MICELLE FORMATION IN AQUEOUS SOLUTIONS OF BILE SALTS

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The solubility of fatty acids and cholesterol in bile does not appear to be due to the formation of soluble compounds of the choleic acid type. The bile is made up chiefly of the conjugated bile salts and such compounds with conjugated bile acids or salts have not been isolated (1). It is generally agreed that the solution of oils in aqueous solutions of soaps is associated with the micelle formation of the soap, the oil being dissolved, most likely, in the lipid center of the micelle (2-4). The bile salts are similar to the soaps in that they consist of a relatively large hydrophobic group and an ionic group. They might be expected also to form micelles and thus to be able to take up water-insoluble substances in a manner comparable to that of the soaps.

Bashour and Bauman (5) were unable to find a sharp change of slope in the equivalent conductivity-concentration curve of sodium desoxycholate, usually taken as an indication of micelle formation, although freezing point determinations on the more concentrated solutions indicated some degree of aggregation. It seemed likely that measurement of the osmotic coefficient as well as of the equivalent conductivity at various concentrations would offer some evidence as to the state of aggregation of bile salts.

EXPERIMENTAL

Cholic acid was obtained from the Sandoz Chemical Works, Inc. Its moisture content was determined as 2.2 per cent. Glycocholic and glycodesoxycholic acids were prepared by the methods of Cortese and Bauman (6). Sodium oleate was obtained from

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Merck and Company, Inc., and used without further purification. The preparation gave a slightly cloudy solution at the higher concentrations (0.19 to 0.43 M per liter). The bile acids were dissolved in 0.5 N NaOH and adjusted to pH 7.8 to 8.0. The more dilute solutions were obtained by adding various amounts of water to the original solution. The solutions were made up by weight and, from density measurements, the concentration was expressed both on a weight and volume basis. In calculation of the osmotic coefficient, the concentration was expressed as moles of salt per kilo of water, whereas in the calculation of equivalent conductivity the concentration was expressed as moles of salt per liter of solution.

By means of the thermoelectric method as modified by Baldes (7) and by Baldes and Johnson (8), the vapor pressures of the bile salt solutions were compared with those of reference NaCl solutions. The osmotic coefficients of the bile salts were then calculated from the osmotic coefficients of NaCl which were derived from the freezing point data given in International Critical Tables (9). Calculations were made by means of the relation 

$$g_b = \left( m_{NaCl} \times g_{NaCl} \right) / m_b$$

where $g_b$ is the osmotic coefficient of the bile salt at the concentration $m_b$, $m_{NaCl}$ is the concentration of NaCl solution which has the same vapor pressure as the bile salt solution, and $g_{NaCl}$ is the osmotic coefficient of sodium chloride at that concentration, the concentrations being expressed as moles per kilo of water. The osmotic coefficient of sodium chloride was calculated from the relation, $g = \Delta t / (2 \times 1.858 \times m)$, where $\Delta t$ is the lowering of the freezing point and $m$ is the molality. Several measurements with four to six thermocouples permit the determination of the vapor pressure of a solution with an accuracy equivalent to 0.0001 to 0.0002 mole of NaCl per kilo of water. Hence, the determination of the osmotic coefficient can be made with a fair degree of accuracy at a concentration as low as 0.005 M.

Conductivity measurements were made with a Wheatstone bridge obtained from Leeds and Northrup, a Kohlrausch slide wire and a microphone hummer being used. The conductivity cell was made of Jena glass and had a cell constant of 0.5674 cm$^{-1}$. Since the water used in making up the solution had a relatively high conductivity ($2.5 \times 10^{-5}$), conductivity measurements were made only on dilutions down to 0.003 M. All measurements were made at a temperature of 25°.
From the results of the measurement of osmotic activity and of equivalent conductivity it is evident that the bile salts which were studied undergo micelle formation in aqueous solutions. In the case of sodium glycocholate (Fig. 1) the osmotic coefficient is decreased by 50 per cent as the concentration is increased from 0.0047 M to 0.098 M, while the equivalent conductivity is decreased only 19 per cent over the same range of concentration, thus indicating an appreciable association of the bile acid ions. With sodium glycodeoxycholate (Fig. 2) the osmotic coefficient is decreased by 56 per cent and the equivalent conductivity by 19 per cent as the concentration is increased from 0.0047 to 0.093 M. Similar results were obtained with solutions of sodium cholate (Fig. 3).

The results obtained with the bile salts differ somewhat from those obtained with sodium oleate (Fig. 4). In the range of concentration in which micelle formation of sodium oleate occurs,
there is a sharp drop in the equivalent conductivity as well as in the osmotic coefficient. This marked decrease in equivalent conductivity with increase in concentration appears to be true generally for the paraffin chain salts and is the result of decreased ionization due to the local concentration of the ions making up the micelle (2). With sodium glycodesoxycholate (Fig. 2), however, the equivalent conductivity remains nearly constant over a short range of concentration in which the osmotic coefficient decreases markedly. This difference might be explained by postulating that, at least at the lower concentrations, the bile salt micelles consist, on an average, of a smaller number of anions than do the soap micelles. However, definite conclusions concerning this difference between the bile salts and soaps are not justified with the data at hand.

The slight initial rise in the osmotic coefficient curves for sodium cholate and sodium olate (Figs. 3 and 4) is probably due to experimental error. Measurements on these solutions were made at concentrations down to 0.003 M at which concentrations the error
Fig. 3. Osmotic coefficient ($\gamma$) and equivalent conductivity ($\lambda$) of sodium cholate at 25$^\circ$.

Fig. 4. Osmotic coefficient ($\gamma$) and equivalent conductivity ($\lambda$) of sodium oleate at 25$^\circ$.
in the determination of the osmotic concentration may be appreciable. With sodium glycocholate and sodium glycodesoxycholate, osmotic coefficient measurements were made at concentrations of not less than about 0.005 M.

While it cannot be definitely stated at this time that the solution of water-insoluble substances by the bile is associated with aggregation of the bile salt ions, such a conclusion appears reasonable. Bashour and Bauman (5), using pure solutions of the bile salts at 37.3°, found that the amount of cholesterol dissolved per gm. of bile salt increased with the concentration of the bile salt. In the case of sodium glycodesoxycholate, the weight of cholesterol dissolved per gm. of bile salt increased to a constant value of 40 mg. at a concentration of about 3 per cent (0.064 M) of bile salt. As seen from Fig. 2, this is in the range of concentration at which the slope of the osmotic coefficient curve of this salt is rapidly decreasing. It would be of interest to compare the osmotic coefficient-concentration curves of the various bile salts with their solvent properties at the same temperature and pH.

**SUMMARY**

Osmotic coefficient-concentration and equivalent conductivity-concentration curves were determined for sodium glycocholate, sodium cholate, sodium glycodesoxycholate, and sodium oleate at 25°. The results indicate that the bile salts form ionic micelles above a critical concentration. It is suggested that solution of water-insoluble substances by the bile may be associated with micelle formation of the bile salts in a manner similar to the solution of oils by soap solutions.

**BIBLIOGRAPHY**

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