[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. X. The Solubility of Cystine in Solutions of Chlorides and Sulfates

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Cystine was the first of the amino acids to be discovered. Though not known to be a constituent of proteins until eighty years later, its presence in cystine stones was noted by Wollaston in 1810. Cystine has by far the lowest solubility in most solvents of any amino acid. It is, however, more soluble in urine than in water, and the causes of this effect were considered by Blix.[1] He noted that not only urea but certain salts, such as calcium chloride, had a pronounced solvent action.

As a part of our investigations of the interaction between amino acids and electrolytes, we have confirmed and extended these observations. Thus the solvent action of calcium chloride has been found to be far greater than that of sodium chloride, as is the case with most other amino acids and proteins. Moreover, salts of bivalent anions such as sodium sulfate and ammonium sulfate have a solvent action in dilute, but a precipitating action in concentrated, solution. This behavior is entirely characteristic of their effects upon proteins, and suggested that an accurate analysis of systems containing this small sulfur-containing tetrapole might throw light on the behavior of the far larger, more complex, proteins.

I. Materials and Methods.—Cystine is not only the least soluble of the amino acids, but contains the S–S linkage also present in proteins. By virtue of the S–S group cystine readily can be estimated quantitatively by a variety of methods in the presence of other amino acids.

Preparation of *l*-Cystine.—Reports of the solubility of *l*-cystine in water vary widely. Loring and Du Vigneaud[2] recently have reviewed this literature and also given the solubilities of the stereoisomers of cystine. The many different values for the solubility of *l*-cystine indicate that it is difficult to prepare the pure isomer. That used in the solubility determinations reported in this and the subsequent papers of this series was prepared from wool by the method of Folin.[3] Four crystallizations were required before a product was obtained which gave a constant solubility independent of the amount of saturating body. Crystallization was accomplished by dissolving in normal hydrochloric acid and neutralizing with sodium acetate. The crystalline product was washed with a large excess of water in order to free it completely from salt. The *l*-cystine prepared by this method gave a solubility of 0.109 g. per liter at 25°, in excellent agreement with the value 0.109 taken by Blix,[1] with the recent value of 0.108 g. per liter of Loring and Du Vigneaud,[2] and of 0.1095 of Dalton and Schmidt.[4] The purified *l*-cystine in a 1% solution in normal hydrochloric acid gave a specific rotation $[\alpha]^{20}D$ 222. The nitrogen content was found to be 11.55%, the theoretical being 11.66%.[5]

Solvents.—All of the salts employed were purified by recrystallization and then dried *in vacuo*. Sodium chloride and sodium sulfate were crystallized from water, while ammonium sulfate was crystallized from alcohol–water mixtures. Calcium chloride was dissolved in water and precipitated as the carbonate by the addition of a solution of ammonium carbonate. After washing the precipitate free from ammonium ions, it was dissolved in an equivalent quantity of redistilled hydrochloric acid. The concentration of calcium chloride was determined by analyzing both for chloride and calcium.

The salts were weighed out and made up to volume with distilled water, or ethanol and water, according to the procedures previously reported.[6] The hydrogen ion activity of the solvents was always tested. In each case the *p*H was very close to 6.0.

Determination of Solubility.—From 0.2 to 0.3 g. of pure *l*-cystine was shaken with the solvent by the method previously described.[7] It was found that twenty-four hours were sufficient to reach equilibrium and that the solubility did not vary with the amount of *l*-cystine used as saturating body.

The concentration of cystine was generally determined either (1) by the Kjeldahl nitrogen method, or (2) colorimetrically by an adaptation of the method of Folin and Marenzi.[8] The latter method is based on the capacity of cystine to be reduced to cysteine, and the color was determined either by means of a Bausch and Lomb colorimeter or by means of a Koenigs–Martin spectrophotometer. Differences in color intensity were best determined with the spectrophotometer with light of 674 mμ wave length. The results obtained by these different methods were in good agreement.

When the analysis was made by the Folin and Marenzi method, a sample was taken which contained approximately 1.5 mg. of cystine. The volume was adjusted to 20 cc. before developing the color. This was accomplished by diluting with water in the case of the more concentrated solutions and by evaporating *in vacuo* when

(1) Blix, *Z. physiol. Chem.*, **178**, 109 (1928).
(2) Loring and Du Vigneaud, *J. Biol. Chem.*, **107**, 267 (1934).
(3) Folin, *ibid.*, **8**, 9 (1910–11).
(4) Dalton and Schmidt, *ibid.*, **109**, 241 (1935).
(5) When cystine, having a solubility of 0.109 g. per liter, was crystallized from hot water, a crystalline product was obtained which had a solubility of 0.152 g. per liter. It would appear that some of the *l*-cystine had been racemized by boiling water, since this is the order of solubility of a mixture of *l*-cystine and *dl*-cystine according to the work of Loring and Du Vigneaud on the *solubility* of the stereoisomers of cystine.
(6) Cohn, McMeekin, Greenstein and Weare, THIS JOURNAL, **58**, 2365 (1936).
(7) Cohn, McMeekin, Edsall, and Weare, *ibid.*, **56**, 2270 (1934).
(8) Folin and Marenzi, *J. Biol. Chem.*, **83**, 103 (1929).

the concentration was small. The same amount of acid was added to the solution to be analyzed as was present in the standard. The following gives the procedure: to the sample was added 2 cc. of 20% sodium sulfite, followed after an interval of two minutes by the addition of 10 cc. of 15% sodium carbonate and 8 cc. of uric acid reagent. After standing for four minutes the solution was brought to volume with a 3% solution of sodium sulfite. Comparison in the colorimeter was made after thirty minutes. For spectrophotometric analyses it was necessary to wait for four and one-half hours before making the readings, since the rate of change of color with time, when a shorter period was used, was too great for the attainment of the accuracy desired.

The reliability of these methods of analysis for cystine is to a large extent dependent upon the accuracy of the determination of the influence of the solvent on the reagents. This source of variation has been corrected for by analyzing known amounts of cystine in each solvent investigated. When the solution contained alcohol, the alcohol was removed by evaporation before analysis. When it contained calcium, this ion was removed as oxalate, which was carefully washed, and the filtrate again concentrated for analysis.

Table I contains the results of a solubility experiment, illustrating the constancy of the solubility upon repeated equilibration of the saturating body in fresh aliquots of solvent. It also gives a comparison of the results obtained by the total nitrogen and the colorimetric methods of analyzing for cystine. The density was always determined on at least two successive days.

TABLE I

THE SOLUBILITY OF CYSTINE IN WATER AND SODIUM CHLORIDE SOLUTIONS AT 25°

Concn. of NaCl	Day of equilibration	Density of solution	Soly. of cystine, g. per liter: Total nitrogen method	Soly. of cystine, g. per liter: Colorimetric method
0	2	0.9972	0.1096	0.1081
	3	.9972	.1094	.1093
	4	.9971	.1094	.1087
	5		.1094	.1099
0.05	2	.9993	.1147	.1154
	3	.9993	.1153	.1148
	4		.1138	.1146
.10	2	1.0014	.1186	.1176
	3	1.0014	.1201	.1183
	4		.1190	.1190
	5			.1183
.20	2	1.0054	.1271	.1234
	3	1.0054	.1249	.1258
	4		.1249	.1258
	5			.1250

Average results for density and solubility in these and other systems are reported in Tables II and IV.

II. Experimental Results.—The interaction of ions and dipolar ions is determined, under certain circumstances, by the ionic strength of the solution.[9] The various salts that have been studied have, however, far different solvent actions upon cystine than might have been expected from this principle (Table II). Moreover, the order in which these salts increase solubility is that which has generally been ascribed to the

TABLE II

THE SOLUBILITY OF CYSTINE IN AQUEOUS SALT SOLUTIONS AT 25°

Ionic strength $\Gamma/2$	Density of soln. ρ	Solubility: Moles per liter C	Solubility: Mole fraction N	Logarithm of soly. Ratio $\log N/N'$
		Water		
0.0	0.9972	0.000454	0.00000820	0.0
		Sodium Chloride: $K_s = 0.14$		
0.05	0.9993	0.000478	0.00000864	0.023
.10	1.0014	.000494	.00000892	.036
.20	1.0054	.000522	.00000943	.061
.50	1.0176	.000578	.0000104	.103
1.00	1.0372	.000650	.0000118	.157
2.00	1.0737	.000714	.0000130	.200
4.00	1.1475	.000845	.0000155	.270
		Calcium Chloride: $K_s = 0.06$		
0.188[a]	1.0030	0.000532	0.00000963	0.070
.30	1.00627	.000580	.0000105	.107
.375[a]	1.0083	.000599	.0000108	.119
.45	1.01083	.000633	.0000114	.143
.750[a]	1.0195	.000720	.0000130	.200
1.500[a]	1.0421	.000895	.0000162	.296
3.000[a]	1.0840	.001140	.0000207	.402
		Sodium Sulfate: $K_s = 0.18$		
0.30	1.0091	0.000533	0.00000963	0.070
.75	1.0280	.000579	.0000105	.107
1.50	1.0587	.000616	.0000111	.131
3.00	1.1157	.000645	.0000117	.154
3.30	1.1273	.000649	.0000118	.158
3.75	1.1441	.000621	.0000113	.139
4.23	1.1632	.000612	.0000112	.135
4.50	1.1652	.000595	.0000109	.123
		Ammonium Sulfate: $K_s = 0.152$		
0.30	1.0047	0.000529	0.00000960	0.068
.75	1.0164	.000591	.00001078	.119
1.50	1.0345	.000666	.00001228	.176
3.00	1.0690	.000720	.00001359	.219
4.50	1.1015	.000699	.00001354	.216
6.00	1.1322	.000662	.00001319	.207
7.50	1.1618	.000579	.00001190	.161
7.89	1.1670	.000545	.00001133	.139
9.00	1.1901	.000475	.00001010	.090
10.50	1.2145	.000416	.00000920	.050
12.00	1.2409	.000316	.00000726	−0.053

[a] These measurements were made by Blix[1] and the results at low concentrations have been confirmed by us. The densities are from "International Critical Tables." The highest point is not plotted in Fig. 2.

(9) Cohn, *Chem. Rev.*, **19**, 241 (1936).

Hofmeister series: $CaCl_2 > NaCl > (NH_4)_2SO_4 > Na_2SO_4$. Whereas sodium and calcium chloride increase the solubility at all concentrations studied, solubility is greater in a molal solution both of sodium and ammonium sulfate than either in water or in more concentrated solutions.

Measurements in Concentrated Ammonium Sulfate Solutions.—In sufficiently concentrated solutions the precipitating action of ammonium sulfate may be defined by the "salting-out" expression which applies equally to gases, amino acids and proteins.

$$\log N = \beta' - K_s'(\Gamma/2) = \beta'' - K_s''\mu \qquad (1)$$

Values of $\Gamma/2$, of μ and of cystine solubility are given in Table II. Taking the ionic strength range from 6.0 to 12.0—although solubility is least accurately determined in these concentrated salt solutions—and solving by simultaneous equations, we have the results listed in Table III.

TABLE III

THE SOLUBILITY OF CYSTINE IN CONCENTRATED AMMONIUM SULFATE SOLUTIONS DEFINED BY THE EQUATIONS: $\log N = \beta' - K_s'(\Gamma/2)$ $\log N = \beta'' - K_s''\mu$

$\Gamma/2$	β'	K_s'	μ	β''	K_s''
6.00	−4.66	0.046	6.915	−4.70	0.027
7.50	−4.60	.043	9.021	−4.69	.026
7.89	−4.60	.044	9.630	−4.70	.027
9.00	−4.63	.044	11.340	−4.70	.027
10.50	−4.61	.041	13.965	−4.67	.025
12.00	−4.53	.045	16.848	−4.70	.027
Averages	−4.61	0.044		−4.69	0.027

Any solubility equation for cystine, as for proteins, must therefore have the characteristic that $\log N$ becomes linear in the ionic strength in sufficiently concentrated salt solutions. These results are somewhat more consistent in terms of ionic strength per thousand g. of water, μ. The values of K_s'' and β'' in these terms are given in the two last columns of Table III. The difference between β'' and the logarithm of solubility of cystine in water ($\log N' = -5.086$) is 0.396. An adequate expression for the activity coefficient of cystine in ammonium sulfate solutions more concentrated than two molal would therefore be

$$-\log \gamma = \log N/N' = 0.396 - 0.027\,\mu \qquad (1a)$$

The large "salting-out" effect observed in these concentrated ammonium sulfate solutions might, it was thought, be manifesting itself also at lower ionic strength. The principle of the ionic strength may be expected to obtain only when the "salting-out" effect is small in comparison with Coulomb forces. That part of the logarithm of the activity coefficient which depends upon Coulomb forces increases inversely as the second power of the dielectric constant.[9,10] It therefore seemed possible that investigations in ethanol–water mixtures might indicate whether the specific action of different salts, greater than could be accounted for in terms of the differences in their size, would not largely diminish at lower dielectric constants.

Measurements in Ethanol–Water Mixtures.—Cystine was therefore studied in 15 and 30% ethanol containing sodium chloride and in 30% ethanol containing calcium chloride (Table IV).

TABLE IV

THE SOLUBILITY OF CYSTINE IN 15 AND 30% ETHANOL SOLUTIONS CONTAINING NaCl AND $CaCl_2$

Ionic strength $\Gamma/2$	Density of soln. ρ	Solubility moles per liter C	Logarithm of solv. $\log N$	$\left(\frac{D}{D_0}\log\frac{N}{N'}\right)\Big/\left(\frac{D_0}{D}\frac{\Gamma}{2}\right)$
NaCl and 15% Ethanol: $D = 71.69$				
0.0	0.97764	0.000161	−5.495	0.0
.05	.97972	.000169	−5.474	.350
.10	.98183	.000178	−5.451	.367
.25	.98777	.000197	−5.409	.289
.50	.99791	.000220	−5.359	.226
NaCl and 30% Ethanol: $D = 64.45$				
0.0	0.95980	0.0000507	−5.951	0.0
.05	.96223	.0000544	−5.921	.403
.10	.96388	.0000582	−5.893	.390
.25	.97046	.0000685	−5.821	.350
.25	.97046	.0000690	−5.818	.358
.50	.97937	.0000866	−5.719	.313
$CaCl_2$ and 30% Ethanol: $D = 64.45$				
0.05	0.96124	0.0000550	−5.916	0.470
.10	.96267	.0000597	−5.880	.478
.15	.96418	.0000649	−5.844	.480
.30	.96857	.0000801	−5.752	.447
.75	.98155	.000128	−5.547	.363

Measurements could not be carried out readily at such low dielectric constants as were employed in our studies upon glycine,[9–11] because the solubility of cystine in such solvents is so low that the errors of analysis would be formidable. The results of these experiments are represented graphically in Fig. 1, which is so plotted that all points should fall on the same curve if only Coulomb forces were involved.[9–11] They demonstrated that in 30% ethanol, at given ionic strength: (1) *the solvent action of calcium chloride was far greater than that of sodium chloride* (Curves 4 and 5), suggesting that under these conditions, as in water (Curves 1 and 3), the "salting-out" effect,

(10) Cohn, *Naturwissenschaften*, **20**, 663 (1932).
(11) Kirkwood, *J. Chem. Phys.*, **2**, 351 (1934).

though smaller, must still be taken into account.

(2) *The change in solvent action with change in dielectric constant could not completely be accounted for by Coulomb forces*, for the limiting slope at very low ionic strengths

$$[(D/D_0) \log N/N']/(D_0/D)(\Gamma/2) = K_R' \qquad (2)$$

appeared to be greater in 30% ethanol than in water, whereas according to the theory it should be unchanged if Coulomb forces alone were responsible for the observed effects.

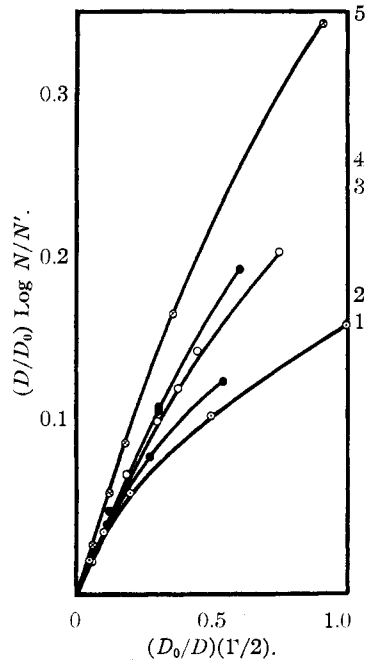

Fig. 1.—Solubility of cystine in ethanol–water mixtures: (1) NaCl ⊙, and (3) $CaCl_2$ ○ in water, (2) NaCl in 15% ●, and (4) in 30% ethanol ●, and (5) $CaCl_2$ in 30% ethanol ⊗.

(3) Comparison with the results previously reported for glycine[6] indicate that *the solvent action of salts upon an α-amino acid*, in 80% ethanol, *is not as great as upon cystine* in 30% ethanol. This observation reflects the influence of the two permanent dipoles, each with a moment equal to that of glycine, which in cystine are bound together by the S–S linkage.

(4) At the lowest salt concentrations at which measurements were made, *change in solubility with change in ionic strength was essentially linear in the ionic strength* (Table IV, column 5), the extrapolated slope being slightly greater than 0.40 for sodium chloride and 0.48 for calcium chloride in 30% ethanol. These experiments thus confirm those previously published upon glycine and are in agreement with the theory of Scatchard and Kirkwood[12] that in the case of dipolar ions change in free energy with change in ionic strength is not linear in the square root of the ionic strength, $\Gamma/2$, as is the case with ions, but in its first power. At the lowest ionic strengths at which cystine has been studied, as at the highest (Table III), log N is nearly linear in Γ, curvature occurring at intermediate salt concentrations.

Measurements in Aqueous Salt Solutions.—In the case of ions it has been found practicable to make measurements at low concentrations in order to distinguish between Coulomb forces and the "salting-out" effect. For dipolar ions, both Coulomb forces and the "salting-out" effect are, as we have seen, linear in the ionic strength. We may, therefore, attempt an analysis of the data in Table II by assuming that, as a first approximation, differences in the solubility curves in the various solvents are completely ascribable to differences in the "salting-out" constant, K_s.

Provided K_s be assumed to be 0.06 for calcium chloride, 0.14 for sodium chloride, 0.152 for ammonium sulfate and 0.18 for sodium sulfate, and we plot log $N/N' + K_s\mu$ against $\Gamma/2$, all of the results with these different salts fall very closely upon the same curve (Fig. 2). This is true although sodium chloride and calcium chloride have a solvent action upon cystine even in concentrated aqueous solution, whereas ammonium sulfate, as we have seen, is a precipitant under these conditions. The uncorrected values of log N/N' are given by the dotted lines. It should be noted, moreover, that the difference between the solubility ratio observed at finite ionic strengths for cystine in sodium chloride and calcium chloride in 30% ethanol, (0.48 − 0.40 = 0.08), is close to the difference in the "salting-out" constants assumed, (0.14 − 0.06 = 0.08). The limiting slope, determined by Coulomb forces alone, may be considered equal to that given by the solid line in Fig. 2, and to have a value close to 0.56.[13]

The shape of the curve in Fig. 2 is different from that expected on the basis of previous studies. It has the property of being linear at very low, and again at very high, values of the ionic strength. An empirical expression on the

(12) Scatchard and Kirkwood, *Physik. Z.*, **33**, 297 (1932).

(13) This is only slightly greater than the sum of the solubility ratios observed in 30% ethanol and the values of K_s.

basis of which the curve in Fig. 2 has been constructed may be written as follows

$$\log \frac{N}{N'} + K_s\mu = \frac{\Gamma}{2}\left(\frac{K_R + A(\Gamma/2)}{1 + B(\Gamma/2)}\right) \qquad (3)$$

The same value of K_R, 0.56, is assumed tentatively for all salts, though the value of the limiting slope may ultimately prove to be greater and slightly different for different salts. The values of K_s are those given above. The constants A and B are less important numerically than is their ratio. At sufficiently high concentrations the terms in A and B will be large in comparison with the first term both of the numerator and of the denominator of the above equation. At the limit of high ionic strength, therefore, the ratio A/B will determine the slope of the curve. Our results are satisfactorily described, up to ionic strengths of 4.5, by a value for A of 0.36 and for B of 2.1 if K_s is multiplied by $\Gamma/2$ in the "salting-out" term. If K_s is multiplied by μ, still higher ionic strengths are described by equation (3), but the ratio A/B must be somewhat greater and B may be taken as 2.0. The solid curve in Fig. 2 is constructed on this basis. At the highest ionic strengths studied the curvature is slightly smaller than that given by the above equation and appears to have a nearly constant slope of approximately 0.21 in such concentrated salt solutions that "salting-out" is the predominant factor and equation (1) is an adequate description of the data. The form of the above equation thus gives us, as a first approximation, a method of expressing the complicated relations that obtain between ions and such dipolar ions as amino acids and proteins.

Further analysis in which certain of these constants are independently evaluated will be reported subsequently. It ultimately should be possible to ascribe differences in the sizes and other properties of the ions and dipolar ions present to the constants in the above equation.

III. Discussion of Results

The "Salting-out" Constant.—The "salting-out" constants that have been reported previously for amino acids and proteins have been derived from direct experiment in terms of equation (1). In the case of cystine in sufficiently concentrated ammonium sulfate equation (1) appears to hold and yields a value for K_s' approximately one-fifth as great as that deduced for K_s by means of equation (3).

Kirkwood[14] has suggested that provided the "salting-out" effect depended entirely upon dipolar ions displacing a certain quantity of solvent, and reducing the polarization of the solvent by the salt ions, K_s should have a value close to that given by the relation

$$K_s = \frac{4\pi N\epsilon^2}{2303\, D_0 KT}\,\frac{b^3}{a}\,\frac{D-1}{2D+1} \qquad (4)$$

where b is the radius of the dipolar ion, a the sum of the radii of ions and dipolar ions, and D the dielectric constant.

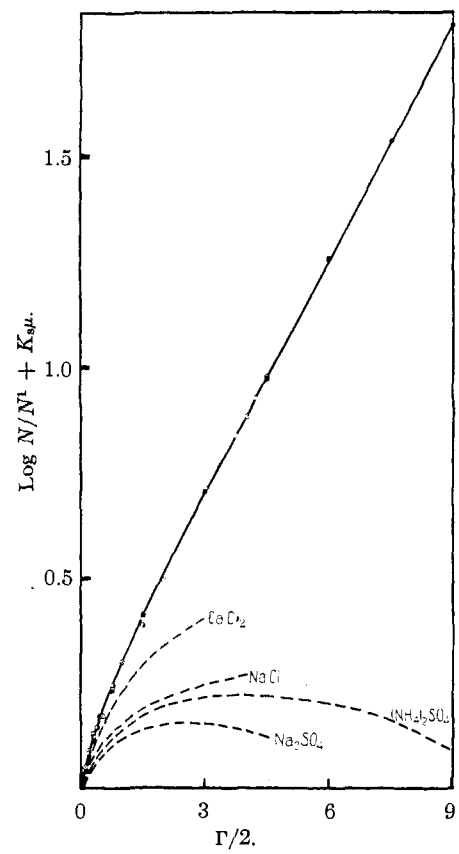

Fig. 2.—Solubility of cystine in aqueous salt solutions: NaCl ⊗; $CaCl_2$ (new data) ◐; Blix ◑; Na_2SO_4, ○; $(NH_2)_4SO_4$ ●.

For the case of cystine and sodium chloride all of the constants in the equation may be considered well known. The radius of cystine may be calculated from its molal volume. Taking the volume of the S–S group as 29.8 cc., as in α,α'-dithiodiacetic acid,[15] and the volume of the other groups as previously given, the molal volume of cystine may be estimated to be 156 cc.

(14) Personal communication; see also "Annual Review of Biochemistry" Vol. V, p. 137. The equation given previously should be multiplied by D_0/D to give the influence of the dielectric constant on K_s, and results in equation (4).

(15) Greenstein, Wyman and Cohn, THIS JOURNAL, **57**, 637 (1935).

and its radius b to be 3.94 Å. The sum of the radii, a, of cystine and sodium chloride[16] is therefore 5.17 Å. Solving equation (4) for cystine and sodium chloride in water at 25°, we have

$$K_s = 0.01165\ (b^3/a) = 0.138$$

The agreement with the value of K_s experimentally determined is excellent.

The sizes of the other ions deduced from values of K_s and the above relation appear to be far too small in certain cases, namely, 0.16 Å. for sodium sulfate, and 0.59 for ammonium sulfate, and far too large for calcium chloride, namely, 8.14 Å., suggesting that other effects must be taken into account for these salts. It is also probable that not only the radius but the number and position of the polar and non-polar groups of dipolar ions influence the "salting-out" effect. The agreement in the case of sodium chloride and cystine is, however, noteworthy and will subsequently be considered in connection with comparable investigations, now being carried out with other dipolar ions and salts.

The Kirkwood Equation for the Interaction of Ions and Dipolar Ions.—Kirkwood has developed an equation for the interaction between ions and spherical dipolar ions which may now be compared[17] with the measurements that are reported. Equations 29 and 30 of Kirkwood[11] may be written in the following form for 25°

$$\frac{D}{D_0}\log\frac{N}{N'} = 0.125\,\frac{D_0}{D}\,\frac{\Gamma}{2}\left[\frac{R^2}{a}\left(\frac{1}{1+\left(0.108a^2\,\frac{D_0}{D}\,\frac{\Gamma}{2}\right)^{1/2}+\left(0.036a^2+0.018\,\frac{b^3}{a}\right)\frac{D_0}{D}\,\frac{\Gamma}{2}}\right)+\right.$$
$$\left.\frac{60}{81}\,\frac{R^2}{a^3}\left(\rho^2-\frac{R^2}{4}\right)\left(\frac{1+\left(0.108a^2\,\frac{D_0}{D}\,\frac{\Gamma}{2}\right)^{1/2}}{1+\left(0.108a^2\,\frac{D_0}{D}\,\frac{\Gamma}{2}\right)^{1/2}+\left(0.0432a^2+0.048\,\frac{b^5}{a^3}\right)\left(0.00237+0.00158\,\frac{b^5}{a^5}\right)\left(\frac{D_0}{D}\,\frac{\Gamma}{2}\right)^{3/2}}\right)\right] \quad (5)$$

The first terms on the right-hand side give the effect ascribed to the dipole, the second to the quadrupole moment. Still higher moments have not been computed.[18]

The moment of each dipole may be taken as equal to that of glycine, 3.17 Å.[11] The distance of each charged group from the edge of the molecule may also be taken as equal to that for glycine, or 1.24 Å. The distance of the charged groups from the center of the molecules, ρ, is, in terms of the above constants, (3.94 − 1.24) = 2.70 Å. Since there are four charged groups assumed to be the same distance from the center of the sphere, instead of the term 0.125 (R^2/a), we have for the dipole term 0.125 $(2R^2/a)(1 - \cos\varphi)$, where φ is the angle of rotation of the dipoles from the antiparallel position, and for the quadrupole term instead of 0.125 $(60/81)(R^2/a^3)$ we have 0.125 $(120/81)(R^2/a^3)(\rho^2 - R^2/4)(1 + \cos\varphi)$.

In terms of the above constants the equation for cystine and sodium chloride at 25° may be written

$$\frac{D}{D_0}\log\frac{N}{N'} = \frac{D_0}{D}\,\frac{\Gamma}{2}\left[\left(\frac{0.486\ (1-\cos\varphi)}{1+1.7\sqrt{\frac{D_0}{D}\,\frac{\Gamma}{2}}+1.17\left(\frac{D_0}{D}\,\frac{\Gamma}{2}\right)}\right)+\right.$$
$$\left.\left(\frac{0.0643\ (1+\cos\varphi)\left(1+1.7\sqrt{\frac{D_0}{D}\,\frac{\Gamma}{2}}\right)}{1+1.7\sqrt{\frac{D_0}{D}\,\frac{\Gamma}{2}}+1.188\left(\frac{D_0}{D}\,\frac{\Gamma}{2}\right)+0.384\left(\frac{D_0}{D}\,\frac{\Gamma}{2}\right)^{3/2}}\right)\right] \quad (6)$$

Assuming that the dipoles in cystine are in the parallel position, the quadrupole term vanishes. Assuming them to be in the antiparallel position, the dipole term vanishes. On the basis of free rotation around the S–S group the dipoles might be considered to be at an angle of 90° with respect to each other, since the average value of cos φ

(16) Pauling, THIS JOURNAL, **50**, 1036 (1928).

(17) We are indebted to Dr. Kirkwood for carrying out certain of these calculations.

(18) Dr. Kirkwood has now computed the octupole contribution which, as will appear below, is not very important. He obtains as an increment to log N/N' the following values:

$\Gamma/2$	Dipoles at 90° $\Delta\log N/N'$	Dipoles parallel $\Delta\log N/N'$
0.1	0.001	0.002
.2	.002	.003
.5	.004	.008
1.0	.007	.014
2.0	.012	.024
4.0	.020	.039

TABLE V

COMPARISON OF THE ACTIVITY COEFFICIENTS CALCULATED FROM THE KIRKWOOD EQUATION WITH THE OBSERVED SOLUBILITY RATIOS

	Log N/N_0 (calculated)				Log N/N' +0.14 (Γ/2)
$\Gamma/2$	Dipoles anti-parallel	Dipoles parallel	Dipoles at 90°	Dipoles at 45°	observed
	(0.129)	(0.972)	(0.550)	(0.849)	
0.05	.006	.034	.020	.030	0.030
.10	.011	.059	.035	.052	.050
.20	.022	.098	.060	.087	.089
.50	.048	.174	.111	.156	.173
1.00	.081	.252	.167	.227	.297
2.00	.128	.338	.233	.307	.480
4.00	.185	.428	.306	.392	.830

The limiting slopes are given by the values in parentheses.

vanishes. The activity coefficients of cystine and sodium chloride calculated on these assumptions are given in the accompanying table and compared with the experimental values corrected with the value 0.14 for the "salting-out" constants (Table V).

Although there is good agreement between observed values of log N/N' (Table II) and those calculated from the Kirkwood equation on the assumption that the two dipoles in cystine are at an angle of 90°, the measurements reported render it certain that a "salting-out" term must be added to the observed solubility results in order that they represent those changes in free energy due to Coulomb forces which Kirkwood's equation should yield.

Corrected for the "salting-out" effect our solubility ratios at low ionic strengths are appreciably greater than those calculated on the assumption that the dipoles in cystine are at 90°. The agreement at lower ionic strengths is better if they are considered to be more nearly parallel, and at an angle close to 45°, as is indicated by comparison of the last two columns in Table V. Indeed, on these assumptions Kirkwood's equation gives curvature within the experimental errors up to an ionic strength of 0.2. The limiting slope on this assumption is, however, 0.85, as contrasted to the limiting slope of 0.56 derived from equation (3). The smaller limiting slope corresponds to an electric moment of 23, the latter of 28.5×10^{-18}, electrostatic units.

Although the first term in the Kirkwood equation is linear in the ionic strength, a very rapid decrease in slope with increase in ionic strength is demanded by the term in the square root of the ionic strength in the denominator. The present experiments, moreover, cannot be used to test this equation at very low ionic strengths. Thus the difference in the expectation from graphical extrapolation of our measurements with the limiting slope 0.56, or by means of equation (6) with the limiting slope 0.85, would be less than 0.8% at an ionic strength of 0.02.

Kirkwood's equation thus appears to give a satisfactory estimate of the limiting slope for the interaction between sodium chloride and cystine as for that between lithium chloride and glycine.[11] That it should give the correct curvature was hardly to be expected from the simplifying assumptions on which it was derived. It is for this reason that we have tentatively adopted an empirical equation (3) for the description of our data.

The shape of the solid curve in Fig. 2 results, it is true, from assuming K_s to be constant for all concentrations of salt. The investigations upon the activity coefficients of amino acids and proteins in salt solutions that have thus far been carried out appear to demonstrate that at very low ionic strengths the logarithm of solubility is linear in the concentration[6,9,19] and that at sufficiently high salt concentrations this is again the case, and equation (1) suffices to describe the latter phenomenon.[20] To meet these conditions either K_s must increase with increase in concentration, or the term K_R approach a constant slope (as in equation 3) at such salt concentrations that equation (1) appears to obtain.

Summary

1. The solubility of the tetrapolar amino acid cystine has been studied in water and in ethanol–water mixtures containing neutral salts.

2. Chlorides have a greater solvent action and sulfates a greater precipitating action on cystine at the same ionic strength.

3. The interaction between cystine and the salts studied is described by assuming that as a first approximation solvent action is a function of the ionic strength, and by ascribing differences in solubility at the same ionic strength to the "salting-out" effect.

4. The value of the "salting-out" constant K_s has been taken as 0.06 for calcium chloride, 0.14 for sodium chloride, 0.152 for ammonium sulfate, and 0.18 for sodium sulfate.

5. The value of the limiting solvent slope K_R, which depends upon the method of extrapolation to zero ionic strength, indicates that the dipole moment of cystine lies between 23 and 28.5×10^{-18} electrostatic units.

6. Empirical equations have been developed for the interaction between ions and dipolar ions which describe the shape of the solubility curve at all concentrations of the salts studied.

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(19) Ferry, Cohn and Newman, THIS JOURNAL, **58**, 2370 (1936).

(20) Cohn, *Physiol. Rev.* **5**, 349 (1925); Florkin, *J. Biol. Chem.*, **87**, 629 (1930); Green, *ibid.*, **93**, 495, 517 (1931); *ibid.*, **95**, 47 (1932); Palmer, *ibid.*, **104**, 359 (1934).