tion. Since this shift is gradual and is unaccompanied by any sudden change in shape, it argues against the formation of higher ratios. The third line of evidence is furnished by the synthesis of a curve assuming dissociation which is almost identical with an experimental curve. The maxima of the curves are at the same wave length, 630 mμ, while there is a secondary maximum in each case at 580 mμ. The difference in height can be explained as a departure from Beer's law, which is attested by the data of Figs. 4 and 5.

In the construction of the "dissociation" curve, two assumptions were made. The first is that cupric acetate in trichloromethane exhibits the same spectrum as in trichloromethane–ethanol. The shape and position of the curves have been experimentally proved identical, and a slight difference in height would not alter the conclusion of dissociation, but would require only that a different percentage of dissociation be assumed. The second assumption, that the 6:1 curve represents the spectrum of the undissociated complex, is justified by the fact that curves obtained from solutions containing higher ratios of amine to copper are little different from the 6:1 curve. It is not contended that exactly 10% dissociation of this complex in trichloromethane exists at a 0.04 *M* concentration, but the actual percentage dissociation is in that neighborhood. It may quickly be demonstrated that calculated dissociations of 5 and 15% do not give curves which resemble the experimental curve very closely. At the higher dissociation, the secondary maximum disappears, while at the lower, this maximum exceeds the true maximum.

Summary

1. A spectrophotometric study of bisdodecylamino-cupric acetate has disclosed its dissociation in trichloromethane solution. At room temperature, a 0.04 *M* solution in trichloromethane is approximately 10% dissociated into dodecylamine and cupric acetate.

2. Due to its dissociation, this compound does not obey Beer's law except in a special instance.

CHICAGO, ILLINOIS RECEIVED NOVEMBER 21, 1945

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ARMOUR AND COMPANY]

The Solubilities of Hexyl- and Dodecylammonium Chloride in Various Dilutions of Aqueous Ethanol

BY A. W. RALSTON AND C. W. HOERR

It has been observed by several investigators[1-4] that the solubility of colloidal electrolytes in water is unusual in that, beyond a certain critical concentration, the solubility increases enormously within a small temperature interval. For example, the solubility of dodecylammonium chloride in water increases from 0.4% by weight to 26.5% within the temperature range 28.0 to 30.0°. This solubility increase occurs at a concentration coincident with marked changes in the electrical behavior of the system, namely, an abrupt decrease in the equivalent conductance of the solutions and a sudden increase in the transference numbers of the colloidal ions. In view of this behavior, it would appear that micelle formation is accompanied by a marked change in the solubilities of the paraffin chain salts.

The solubilities of a number of the primary alkylammonium chlorides in 95% ethanol have recently been reported.[5,6] These salts present a quite normal behavior in this solvent in that their solubilities increase uniformly with increased temperatures. The comparison of this regular behavior in alcohol with the abnormal behavior of these salts in pure water suggested the investigation of both the electrical properties and the solubility characteristics of the alkylammonium chlorides in various mixtures of ethanol and water. Such studies should demonstrate the influence of the solvent upon micelle formation and may contribute to the better understanding of this phenomenon.

We have, therefore, determined the solubility of a typical colloidal electrolyte, dodecylammonium chloride, in various dilutions of aqueous ethanol, and have compared its behavior with that of a lower homolog, hexylammonium chloride. This latter salt can be assumed to function as an ordinary uni-univalent electrolyte.[7,8] The electrical properties of these salts in aqueous ethanol will be the subject of a subsequent report.

Experimental

Hexyl- and dodecylammonium chlorides were prepared from the corresponding primary amines, which had been previously purified by vacuum distillation in a Stedman-packed column. The salts were obtained by treating a benzene solution of the amine with concentrated hydrochloric acid and subsequent removal of the water by azeotropic distillation. The amine salts were recrystallized three times from benzene and analyzed for alkylammonium

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(1) Tartar and Wright, THIS JOURNAL, **61**, 539 (1939).

(2) Tartar and Cadle, *J. Phys. Chem.*, **43**, 1173 (1939).

(3) Ralston, Hoffman, Hoerr and Selby, THIS JOURNAL, **63**, 1598 (1941).

(4) Hoerr and Ralston, *ibid.*, **64**, 2824 (1942).

(5) Harwood, Ralston and Selby, *ibid.*, **63**, 1916 (1941).

(6) Sedgwick, Hoerr and Ralston, *J. Org. Chem.*, **10**, 498 (1945).

(7) Ralston and Hoerr, THIS JOURNAL, **64**, 772 (1942).

(8) Paquette, Lingafelter and Tartar, *ibid.*, **65**, 686 (1943); Scott and Tartar, *ibid.*, **65**, 692 (1943).

and chloride ion content by the method described elsewhere.[9] The salts employed in these experiments are considered to be of a purity exceeding 99.9%.

The aqueous ethanol mixtures were prepared by diluting commercial "absolute" (99.6% by weight) ethanol with double-distilled water. Their concentrations were accurately determined by measurement of their densities with a 25-ml. pycnometer, and interpolation of these values with those in the "International Critical Tables" [10]

The solubilities of the amine salts were determined by observing sealed samples in small glass tubes rotated in an electrically heated water-bath by the method and with the equipment described elsewhere.[4-6] In view of the polymorphic behavior of these salts,[6] considerable care was exercised to assure observation of the solubilities of only the stable form in all instances.

Results

The solubilities of hexylammonium chloride in aqueous ethanol are shown graphically by the temperature–concentration curves in Fig. 1. The nearly parallel alignment of these curves shows the solubilizing influence of water upon this salt. The change in slope of the solubility curves for the solvents containing less than 80% ethanol by weight suggests the possibility that hexylammonium chloride may exist in several different degrees of hydration.

In contrast to this rather normal behavior, dodecylammonium chloride presents the more irregular pattern of curves shown in Fig. 2. The solvents containing a preponderance of ethanol result in solubility curves which are typical of the ordinary aliphatic compounds in the common organic solvents, while the curves in the solvents

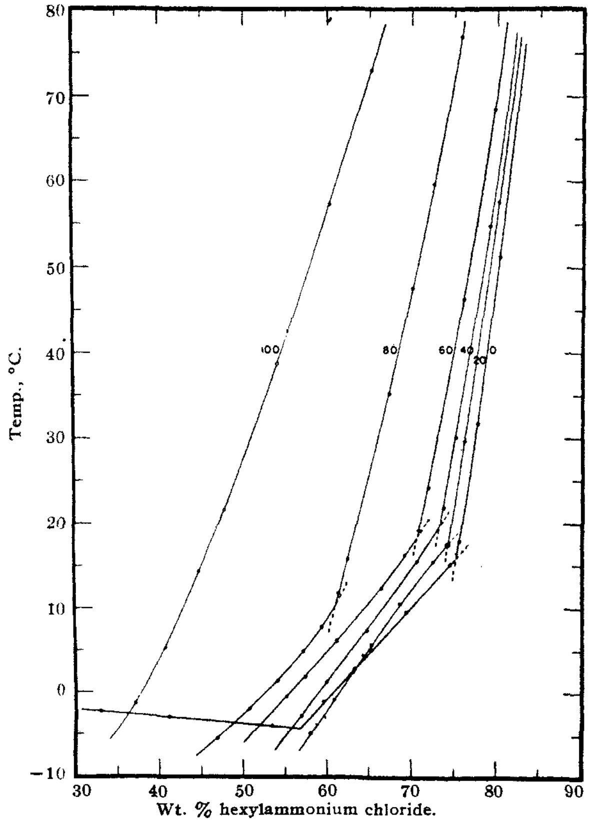

Fig. 1.—Solubilities of hexylammonium chloride in aqueous ethanol. The numbers on the curves refer to the weight percentage of ethanol in the respective solvent mixtures.

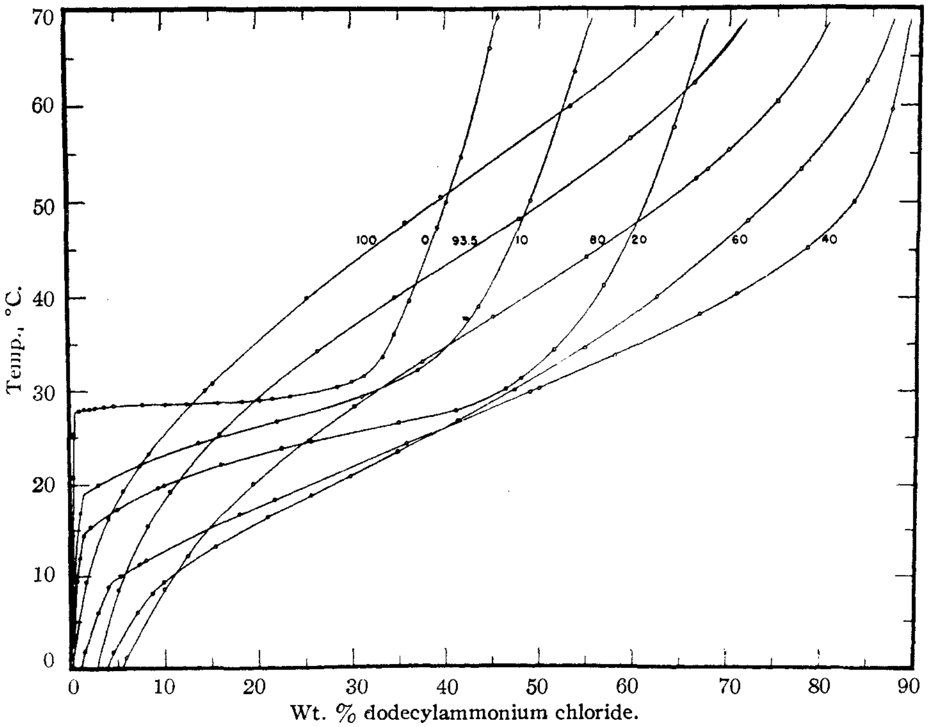

Fig. 2.—Solubilities of dodecylammonium chloride in aqueous ethanol. The numbers on the curves refer to the weight percentage of ethanol in the respective solvent mixtures.

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(9) Ralston and Hoerr, *Ind. Eng. Chem.*, *Anal. Ed.*, **16**, 459 (1944).

(10) "International Critical Tables," Vol. III, 1929, p. 117.

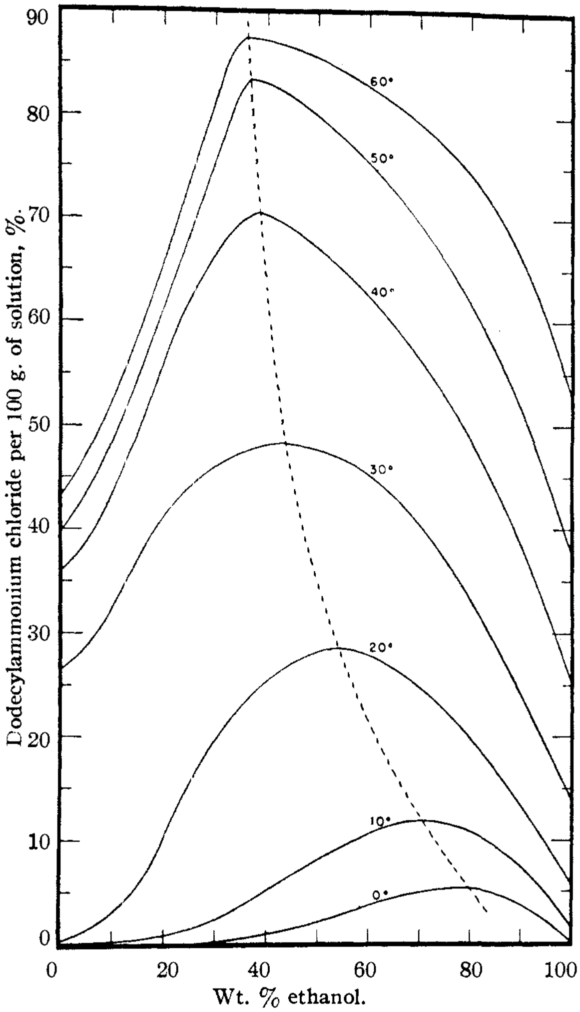

Fig. 3.—Variation of the solubility of dodecylammonium chloride with change of composition of the solvent. The broken curve is drawn through the maxima of the solubility curves and demonstrates the shift of the maxima with elevation of the temperature.

containing less than about 40% ethanol tend to approximate the type of curve which is characteristic of the paraffin-chain electrolytes in water.[1-4] In these latter curves the solubility beyond the critical concentration increases markedly with slight increases in solution temperature, whereas, below this point appreciable temperature increases exert only a small influence upon the solubility.

The variation in the solubility of dodecylammonium chloride with change of composition of the solvent is shown in Fig. 3. It will be noted that each of these curves shows a distinct maximum and that this maximum is shifted toward higher water concentrations with increase in temperature. That the maxima in these curves is in some way correlated with the micelle-forming tendency of the dodecylammonium chloride is shown by the absence of any similar maxima in the curves for hexylammonium chloride in Fig. 4.

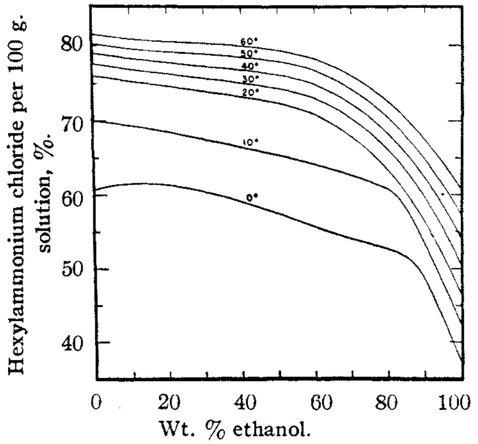

Fig. 4.—Variation in the solubility of hexylammonium chloride with change of composition of the solvent.

The most significant feature of the solubility curves of dodecylammonium chloride is the large increase in solubility with small increase in temperature in those solvents which contain a preponderance of water. This phenomenon decreases with progressive increases in the alcohol content of the solvent and disappears in those solvents in which the concentration of alcohol is high. This marked change in solubility can be attributed to a great increase in "molecular" weight of the solute which may even involve the formation of particles of colloidal size. This tendency toward association begins at a certain critical concentration and continues over a wide range. Such particles have been termed micelles and their formation has been shown to be attended by abrupt changes in the electrical and osmotic properties of the system. It is interesting, however, to note that the formation of an associated particle at a critical concentration is indicated by solubility considerations alone. It is the fact that an associated particle is formed, and not the type of associated particle formed, which is significant as regards this solubility effect. Since the proportion of solute and solvent cannot be varied arbitrarily at a fixed temperature and pressure, the associated particles present in such solutions cannot be considered as a separate phase.

The effect of increasing amounts of alcohol in the solvent upon the solubility of dodecylammonium chloride is extremely interesting. It appears that the addition of small amounts of alcohol results in enhanced solvent properties without materially decreasing the micelle forming properties of this solute. Higher concentrations of alcohol, however, inhibit micelle formation and result in lower solubilities. This could account for the maxima shown on these curves and also for the shifting of the maxima toward higher water content of the solvent at higher temperatures. Studies which we have made upon the electrical properties of these systems lend considerable support to this interpretation.

Summary

The solubilities of hexyl- and dodecylammonium chloride have been determined in various mixtures of water and ethanol.

The correlation between solubility and micelle-forming ability is noted.

CHICAGO, ILLINOIS RECEIVED DECEMBER 7, 1945

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

Optical and Crystallographic Properties of Lysozyme Chloride

BY FRANCIS T. JONES

In view of the general interest in antibiotic substances and in crystalline proteins, it seems desirable that the optical and crystallographic properties of lysozyme chloride be recorded in order to facilitate the work of others engaged in research on this material.

Although much work has been done on such crystalline proteins as insulin, hemoglobin, β-lactoglobulin, and the enzymes, all of which is well summarized in books by Schmidt[2] and by Cohn and Edsall,[3] most of the work has been by X-ray methods, and little except qualitative optical data are given. Crowfoot[4] gives index values for insulin, and Fankuchen[5] has recently given some data for β-lactoglobulin. This lack of optical data is due at least in part to the difficulty of obtaining crystals which are constant in composition and which do not disintegrate when exposed to air. It is well known that crystals of some protein substances may lose as much as 30% of their weight when dried, and regain part or all of it, depending on the humidity, when placed in a humid atmosphere. Crystals of some substances, *e. g.*, β-lactoglobulin, may contain salts. The quantity of salt may vary with the composition of the solution from which the crystals separate.[6] Such changes in composition cause changes in density and refractive indices. Values obtained on air-dried crystals will not be constant unless the humidity is constant and unless immersion media that will not affect the composition of the crystals are used. The most useful index values should be those obtained from crystals in equilibrium with the saturated solution from which they separated because they will then have the maximum water content for that preparation and there will be no doubt about the water content or the influence of strain due to partial drying.

The determinations recorded here were made on crystals regarded as lysozyme chloride, grown in acid solutions of *p*H 3–6 by the method of Alderton, Ward and Fevold.[7] These authors point out that crystals that are different from those described here are obtained at the isoelectric point (*p*H 10.8) or when chloride ions are replaced with other anions. In order to test for the presence of sodium chloride in the air-dried crystals several that appeared to be free from encrusting salt were ashed on platinum foil. This treatment produced only a trace of ash which gave a fleeting sodium flame. The crystals evidently contain very little salt, and this is probably occluded or contamination from the film of solution which must dry on the surface of the crystals, leaving sodium chloride crystals. Examination with a dark-field illuminator showed patches of bright particles on the surface of some lysozyme crystals but no appreciable Tyndall effect inside the crystals which were mounted in a liquid of nearly their own refractive index.

Preparation

The preparation was as follows: 4 g. of isoelectric lysozyme, which had been dissolved in dilute acetic acid at *p*H 6 and dried in the frozen state, was dissolved in 60 cc. of 0.2 *M* acetic acid. Saturated sodium chloride solution was then added to bring the composition to 0.86 *M* sodium chloride, and the *p*H was adjusted to 4.5 by adding a few drops of potassium hydroxide solution. Well-defined crystals up to 0.5 mm. in diameter developed in this solution at room temperature during the first twelve hours, and crystallization appeared to be complete within forty-eight hours. Many of the crystals developed at the surface of the solution, hanging from an edge or corner as shown in Figs. 1 and 2.

Crystallographic Description

The crystals of lysozyme chloride are colorless, tetragonal, tabular bipyramids of the first order, usually showing a short prism of the second order (Figs. 2 and 3). In Fig. 1 the prism is absent. Large crystals often develop cracks which radiate from the center. If the crystals are removed from their mother liquor they will dry out quickly and many of them will crack into smaller fragments. Some of the resulting cracks are parallel to the prism faces and appear to be cleavage cracks, but the cracks that develop perpendicularly to the *c* axis are so irregular that the crystals may be said to fracture rather than cleave perpendicularly to the *c* axis. Cracking of the

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(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Schmidt, "The Chemistry of Amino Acids and Proteins," C. C. Thomas Co., Springfield, Ill., 1944.

(3) Cohn and Edsall, "Proteins, Amino Acids and Peptides," A. C. S. Monograph 90, Reinhold Publishing Corp., New York, N. Y., 1943.

(4) Crowfoot, *Proc. Roy. Soc.* (London), **4164**, 580 (1938).

(5) Fankuchen, THIS JOURNAL, **64**, 2504 (1942).

(6) McMeekin and Warner, *ibid.*, **64**, 2393 (1942).

(7) Alderton, Ward and Fevold, *J. Biol. Chem.*, **157** (1), 43–58 (1945).