carbon chain. At high temperatures (greater than 270°), however, the saturated solution is concentrated and the environment of a given soap molecule is not so "unfriendly." Here the order of solubility seems to be related to the strength of the neat soap "structure" and is the same as the order of melting points.[3] Consequently, an isotropic solution of sodium myristate in which neat soap just forms is more concentrated than is the corresponding solution of sodium laurate, the effect of increasing chain length here being just opposite to that at lower temperatures.

Summary

A large portion of the phase diagram of the system sodium myristate–water has been constructed from data obtained by visual and microscopic observations of the temperatures at which phase changes occur, supplemented by a few dilatometric determinations and by viewing experiments at selected temperatures. The phase behavior of this system is qualitatively similar to that of systems of the other saturated soaps and of sodium oleate with water.

The effect of the chain length of the soap molecule on the phase diagram involves at least two opposing factors, the longer chain soaps being less soluble at low temperatures and low concentrations of soap while at higher temperatures and high concentrations of soap the shorter chain soaps are the less soluble.

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[FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, CALIFORNIA]

The Solubility Curve of Sodium Deoxycholate in Water

BY ROBERT D. VOLD AND JAMES W. MCBAIN

Determinations of the solubility of sodium deoxycholate in water at different temperatures have been made and used to establish the type of interaction of this substance with water.

An important physiological function ascribed to the bile acids—a generic term which also includes their sodium salts—is to promote transfer of water-insoluble materials.[1] In addition to emulsification, two other mechanisms have been suggested for this process. One[2] (p. 110) is the formation of addition compounds between the bile salt and the water-insoluble substance, the so-called choleic acids. Recently[3–7] it has been recognized that the action may be much less specific, the bile salts simply acting like soap solutions which can solubilize water-insoluble materials by retention on or in the colloidal micelles.[8,9]

The present work was undertaken to determine whether a representative bile salt would form addition complexes with water and whether it would give a phase rule diagram similar to that of the soaps, since both these substances have the same solubilizing action in dilute aqueous solution.[5–7] Sodium deoxycholate was chosen for this study since physico-chemical measurements have been made on its solutions[5] and since it, together with sodium apocholate, has the greatest tendency to form coördination compounds (ref. 2, p. 122) in the solid state.

Experimental

Material.—Sodium deoxycholate purchased from Riedel-de Haen in 1939 was used without purification. It contained 10.5% water[10] as judged by the loss of weight at 105°. The specific rotation, $[\alpha]^{20}\text{D}$, of a 2.57% solution was 39.40° comparing with Josephson's value of 45.70° for a 2.5% solution.[11] The *p*H of a 2.57% solution was 9.63 while that of a 26.5% solution was 10.04. The melting point of the anhydrous material was 357–365° (cor.). The failure to melt sharply may be due to impurities or to decomposition during melting, since repeated melting of the same sample resulted in progressive lowering of the melting point with eventual formation of a non-crystallizable glass.

Although the sodium deoxycholate was a white powder, the solutions were yellowish in color. Solutions prepared

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[1] B. Moore and D. P. Rockwood, *J. Physiol.*, **21**, 58 (1897); B. Moore and W. H. Parker, *Proc. Roy. Soc.* (London), **B68**, 64 (1901); F. B. Kingsbury, *J. Biol. Chem.*, **29**, 367 (1917); A. C. Frazer, *Analyst*, **63**, 308 (1938).

[2] H. Sobotka, "The Chemistry of the Sterids," Williams and Wilkins Co., Baltimore, (1938). Also see H. Sobotka and A. Goldberg, *Biochem. J.*, **26**, 555 (1932), for the "choleic acid principle."

[3] J. W. McBain, *J. Chem. Education*, **17**, 109 (1940).

[4] J. W. McBain, R. C. Merrill and J. R. Vinograd, THIS JOURNAL, **62**, 2880 (1940).

[5] J. W. McBain and S. A. Johnston, to be communicated to THIS JOURNAL.

[6] R. R. Roepke and H. L. Mason, *J. Biol. Chem.*, **133**, 103 (1940).

[7] M. L. Anson, *J. Gen. Physiol.*, **23**, 239 (1939).

[8] J. W. McBain and M. E. L. McBain, THIS JOURNAL, **58**, 2610 (1936); J. W. McBain and T. M. Woo, *J. Phys. Chem.*, **42**, 1099 (1938).

[9] G. S. Hartley, *J. Chem. Soc.*, 1968 (1938).

[10] All concentrations are given in terms of weight per cent.; that is, g./100 g. total system, unless otherwise stated.

[11] Ref. (2), p. 134.

at room temperature were fully as colored as those prepared by heating to high temperatures. All samples contained small amounts of a very faintly anisotropic, insoluble sludge, increasing in amount with the concentration of sodium deoxycholate, but not sufficient to interfere with determination of the temperatures at which solution occurred. Although the solutions foamed readily, they felt "sticky" rather than soapy.

Methods.—The technique used was the same as that previously employed with systems of soap and water.[12,13,14] T_c, the temperature at which the last trace of crystal disappears, was determined on slow heating. All temperatures recorded are corrected and were determined with thermometers which had been calibrated under conditions of use.[15]

No liquid crystalline phases were detected, *i. e.*, states in which the material though non-rigid is still spontaneously doubly refracting.

The melting point of the anhydrous salt was determined by visual observation of a 4-g. sample heated in a sealed tube in the electric oven. This visual value, 356–364° and 359–366° on two separate samples, is reasonably confirmed by the value 352° for the temperature of initial melting obtained by a differential calorimetric method. The calorimetric results, details of which will be described elsewhere,[16] also showed that sodium deoxycholate apparently undergoes no transitions accompanied by an appreciable heat effect between room temperature and the melting point.

Results.—The experimental data are tabulated in Table I and plotted in Fig. 1. It is at once clear that the solubility curve is divided into three segments. Up to 48% sodium deoxycholate, ice is the solid phase which separates. In accord with the colloidal nature of the solution the lowering of the freezing point is far less than would be calculated from the composition on the basis of a perfect solution. Between 48 and 71% the solid phase appears to be a hydrate of sodium deoxycholate, commonly occurring as a spherulitic, cottony-looking crystalline mass. Between 71 and 100% the separating phase is presumed to be anhydrous sodium deoxycholate.

All the solutions undercooled markedly, several days sometimes being required before crystals formed.

Systems containing from 74.9 to 89.5% deoxycholate on cooling from the melt commonly formed an isotropic transparent glass, a property not possessed by either more or less concentrated systems. On chilling, they frequently cracked with a typical conchoidal fracture, but neither prolonged nor sudden exposure to low temperatures (dry-ice) induced crystallization. The composition for maximum stability of the glass appears to be around 77%, this system having never been obtained with more than a trace of crystal, and apparently remaining indefinitely as a glass at room temperature (observed over three months). Approximate softening temperatures of these glasses, determined by visual observation, are lower than T_c and are given in Table I. Sufficient crystallization to permit determination of T_c was achieved by maintaining the glass for a day or longer at higher temperatures somewhat under the softening point.

TABLE I

TEMPERATURES OF FORMATION OF ISOTROPIC LIQUID

Concn., wt. % NaD	T_c, °C.	Concn., wt. % NaD	T_c, °C.
0	0	69.8	63.8
16.0	−0.1	74.9[a]	114
29.6	−0.9	76.7[a]	. . .
38.3	−1.5	81.4[a]	183
47.8	−2.9	89.5	271
49.9	+35.6	96.0	338
53.0	42.9	100.0	356–364
56.2	47.2	100.0	359–366
63.2	60.2		

[a] Glasses of these compositions melted at about 70, 80 and 113°, respectively.

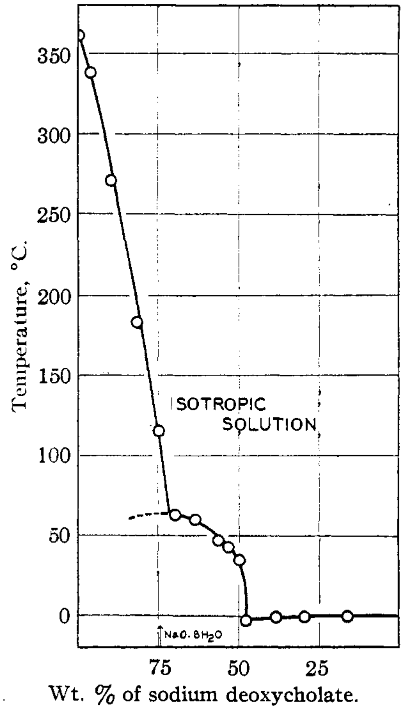

Fig. 1.—The solubility of sodium deoxycholate in water: O, temperatures of formation of isotropic liquid.

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(12) R. D. Vold, *J. Phys. Chem.*, **43**, 1213 (1939).

(13) J. W. McBain, G. C. Brock, R. D. Vold and M. J. Vold, THIS JOURNAL, **60**, 1870 (1938).

(14) R. D. Vold, R. Reivere and J. W. McBain, *ibid.*, **63**, 1293 (1941).

(15) R. D. Vold and M. J. Vold, *ibid.*, **61**, 808 (1939).

(16) R. D. Vold, to be communicated to THIS JOURNAL.

The solubility of sodium deoxycholate in water was found to be about 51% at 37° and 49.5% at 25°.

Discussion

Phase rule diagrams have been used before[17,18] to investigate the nature of the interaction between anhydrous bile salts and various solvent media. The general conclusion that crystalline addition compounds, choleic acids, are frequently formed seems probably justified within the error of the determinations, in spite of thermodynamic impossibilities in some of the published diagrams. It was assumed that the solubilizing effect of the bile salts is due to their ability to enter into such coördination complexes with water-insoluble substances. However, even though such compounds may exist in the crystalline state they can scarcely be responsible for solubilizing action if they are completely dissociated in solution.[19]

Apparently, sodium deoxycholate can crystallize from solutions in a certain concentration range in the form of an octahydrate, as judged from the extrapolated maximum in the solubility curve at about 74.5%. This hydrate melts incongruently, *i. e.*, the compound decomposes on formation of the solution.

Solutions of sodium deoxycholate closely resemble soap solutions in their colloidal properties and in their ability to solubilize water-insoluble substances. In the case of soaps the mechanism involves incorporation in or on a micelle which itself is probably liquid crystalline in nature.[20,21] At higher concentrations of soap a liquid crystalline solution phase separates at saturation rather than an ordinary crystalline phase. In view of the similarity of the two solutions, it was thought that liquid crystalline phases might also be encountered in the case of sodium deoxycholate and water, particularly since cholesterol caprate, a compound with a rather similar structure, is known to have a liquid crystalline form. However, no such phases were found in the present instance.

The structural requirement for the existence of a liquid crystalline form seems to be that the molecule should be anisometric and have neither too many nor too strong polar groups[22]; preferably it should have a long-chain or long-ring non-polar portion with a terminal polar group. Sodium deoxycholate has three polar groups, two hydroxyls and a carboxylate, located rather symmetrically along the molecule. It is easy to imagine that these hold the crystal lattice together very uniformly and tightly, thus accounting for the very high melting point and for the apparent absence of mesomorphic phases.

Summary

The temperatures of complete solution of sodium deoxycholate in water have been determined over the whole range of composition. The solubility curve obtained in this manner shows that an octahydrate exists which, however, is completely dissociated in the dissolved state.

No liquid crystalline phases were found in this system.

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[17] Ref. 2, p. 119.

[18] H. Sobotka and A. Goldberg, *Biochem. J.*, **26**, 557 (1932).

[19] Reference 2, p. 118.

[20] H. Kiessig and W. Philippoff, *Naturwissenschaften*, **35**, 593 (1939).

[21] J. Stauff, *Kolloid Z.*, **89**, 224 (1939).

[22] J. D. Bernal and D. Crowfoot, *Trans. Faraday Soc.*, **29**, 1032 (1933).