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The Conductance of Copper *m*-Benzenedisulfonate, an Unassociated 2,2 Electrolyte, and Some Related Salts

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The conductances of copper *m*-benzenedisulfonate (CuBDS), H₂BDS, Na₂BDS, K₂BDS and lanthanum 1,3,6-naphthalenetrisulfonate (LaNTS), H₃NTS, Na₃NTS and K₃NTS have been measured in water at 25°. All data are analyzed by means of the extended Fuoss-Onsager theory. It is demonstrated that, unlike previously measured 2-2 salts, CuBDS is not associated in solution. A new criterion for unassociated high-charge type electrolytes is derived from the theory and exhibited experimentally. Although the LaNTS data are more ambiguous, they exhibit qualitatively the behavior of an unassociated salt also. The 2-1 and 3-1 salts and acids behave as strong electrolytes of their respective charge types.

The form of the distribution function used in the derivation of most modern electrolyte solution theories leads us to expect that such theories should work best for symmetrical electrolytes.¹ That this expectation is borne out in fact is easily ascertained.² Consequently, a vast proportion of the experimental effort in this field has gone into the investigation of such electrolytes. Unfortunately, the greatest part of this work is on the 1-1 electrolytes. The data on the 2-2 salts are scattered and sparse and on 3-3 or higher types almost non-existent. The main reason for this seeming over-emphasis has been the lack of well-behaved salts of the higher charge types. The dual effects of hydrolysis and strong ion-association have defeated all attempts to obtain data comparable to the 1-1 electrolyte data. This fact has both left the actual validity range of the theories in doubt and created much confusion on the subject of ion-association. The first problem is exemplified in the conductance field by the statement: "no 2-2 electrolyte has yet been found to have a conductance curve which approaches the limiting law from above."³ The second problem is illustrated both by the diversity of names used to describe the problem⁴ and by the widely varying estimates of the magnitude of the association constant for a given electrolyte. For example, we can find K_A for CuSO₄ listed variously as 4.3×10^{-3} ,⁵ 6.0×10^{-3} ,⁶ and 8.0×10^{-3} .⁷ For LaFe(CN)₆ we can find⁸ 1.82×10^{-4} and 3.50×10^{-4} .⁹

There are two main reasons for the differences of the type given above. The first is simply the difference in inherent sensitivity of the various experimental methods; an interaction that is of measurable strength in a conductance experiment may not be noticed in spectrophotometric work. The second reason, which is of more immediate concern to us, is the lack of a well-defined baseline

for the calculation of association constants in each of the methods. That is, since we do not know in fact how CuSO₄ would behave in conductance if completely dissociated, we must assume some behavior in order to calculate association. As the various experimental methods are dependent to various degrees on the different parts of equilibrium and non-equilibrium theory, the particular assumption made will have a profound and unknown effect on our "measured" association constant.

This problem has become more intriguing recently with the extension of the Fuoss-Onsager equation¹⁰ and the corresponding treatment of Falkenhagen and Kelbg.¹¹ The extended Fuoss-Onsager equation has been shown^{12,13} to work very well for 1-1 electrolytes with reasonable \hat{a} and Λ^0 parameters and is thought to be in essentially complete form.¹⁴ It also points out previously inexplicable "fine structure" in conductance data and provides a gradual and reasonable transition between unassociated and associated electrolytes.

Therefore, if 2-2 and 3-3 salts could be found that would obey the extended theory with reasonable parameters, a baseline could be established for the more rational discussion of association in salts like CuSO₄ and LaFe(CN)₆. It has been said¹⁵ that a 2-2 salt in water is analogous to a 1-1 in liquid ammonia, implying that an unassociated 2-2 electrolyte is essentially impossible. Although this statement is consistent with the simple coulombic force law used in the Debye and Onsager theories, it ignores the short range ion-solvent and ion-ion forces that probably are operative in ion-association.

We speculated that if the -2 charge on an ion like SO₄⁼ could be split up into two -1 charges separated by an inert framework an interesting ion type could be investigated. In this way we hoped to decrease short range interactions while maintaining the long range properties of a divalent ion. The first such ion to be explored was the well-characterized *m*-benzenedisulfonate (to be abbreviated BDS). An analogous trivalent anion to be investigated was 1,3,6-naphthalenetrisulfonate (NTS).

(1) E.g. R. M. Fuoss and L. Onsager, *J. Phys. Chem.*, **61**, 668 (1957).

(2) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd Ed., Reinhold Publishing Corp., New York, N. Y., 1959.

(3) Ref. 2, pp. 206.

(4) E.g. E. A. Guggenheim, *Discussions Faraday Soc.*, **24**, 53 (1957).

(5) B. B. Owen and R. W. Gurry, *J. Am. Chem. Soc.*, **60**, 3074 (1938).

(6) P. G. M. Brown and J. E. Prue, *Proc. Roy. Soc. (London)*, **A232**, 320 (1955).

(7) R. Nasanen, *Acta Chem. Scand.*, **3**, 179 (1949).

(8) C. W. Davies and J. C. James, *Proc. Roy. Soc. (London)*, **A195**, 116 (1948).

(9) D. Berg and A. Patterson, Jr., *J. Am. Chem. Soc.*, **75**, 1484 (1953).

(10) R. M. Fuoss, *ibid.*, **81**, 2659 (1959).

(11) H. Falkenhagen and G. Kelbg, Chapter 1 in "Modern Aspects of Electrochemistry," ed. J. O'M. Bockris, Butterworths, London, 1959.

(12) R. M. Fuoss and F. Accascina, "Electrolyte Conductance," Interscience Publishers, Inc., New York, N. Y., 1959.

(13) R. L. Kay, *J. Am. Chem. Soc.*, **82**, 2099 (1960).

(14) Ref. 1, p. 680.

(15) Ref. 1, p. 679.

TABLE I

CuBDS		H ₂ BDS		Na ₂ BDS		K ₂ BDS	
10°C	A	10°C	A ^a	10°C	A	10°C	A
0.9035	109.57	0.746	408.5	0.5120	108.30	0.7512	131.33
1.4132	108.73	2.438	405.6	1.3672	106.90	1.5726	130.25
1.8535	107.74	5.372	302.1	3.6213	105.25	2.4585	129.53
4.6921	103.40	12.007	397.7	5.4140	104.27	4.3921	128.12
5.6935	102.05	18.576	394.8	7.2115	103.27	8.2340	126.32
7.2820	100.61	25.890	392.1	10.304	102.52	12.881	124.68
13.158	97.15	37.558	388.6	14.455	101.36	20.344	122.69
17.489	95.63			18.072	100.39	31.483	120.54
21.650	92.99			23.788	99.36	46.236	118.39
26.781	91.80			32.127	98.03	59.180	116.68
31.177	90.31			46.720	96.10		
43.553	87.75						
54.560	85.89						
66.830	84.09						

LaNTS		H ₃ NTS		Na ₃ NTS		K ₃ NTS	
10°C	A	10°C	A ^a	10°C	A	10°C	A
0.2233	127.73	0.928	413.6	1.0199	116.63	1.0574	139.96
.3034	122.06	4.263	405.8	1.7592	115.38	5.2771	134.39
.5202	118.39	5.283	403.6	4.4823	112.13	9.4206	131.27
1.0450	109.70	9.151	398.9	5.8743	110.97	15.021	128.19
1.8211	101.23	10.528	397.1	9.4072	108.54	24.905	124.36
3.5221	90.65	17.069	392.0	11.449	107.45	34.682	121.63
5.0143	84.90	24.722	386.8	18.365	104.51	40.437	120.36
6.7760	80.30	28.064	385.4	23.983	102.69		
10.453	73.61			27.905	101.53		
14.037	69.65			31.847	100.62		
21.088	64.40						
32.455	59.65						

^a The acid conductances are *not* corrected for solvent conductance. All concentrations are in moles/liter.

Experimental

CuBDS was prepared in two ways. In the first, the crude commercial H₂BDS¹⁶ was converted to CaBDS with Ca(OH)₂ thus removing the bulk of the large H₂SO₄ impurity. The CaBDS then was converted to K₂BDS using K₂CO₃, and the K₂BDS recrystallized twice from water. It was then reconverted to acid using cation exchange techniques. The acid was used to make the Cu⁺² and Na⁺ salts by reaction with CuCO₃ and NaOH, respectively. The final salts and some of the pure acid were recrystallized twice from conductivity water and dried over CaCl₂ at 25°. A second, less tedious method finally was adopted. This involves the neutralization of the crude acid with Ba(OH)₂, thus removing almost all the SO₄²⁻. The BaBDS then was recrystallized and converted to acid by ion exchange when it was desired to prepare another salt. The pure salts and acid obtained showed no SO₄²⁻ impurity. The isomer purity was checked by converting some of the pure acid to the sulfonyl chloride which melted at 61°, the literature value.¹⁷ All of the electrolytes formed definite hydrates on drying over CaCl₂ at room temperature: NaBDS·4H₂O, K₂BDS·H₂O, CuBDS·6H₂O.

The naphthalenetrisulfonate salts were prepared from Na₃NTS, commercially available in fairly pure form.¹⁸ The technical Na₃NTS was converted to the acid by ion exchange, and the acid treated with La₂O₃. The LaNTS obtained was recrystallized twice from hot water. The much lower solubility of La₂(SO₄)₃ and its retrograde temperature dependence allowed a good separation of the SO₄²⁻ impurity. The pure LaNTS, showing no SO₄²⁻, was then used to prepare the pure acid, Na₃NTS and K₃NTS by ion exchange methods as described for the BDS salts. The La⁺³ salt dries to a definite composition LaNTS·5.5H₂O at 25° over CaCl₂ or can be dried to a dihydrate at 180°. Unfortunately, the Na⁺ and K⁺ salts are somewhat hygroscopic and difficult to weigh in solid form. This problem can be circumvented either by making up the stock solutions in a dry atmosphere or by careful analysis of the stock solutions.

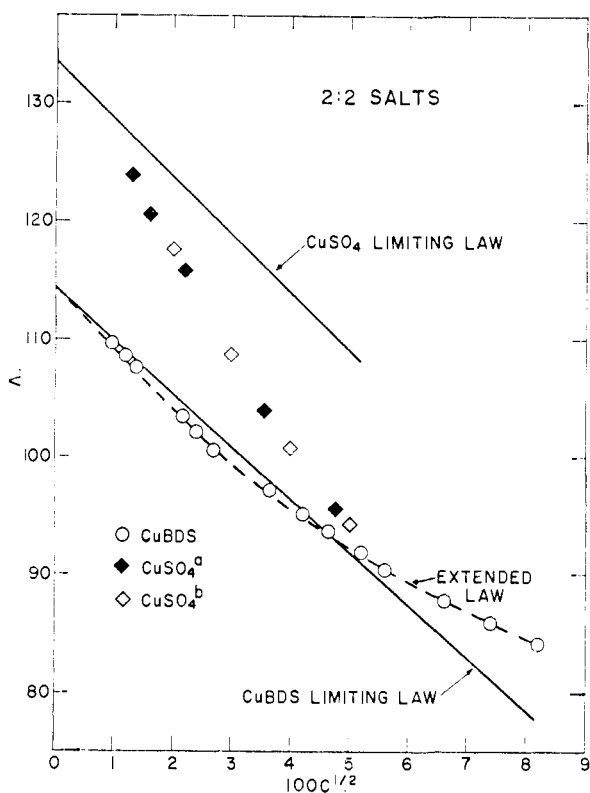
(16) Matheson, Coleman and Bell, Norwood, Ohio, Item T 1164.

(17) J. Träger and W. Meine, *J. prakt. Chem.*, **68**, 313 (1903).

(18) Aldrich Chemical Co., Milwaukee 10, Wisconsin.

Analyses for the BDS and NTS anions were performed by quantitatively converting a weighed amount of the salt to the acid using analytical grade ion-exchange resin and potentiometrically titrating the acid with standard NaOH. Since both H₂BDS and H₂NTS are strong acids, good end-points were obtained and the precision was ± 0.1% or better. A check of the accuracy of the method using electrochemical grade KCl showed no systematic errors. Electrodeposition was used for Cu determination; La was determined by precipitation as oxalate and conversion to oxide. Water of hydration was determined by the Karl Fischer method and by difference. Potassium determinations were made using the sodium tetraphenyl boron technique.

At least two stock solutions were made up by weight for each electrolyte. The concentrations of these stock solutions were checked periodically by the ion exchange technique. Flask type cells with smooth Pt electrodes were used for all measurements. The cell constants were known to ± 0.01% as a function of frequency and measured conductance. Two cells were used with infinite frequency, zero conductance cell constants of 0.2635₀ and 0.1765₄. Each concentration was measured at four frequencies and the resistance extrapolated to infinite frequency by an *R* versus *f*^{-1/2} plot. In a typical run, the conductance water was weighed into the cell and its resistance measured after temperature equilibration. Then, six to eight successive aliquots of the stock solution were weighed into the cell, the resistance being measured after each addition upon attaining equilibrium. The solvent correction used in the calculations was thus the right correction for that batch of water on that day in that cell. The conductance water used had a specific conductance of less than 1 × 10⁻⁶ mho for all measurements reported (*L*_{average} = 0.7 × 10⁻⁶ mho). All measurements were made at 25 ± 0.002° in a transformer oil-bath. The measuring equipment consisted of a General Radio 1301A low-distortion oscillator, a Leeds and Northrup Jones bridge and a Fisher TR-1 battery powered preamplifier. The final amplifier and null detector was a Dumont 304H oscilloscope. From two to six runs were made for each electrolyte and all runs are presented here in tables. The complete data are available from the authors on request as well as a more detailed explanation of the data treatment.

Fig. 1.—a, This Laboratory; b, Owen and Gurry.⁵

Calculations and Results

Table I gives the equivalent conductances of the eight electrolytes measured. Since both H_2BDS and H_3NTS are essentially completely dissociated, no correction need be made for anion hydrolysis. For all cations other than the common monovalents, a hydrolysis correction must be made. The data needed for this correction are the hydrolysis constants of the cation and CO_2 and the pH of the solvent and of the solutions over the concentration range of interest. These were obtained in our work by use of a Beckman GS pH meter making a run exactly analogous to the conductance runs, *i.e.*, starting with solvent and adding aliquots of stock solution. The $[H^+]$ of the solvent is assumed to be due entirely to the H_2O-CO_2 equilibrium and the solvent correction is written, $L_s = L_s(CO_2) + L_s(\text{residual})$, the residual conductance being due to unknown but non-protonic impurities. The solvent correction used for the solutions then becomes, in the case of $CuBDS$

$$L_s = L_s(\text{residual}) + L_s(H^+) + L_s(CuOH^+) + L_s(HCO_3^-) + L_s(BDS^-)$$

The BDS^- correction is to take care of the Cu^{++} removed by hydroxide formation. All the above terms can be calculated by a series of successive approximations. The $CuBDS$ concentration is also corrected for the salt removed by hydrolysis and so included in the solvent correction. The hydrolysis constants used were $K_h = 1.5 \times 10^{-8}$ for Cu^{++} ¹⁹ and 1.2×10^{-9} for La^{+3} .²⁰ The La^{+3}

(19) K. J. Pederson, *Kgl. danske Vidensk. Selsk. mat.-phys. Medd.*, **20**, 7 (1943).

(20) T. Moeller, *J. Phys. Chem.*, **50**, 242 (1946).

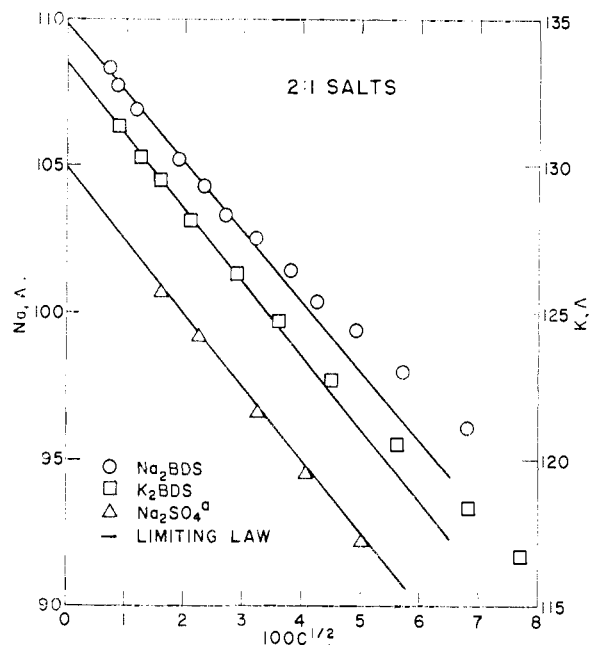


Fig. 2.—a, ref. 2, p. 697.

hydrolysis correction was carried out in exactly the same way. The correction to the equivalent conductance of $CuBDS$ ranged from 0.8% at 10^{-4} molar to 0.2% at 5×10^{-3} molar. That for $LaNTS$ varied from 0.1% at 5×10^{-5} molar to 0.06% at 2×10^{-3} molar.

After these preliminary corrections, the data were analyzed by the Fuoss-Onsager extended equation in the form for completely dissociated electrolytes.²¹

$$\Lambda = \Lambda^0 - SC^{1/2} + EC \log C + JC$$

where Λ^0 = equivalent conductance at infinite dilution

$$S = \alpha\Lambda^0 + \beta \text{ (Onsager limiting slope)}$$

$$E = E_1\Lambda^0 - E_2$$

$$J = \sigma_1\Lambda^0 + \sigma_2 \text{ (size parameter term)}$$

The symbols are further defined in ref. 21. Preliminary Λ^0 values were obtained either from a Shedlovsky plot²² or from a careful extrapolation of the low concentration points on a Λ versus $C^{1/2}$ plot (phoreogram). The final analyses were made by means of the Fuoss Λ' versus C plots where

$$\Lambda' \equiv \Lambda + SC^{1/2} - EC \log C = \Lambda^0 + JC$$

Table II lists the Λ^0 , λ_1^0 (estimated), J and a^0 values for these eight electrolytes.²³ Figure 1 shows phoreograms for $CuBDS$ as contrasted to $CuSO_4$. The $CuSO_4$ data are from both Owen and Gurry⁵ and from this Laboratory. The hydrolysis correction to our $CuSO_4$ data was carried out in the same way as the $CuBDS$ correction including an additional term for the HSO_4^- equilibrium. As can be seen, the two sets of $CuSO_4$ data agree very well. The $CuBDS$ curve definitely approaches the limiting tangent from above in

(21) Ref. 12, chapter XV.

(22) T. Shedlovsky, *J. Am. Chem. Soc.*, **54**, 1405 (1932).

(23) The Fuoss-Onsager treatment was carried out consistently for the unsymmetrical salts as outlined in ref. 12. Although its applicability to such salt types is questionable, as a curve fitting method it is far superior to the older methods (Shedlovsky, Owen, etc.).

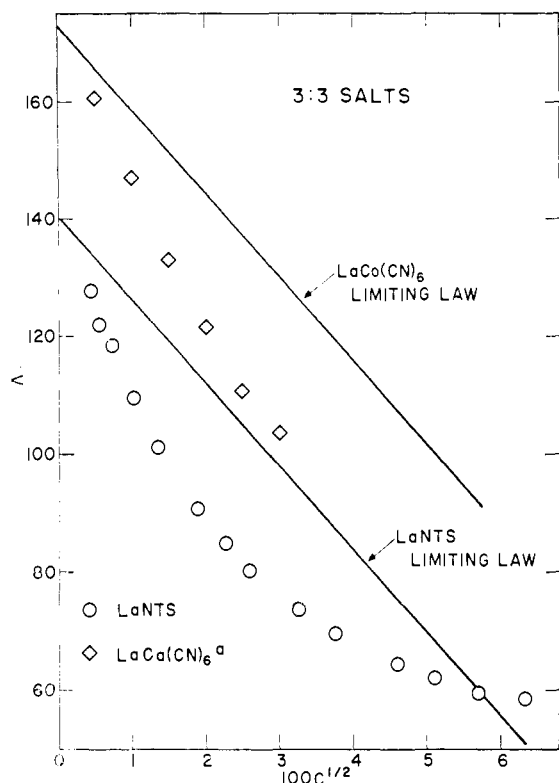


Fig. 3.—a, G. Atkinson, THIS JOURNAL, 82, 818 (1960).

marked contrast to CuSO_4 . Figure 2 compares H_2BDS , Na_2BDS and K_2BDS with a previously measured 2-1 electrolyte. Figure 3 contrasts LaNTS and $\text{LaCo}(\text{CN})_6$. Again the LaNTS approaches the limiting tangent from above. Figure 4 compares H_3BDS , Na_3BDS and K_3BDS with a previously measured 3-1 salt. The unsymmetrical electrolytes were included in this study primarily to give more information on these anions and particularly to give approximate λ^0 values.

TABLE II

Salt	SUMMARY OF PARAMETERS			<i>J</i>	σ^0
	Λ^0	λ^0 (est.)	λ^{0+} (est.)		
H_2BDS	412.3	6,650	4.99
Na_2BDS	109.99	59.88	50.11 ^a	1,775	4.52
K_2BDS	133.52	60.00	73.52 ^a	1,975	4.13
CuBDS	114.54	(59.94)	54.60	8,350	5.02
H_3NTS	421.8	33,800	6.2
Na_3NTS	121.55	71.44	50.11 ^a	9,850	6.2
K_3NTS	145.05	71.53	73.52 ^a	11,950	6.4
LaNTS	140.50	71.44	69.06 ^b	129,700	6.0

^a Ref. 2, pp. 231. ^b F. H. Spedding and G. Atkinson, Chapter 22, "The Structure of Electrolyte Solutions," ed. W. J. Hamer, John Wiley and Sons, Inc., New York, N. Y., 1959.

Closer examination of the CuBDS and LaNTS curves reveals a puzzling behavior. Although both curves approach their limiting law tangents from above (the old criterion for an unassociated electrolyte), they then cross the line and approach Λ^0 from below. This behavior is a consequence of the $C \log C$ term in the extended equation. Over the concentration range of interest ($Ka < 0.2$) $EC \log C$ is always negative and JC positive. For

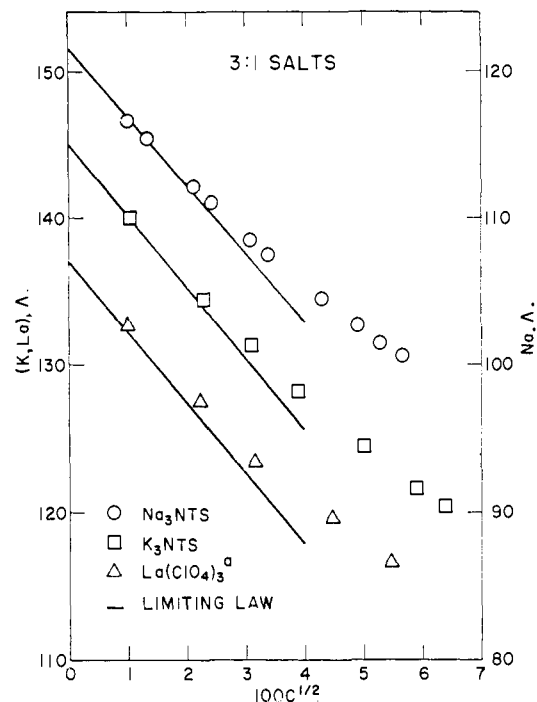


Fig. 4.—a, F. H. Spedding and G. Atkinson, "The Structure of Electrolyte Solutions," W. J. Hamer, ed., John Wiley and Sons, New York, N. Y., 1959, Chapter 22.

these high charge type salts $EC \log C$ becomes important at very low concentrations but is eventually compensated for and finally outweighed by JC . The extended theory, then, says that the limiting law and extended form really have only two points in common; $C = 0$ and $C = C_x$, the crossover point. At the crossover point

$$\Lambda^0 - SC_x^{1/2} = \Lambda^0 - SC_x^{1/2} + EC_x \log C_x + JC_x$$

leading to $\log C_x = -JE$, so that the crossover concentration is characteristic of a given electrolyte. Then between $C = 0$ and $C = C_x$, $\Delta\Lambda = (EC \log C + JC)$ must have a maximum value. This easily is shown to be

$$\log C_M = -(0.4343 + J/E)$$

and at C_M

$$\Delta\Lambda_M = EC_M \log C_M + JC_M$$

Table III gives the C_x , C_M and $\Delta\Lambda_M$ for KCl , CuBDS and LaNTS as calculated from the above equations. The KCl figures are those of Fuoss who first noticed this consequence of the theory.²⁴ As we see, the effect for a 1-1 is so small as to be essentially unmeasurable. For all practical purposes then, the curve for a 1-1 unassociated salt does approach the limiting tangent asymptotically. However, this behavior is very sensitive to charge type as can be expected from the J and E values given. The final columns in Table III give the C_x and $\Delta\Lambda_M$ values as taken from large scale phoreograms. The agreement between the observed and calculated values is quite good for CuBDS giving additional evidence that this is indeed an unassociated 2-2 electrolyte. The agreement is poorer for the LaNTS salt, which is not too surprising. The J and E are very large and hard to evaluate accurately.

(24) Ref. 1, pp. 678.

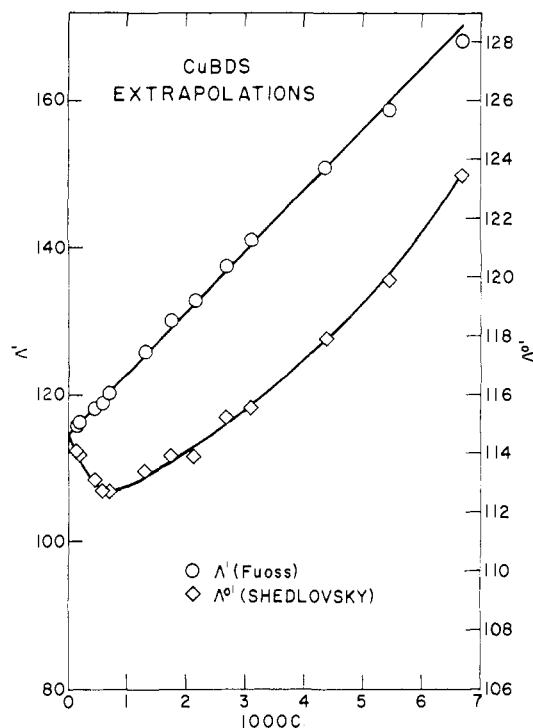


Fig. 5.

Fig. 5 shows the Λ' plot for CuBDS as contrasted with the Shedlovsky plot. It is easily seen that the Shedlovsky plot furnishes a very poor way of evaluating Λ^0 for such high charge type salts. Fuoss has shown that this inadequacy is easily predicted from the extended theory. The CuBDS Λ' is very close to a straight line, possibly curving down a little at the high end. The plots for all the unsymmetricals exhibit this behavior to a marked degree though they still are useful for extrapolation. Various explanations can be advanced for this behavior of which the most prominent are:

1. Association, *i.e.*, we are using JC when we should use $(J - K_A\Lambda^0)C$
2. Non-inclusion of a Stokes law viscosity correction
3. Non-inclusion of higher terms $(O)C^{3/2}$
4. General breakdown of theory for unsymmetricals

At the present time we can only say that the first two reasons do not give corrections with the right magnitude or concentration dependence. The mixed solvent work in progress may aid to our understanding here. An interesting feature of the unsymmetrical salt data is related to the third reason.

If we say that our full equation should be

$$\Lambda = \Lambda^0 - SC^{1/2} + EC \log C + JC + (O)C^{3/2}$$

then we can define

$$\begin{aligned} \Lambda'' &= \{(\Lambda + SC^{1/2} - EC \log C) - \Lambda^0\} / C \\ &= (\Lambda' - \Lambda^0) / C \\ &= J + (O)C^{1/2} \end{aligned}$$

Figure 6 shows a plot of Λ'' versus $C^{1/2}$ for some of these salts. Although this procedure pushes the data to extremes and the error in Λ'' is very large at low concentrations, the lines do appear to be straight. We are offering this more as a matter of

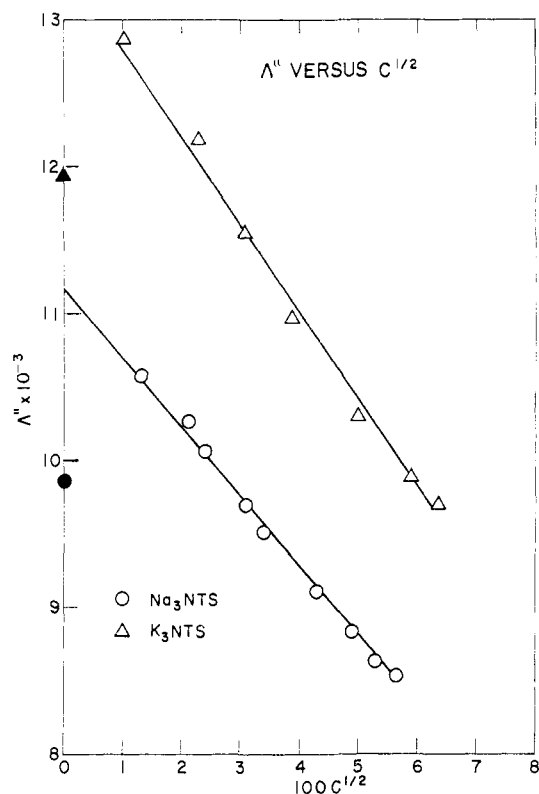


Fig. 6.

interest than as evidence. Much more data are certainly needed.

The Λ' plot for the LaNTS is very puzzling. The initial slope increases then decreases again giving the curve a definite S shape. Although the initial slope gives the most consistent Λ^0 value, the error in these points is the highest. A great problem is, of course, the extreme slope of the line making a small change in slope correspond to a large change in Λ^0 . This uncertainty in Λ^0 causes an uncertainty in E which is large. As a result we are not as confident that LaNTS is unassociated and are working on this and related 3-3 salts in greater detail.

TABLE III

Salt	THE CROSSOVER POINT				Calcd. $\Delta\Delta_M$	Plot
	Calcd. $10^4 C_c$	Plot	Calcd. $10^4 C_M$	Plot		
KCl	1.8	..	0.7	..	0.002	..
CuBDS	26.1	25.5	9.6	10.1	1.3	1.5
LaNTS	22.3	32.5	8.2	6.8	19.8	22.8

Various approaches have been tried in the fitting of the LaNTS data, none of which seem satisfactory. In the first attempt, J is evaluated from the best straight line on the Λ' versus C plot. This gives an δ value consistent with the other NTS³⁺ salts but a Λ^0 value that is inconsistent; and the calculated curve is a poor reproduction of the experimental. In a second approach, a Λ^0 is picked to be consistent with the other NTS³⁺ salts and La³⁺ salts thus fixing S and E . The curve is then fitted in the low concentration range by arbitrarily picking J to minimize the difference between experiment and theory. This yields a J value less

than half that found by the first method and a ridiculous a^0 . Also, it is not possible to find a single J that will make the theoretical and experimental curves coincide over any range of concentration. Permutations and combinations of the above do not give anything much better in the way of a fit. A viscosity correction does not give enough aid and the simple association correction would push the Λ' curve up instead of down. At the present time, we can only hope that further work on this type salt and particularly mixed solvent work will help our interpretive problem.

To illustrate the difficulties involved in adding these "corrective" terms to the original limiting law, Table IV gives the relative size of the terms in the CuBDS and LaNTS extended equations at a few concentrations. To say that we are straining the theory in these cases is certainly an understatement. Approximations of no import for a 1-1 may become very important for a 3-3.

We hope to shed more light on these unsettled questions by the extensive experimental program we have in progress. At present we are exploring both the low-field and high-field conductance of the BDS⁼ salts of the other stable divalent ions as well as other lanthanide NTS⁼ salts. We also have prepared salts of benzenetrisulfonic acid and 4,4'-

TABLE IV

Salt	$C \times 10^4$	Λ^0	$SC^{1/2}$	$EC \log C$	JC	Λ
CuBDS	1	114.54	4.52	- 1.29	0.84	109.57
	10	114.54	14.25	- 9.70	8.35	98.94
	50	114.54	31.95	- 37.20	41.75	87.14
LaNTS	1	140.5	14.1	- 23.5	15.8	118.7
	10	140.5	44.5	-176.3	158.0	77.7
	50	140.5	99.6	-676.6	790.0	154.3

diphenyldisulfonic acid and are doing preliminary work with them. In addition, we are collaborating with workers at other universities in the exploration of the activity coefficients and transference numbers of these salts. It is hoped that this paper will stimulate more interest in the properties of this intriguing new class of strong electrolytes. Finally, one must say that the success of the extended Fuoss-Onsager theory in treating these high charge type salts is a striking demonstration of the essential correctness of the theory.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY, ITHACA, NEW YORK]

Thermodynamic Study of Shrinkage and of Phase Equilibrium under Stress in Films Made from Ribonuclease^{1,2}

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Ribonuclease films were used for studies of shrinkage and phase equilibrium under stress. The films were prepared by coagulating ribonuclease with formaldehyde, compacting the clot to a thin film, partially cross-linking with *p*-benzoquinone, stretching in hot urea solution and further cross-linking to obtain a partially oriented, partially crystalline material. The film is considered to consist of two regions, *i.e.*, amorphous zones which crystallize on stretching and separate zones which contain crystallites in equilibrium with the surrounding amorphous zone. With these films, investigations were carried out on the dependence of the helix-random coil transition temperature on the *pH* of the medium, using measurements of retractive force. The change in force with temperature was reversible in the presence of 2 *M* KCl at low *pH* where no side-chain hydrogen bonding is present. The *pH*-dependence of the transition temperature was adequately accounted for by means of a reasonable model and a recent theory of reversible protein denaturation, in which side-chain hydrogen bonds play a dominant role. The heat and entropy change per peptide residue, accompanying the helix-random coil transition, were obtained from similar experiments on the dependence of the transition temperature on the concentration of urea. The crystalline unit in the assumed model consisted of two helical portions of 12 amino acid residues each, connected at both ends by cross-links. Force-temperature curves as a function of relative length, as well as data on swelling and its dependence on ionic strength, also were obtained, the data being subjected to a thermodynamic analysis from the point of view of phase equilibrium under stress. It was thus possible to obtain estimates of the contributions of the heat of solution, heat of rupture of a backbone hydrogen bond and the heat change accompanying the change of composition of the fiber induced by a change in its length. The model and the results are compatible with known structural features of ribonuclease and the elastic behavior of other fibrous proteins.

Introduction

The effect of side-chain hydrogen bonds on the elastic properties of protein fibers and on the configurations of proteins in solution recently has been treated theoretically⁴ on the basis of earlier work

on elastic mechanisms in proteins⁵ and on the stability of side-chain hydrogen bonds.⁶ The purpose of this paper is to provide experimental evidence for the earlier theoretical considerations⁴ and to obtain further information about the role of side-chain hydrogen bonds in the mechanism of shrinkage. Ribonuclease was chosen for this study since the amino acid sequence⁷ and disulfide bridge positions⁸ are known, and some information is

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