Suitable weights of the bile acid salts were dissolved and the solutions diluted to volume in volumetric flasks at 40°C with water free from carbon dioxide. An amount of each solution was then weighed into the conductance cell. The cell together with the spiral tube was immersed in the thermostat and fastened by a clamp to the stand. The spiral tube was connected to the unit for introducing water into the cell.

The continuous recording of the electrolytic resistance was carried out with an apparatus described by Andersson, Stenhagen and Mellander [1]. A condition that must be fulfilled when this method is employed is that no greater capacitive unbalance develops during the recording. For a discussion of errors in measurement and for a description of a circuit that permits simultaneous compensation and registration of the capacitance, a paper by Andersson, Möhl and Stenhagen may be consulted [9]. On diluting a 0.1 M taurocholate solution to a 0.02 M solution in cell A, the capacitance of the cell increased by only 20 pF while the resistance increased by 335.2 Ω. The null position was re-established eight times by balancing the resistive and capacitive components of the bridge separately while the resistance was being recorded in this experiment. It is thus obvious that the capacitive unbalance developed did not materially affect the recorded resistance values.

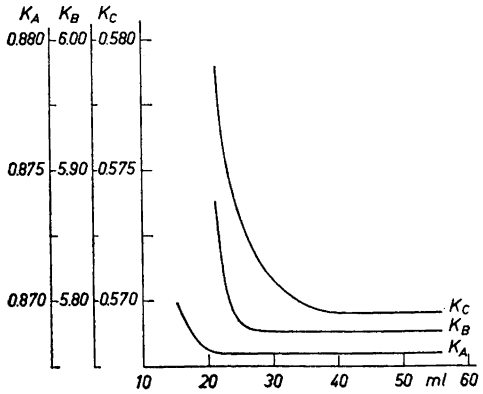

Fig. 3. Variation of cell constants for cells A, B and C as a function of the added volume of 0.01 M potassium chloride solution. Temperature: 40°C. Continuous recording of electrolytic resistance.

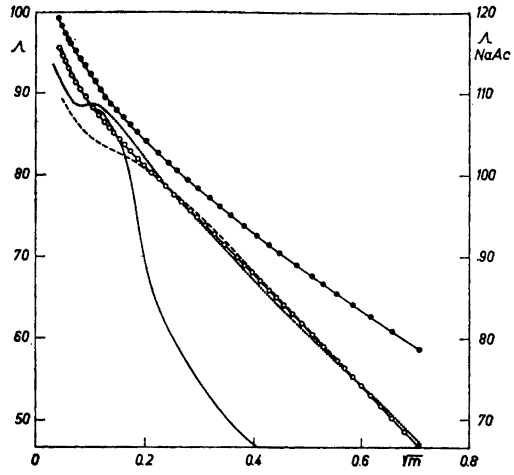

Fig. 4. Equivalent conductance at 40°C. as a function of the square root of the molar concentration for sodium dehydrocholate (O—O), sodium deoxycholate (.....), sodium cholate (— — — —), and sodium acetate (●—●). The values for sodium laurate (———) at 40°C are those reported by Ekwall [14].

RESULTS

The method employed to record the resistance continuously can be applied only to relatively dilute (under 0.05 M) solutions of bile acid salts in which the equilibration of the solution during the addition of water can be effected practically instantaneously. The results of the measurements are plotted in Figs. 5, 7 and 8. When more concentrated solutions are employed, the time that is required before the resistance becomes constant after the addition of a certain quantity of water is so long that a satisfactory accuracy cannot be attained in the measurement of resistance by the continuous recording technique. For example, solutions of the bile salts studied that are between 0.5 and 0.3 M require 10 to 15 min before the value of the resistance becomes constant. Figs. 4, 9 and 10 give the results of the determinations of electrolytic resistance for individual dilution series. Several series of dilutions extending over the same concentration range were prepared from each bile acid salt. Separately recorded curves plotting the equivalent conductance as a function of concentration exhibited satisfactory agreement.

DISCUSSION

The variation of equivalent conductance against root concentration is shown for sodium cholate, deoxycholate, dehydrocholate, acetate and laurate in Fig. 4. The courses of the curves for these compounds in the concentration range 0.002—0.05 M are seen in Fig. 5.

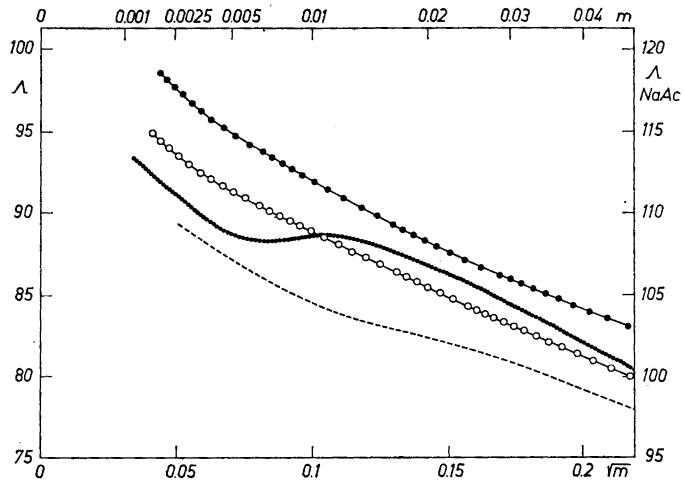

Fig. 5. Equivalent conductance at 40°C as a function of the square root of the molar concentration for sodium dehydrocholate (O—O), sodium deoxycholate (.....), sodium cholate (— — — — —) and sodium acetate (● — ●).

In the concentration range below 0.05 M, the curve for dehydrocholate is similar in form to the curve for a normal uni-univalent electrolyte, *e.g.* sodium acetate. According to McBain [3] the osmotic coefficients of dehydrocholate solutions do not deviate significantly from the values for a normal electrolyte and Ekwall and coworkers [10] were unable to detect any association in dilute solutions of this bile acid salt by solubilization studies. The latter authors found that a low degree of association occurs when the concentration of the salt exceeds 0.15—0.2 M (limit 1) and a higher degree of association at concentrations above 0.7 M (limit 2). Accordingly, no deviation from the normal variation of the conductance was to be expected below 0.15 M.

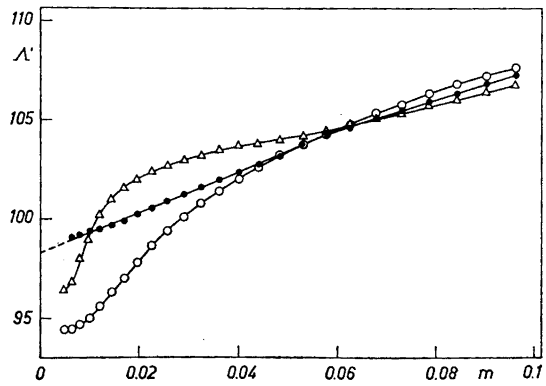

Fig. 6. Plot of Λ' *versus* molar concentration for sodium cholate (O—O), sodium deoxycholate (△—△), and sodium dehydrocholate (● — ●). 40°C.

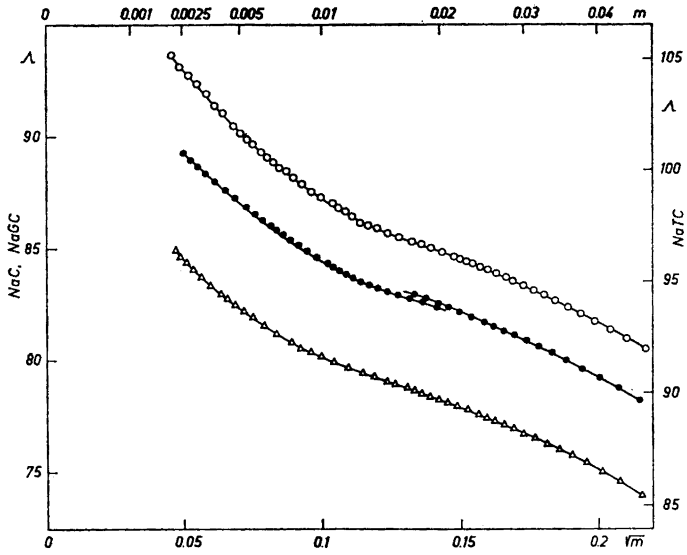

Fig. 7. Equivalent conductance at 40°C as a function of the square root of the molar concentration for sodium cholate (●—●), sodium taurocholate (O—O), and sodium glycocholate (△—△).

Both sodium cholate and sodium deoxycholate exhibit a behaviour that deviates clearly from that of a normal uni-univalent electrolyte. The equivalent conductance of sodium cholate begins to decrease at a slower rate than in the case of a normal electrolyte when the concentration exceeds 0.01 M but when the concentration exceeds 0.04 M the conductance decreases more rapidly. The curve for sodium deoxycholate passes through a minimum at a concentration of 0.005 M and a maximum at a concentration of 0.01 M, while above the latter concentration the equivalent conductance decreases relatively rapidly. The first deviations from the behaviour of a normal electrolyte thus occur with both cholate and deoxycholate at the same concentrations where Ekwall and coworkers [6] detected the first signs of beginning association (limit 1) The second deviations occured at concentrations (limit 2) where the association is, according to Ekwall, of such degree that all the added bile salt anions undergo association.

The deviations from the normal behaviour become more clearly evident when the limiting equivalent conductance Λ_∞ is determined by extrapolating the plot of the function $\Lambda' = \dfrac{\Lambda + A\sqrt{c}}{1 - B\sqrt{c}}$ (where A and B are the constants of the Onsager equation) *versus* concentration (Fig. 6) [11]. The corresponding points for dehydrocholate lie close to a straight line as is the case for a normal electrolyte. The deviations seen at the lowest concentrations are evidently due to hydrolysis and the presence of impurities in the water. The value of the equivalent conductance of sodium dehydrocholate at infinite dilution is

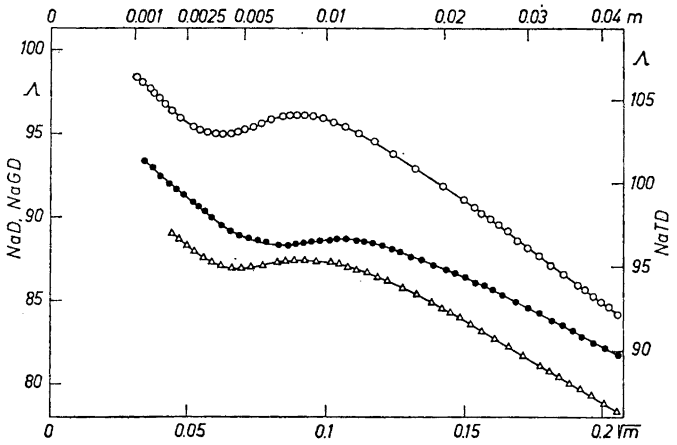

Fig. 8. Equivalent conductance at 40°C as a function of the square root of the molar concentration for sodium deoxycholate (● — ●), sodium taurodeoxycholate (O — O) and sodium glycodeoxycholate (△ — △).

found by extrapolation to be 98.0. The curves plotting Λ' are so abnormal for cholate and, especially, deoxycholate that no value can be obtained for the limiting equivalent conductance by extrapolation.

The equivalent conductance of cholate and deoxycholate solutions between the concentration limits 1 and 2 is higher than the value calculated assuming the existence of single ions only. The increased conductance is possibly due to a higher mobility of the aggregates in accordance with Stokes' law. After the first increase noted in cholate and deoxycholate solutions when association sets in, the conductance decreases more than would be expected for a normal univalent electrolyte as the bile salt concentration increases over the limit 2 (Figs. 4 and 5). The behaviour is similar to that observed in solutions of the sodium salts of higher fatty acids in which a marked decrease in the equivalent conductance occurs above the critical concentration. The decrease in the latter solutions has been mainly ascribed to the binding of gegenions by the micelles. Also the lowering of the conductance with increasing concentration in cholate and deoxycholate solutions above concentration limit 2 may presumably be ascribed to the same factor. Micelle formation in bile salt solutions has only a slight effect on the conductance and hence it is difficult to evaluate the critical micelle concentration of bile salts as in the case of the sodium salts of higher fatty acids.

The curves plotting equivalent conductance against root concentration for solutions of the taurine and glycine conjugates of cholate and deoxycholate (Figs. 7 — 10) all deviate from the corresponding curve for a normal uni--univalent electrolyte but do not differ in this respect essentially from the curves for the unconjugated bile salts. The first deviation from the normal behaviour does, however, occur at a lower concentration (0.003 M) with tauro- and glycodeoxycholate than with unconjugated deoxycholate (0.005 M).

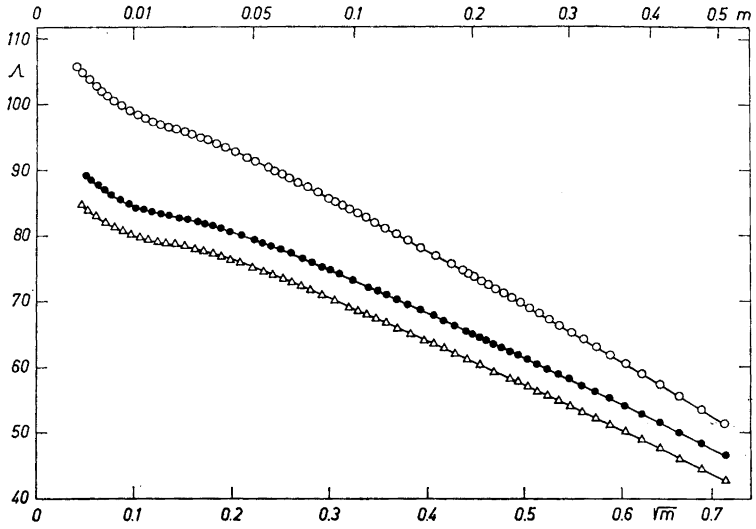

Fig. 9. Equivalent conductance at 40°C as a function of the square root of the molar concentration for sodium cholate (●—●), sodium taurocholate (O—O), and sodium glycocholate (△—△).

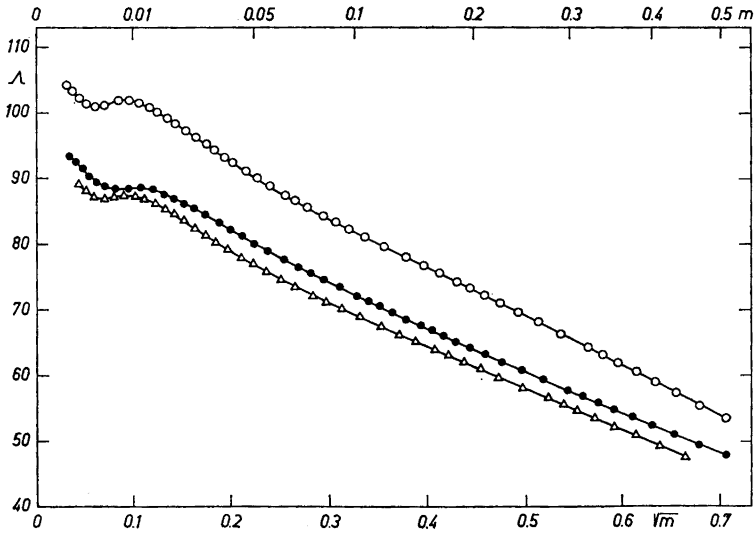

Fig. 10. Equivalent conductance at 40°C as a function of the square root of the molar concentration for sodium deoxycholate (●—●), sodium taurodeoxycholate (O—O), and sodium glycodeoxycholate (△—△).

Judging from the variation of the equivalent conductance with concentration, it seems improbable that there is any greater difference in the nature of the association process between the conjugated and unconjugated compounds. This conclusion is in accord with the results of previous studies of the ability of the taurine and glycine conjugates of cholate and deoxycholate to solubilize xylene [6,12] and 20-methylcholanthrene [13]. These observations suggest that the various phases of the association process, the beginning association and its stepwise development, and the saturation capacities of the aggregates formed are with only minor variations similar for both conjugated and unconjugated bile acid salts.

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