

very fast, and an idea as to the duration of the transient state may be obtained by noting that the half times for the interconversions of the two enzyme substrate complexes is less than a millisecond. The rapid approach to the steady state which is indicated by these calculations justifies the use of the steady-state approximation in deriving equations for the representation of experimental data and shows that the error made in using total elapsed time in integrated equations rather than time after establishment of the steady state is negligible.

Discussion

The minimum values of the second-order rate constants for the reactions of fumarase with its substrates are greater than any which have been measured for reactions of enzymes. Chance²⁰ obtained $1.2 \times 10^8 \text{ sec.}^{-1} M^{-1}$ for the reaction of yeast cytochrome-*c* peroxidase with cytochrome-*c* by the flow method. The second order rate constants^{11,12} for the combination of alcohol dehydrogenase with DPN and DPNH are of the order of $10^6 \text{ sec.}^{-1} M^{-1}$. The high minimum values of the second-order rate constants for the fumarase reaction raise the question, which is to be discussed in a future article, as to how this rate compares with what would be expected from collision theories and diffusion theories of reactions in solution.

The values of the equilibrium constants for the first and last steps of mechanism 11 cannot be calculated from the steady-state kinetic data, but the maximum and minimum values consistent with the experimental data may be calculated using equations 30, 31, 34 and 35 and inequalities 36 and 39. The maximum and minimum factors by which k_2/k_1 differ from K'_F at 0.001, 0.01 and 0.10 ionic strengths are 1.3–0.96, 1.05–0.90, 1.2–0.95, respectively. Thus although the exact values of the equilibrium constants cannot be calculated, it can be stated that they are equal to the Michaelis constants within approximately the experimental error. The same conclusion has been reached for

(20) B. Chance, in J. T. Edsall, "Enzymes and Enzyme Systems," Harvard University Press, Cambridge, Mass., 1951.

chymotrypsin²¹ and urease²² by other methods.

The calculations of specific reaction rate constants in this article are all based upon the assumption of one enzymatic site per fumarase molecule. If there are n sites the calculated rate constants will all be decreased by this factor. Since the titration curve for fumarase has been determined²³ in 0.1 *M* NaCl at 25° it is possible to estimate the maximum number of enzymatic sites per molecule. The kinetic studies with 0.1 ionic strength buffer (0.09 *M* NaCl plus 0.01 *M* "tris" acetate) show that there are two groups per enzymatic site with $pK = 7.4$. The slope of the titration curve at $pH 7.4$ indicates a maximum of 12 groups with this pK value or a maximum of 6 catalytic sites per molecule.

Although the present calculations have been made using data from kinetic studies of both the forward and reverse reactions, similar calculations could be made for reactions which go essentially to completion, provided the inhibition constant of the product has been measured. Since this constant is identical with the Michaelis constant for the product, the maximum initial velocity for the reverse reaction could be calculated using $K_{eq} = V_F/K_M V_M K_F$ provided the free energy change or equilibrium constant for the over-all reaction are known. For reactions involving two reactants or two products the mechanism would have to be known so that the correct form of the relation between kinetic parameters and the equilibrium constant for the over-all reaction could be chosen.²⁴

Acknowledgments.—The authors are indebted to Professor Edward L. King for helpful suggestions and to Professor Preston C. Hamner of the Numerical Analysis Laboratory of the University of Wisconsin for mathematical advice and assistance.

(21) H. T. Huang and C. Niemann, *THIS JOURNAL*, **73**, 1541 (1951).

(22) G. B. Kistiakowsky and W. E. Thompson, *ibid.*, **78**, 4821 (1956).

(23) N. Shavit, R. G. Wolfe, Jr., and R. A. Alberty, unpublished.

(24) R. A. Alberty, *THIS JOURNAL*, **75**, 1928 (1953).

MADISON, WISCONSIN

[CONTRIBUTION NO. 1410 FROM THE STERLING CHEMISTRY LABORATORY OF YALE UNIVERSITY]

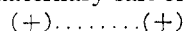
Bolaform Electrolytes. VI. Conductance of Bis-(trimethylammonium)-polymethylene Iodides and Related Compounds in Methanol and in Ethanol

BY ORMOND V. BRODY¹ AND RAYMOND M. FUOSS

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The following conductance data are presented: bis-(trimethylammonium)-trimethylene diiodide, bis-(trimethylammonium)-tetramethylene diiodide, bis-(trimethylammonium)-pentamethylene diiodide, tetramethylammonium iodide, methylpyridinium iodide and dimethylpiperidinium iodide in ethanol at 25°; tetramethylammonium iodide and bromide, methylpyridinium iodide, dimethylpiperidinium iodide and 1,1-methane-*N,N'*-bispyridinium diiodide in methanol at 25°. In ethanol, electrostatic attraction is strong enough to stabilize structures in which two anions associate with the bolaform cations. The association decreases with increasing distance between the cationic sites of the latter.

It has been shown that association of an anion to one end of a bisquaternary salt of the structure



(1) California Research Corporation Postdoctoral Fellow, 1955–1956.

is sensitive to the distance between the cationic sites. Most of our previous work has been in methanol, where the dielectric constant (33.62) is sufficiently high to permit neglect of configurations in which anions associate with both cationic sites, at

least in the range of low concentrations. When the two charges coalesce (as in Mg^{++}), the field is intense enough to stabilize ($Cl' Mg^{++} Cl'$) clusters; the data therefore lead to numerical values for the constants describing association of first one and then two anions with the cation. We would expect binary association to occur in the case of bolaform electrolytes if the dielectric constant of the solvent were significantly lower than that of methanol. A series of bis-(trimethylammonium)-polymethylenes was therefore studied in ethanol ($D = 24.30$). As expected, k_1 , the constant describing the dissociation of $(-)(+) \dots (+)(-)$ clusters into $(-)$ and $(+) \dots (+)(-)$, was found to depend on the distance between the positive charges in the cation, the effect decreasing in accordance with Coulomb's law with increasing distance between the charges.

Experimental

Materials.—1,3-Bis-(trimethylammonium)-trimethylene diiodide ("trimethonium iodide," I), 1,4-bis-(trimethylammonium)-tetramethylene diiodide (II) and 1,5-bis-(trimethylammonium)-pentamethylene diiodide (III) were from stock prepared by Chu.²

1,1-Methane-N,N'-bispyridinium diiodide was prepared by heating a mixture of 24 ml. of pyridine and 8.2 ml. of methylene iodide for 30 min. at 90°. Golden crystals separated; they were washed with cold ethanol and recrystallized from water. Iodine by potentiometric titration, 59.93; theory, 59.60%. Nitrogen, 6.51, 6.36%; theory, 6.58%. The compound has a strong maximum (molar extinction coefficient at $3.07 \times 10^{-5} M$ in water, 9950) at about 259 $m\mu$. The ultraviolet absorption curve parallels that of N-methylpyridinium iodide; the ratio of the molar extinction coefficients is 2.25. Solutions of the salt in methanol were unstable; resistance increased slowly (5% in 10 hr.) on standing. The nitrate (IV) was prepared by ion exchange; Amberlite XE 75 was converted to a nitrate column by concentrated sodium nitrate solution. After thoroughly washing the column, a 2.5% solution of the iodide was run through the column, and eluted with water. Salt was recovered by evaporation, and recrystallized from methanol. *Anal.* Calcd. for $(C_5H_4N)_2CH_2(NO_3)_2$: N, 18.92. Found: N, 18.05, 17.75. The nitrate is white; the iodide thus represents another example of colored-onium iodides.^{3,4}

Tetramethylammonium iodide was prepared from trimethylamine and methyl iodide, and recrystallized from conductivity water. It was analyzed for iodide by potentiometric titration with 0.01 *N* silver nitrate (% iodide found: 63.13, 63.13, 63.18; av. 63.15. Theoretical, 63.12%). The compound is easy to prepare and we plan to use it as a secondary standard for cell calibration, using non-aqueous solvents. A check analysis was therefore made, using 0.1 *N* ceric sulfate which was standardized against Bureau of Standards arsenious oxide.⁵

The ceric ion evidently attacks the quaternary ion, because erratic results and ambiguous end-points were obtained. The tetramethylammonium iodide was therefore quantitatively converted to the equivalent amount of hydriodic acid, which was then titrated potentiometrically (glass electrode vs. a platinum wire) with ceric sulfate solution. End-points were very sharp (ca. 4000 mv./ml. 0.1 solution at the peak). Iodide found: 63.11, 63.18%.

The conversion to hydriodic acid was accomplished by running solutions of weighed samples (ca. 200 mg.) of salt through an Amberlite IR 120 column and washing the column quantitatively. The resin was previously purified by continuous Soxhlet extraction with 6 *N* hydrochloric acid for 12 hr. followed by extraction with several portions of

distilled water until the extract tested halogen-free. Two 24-hr. extractions sufficed.

Other salts were prepared by mixing the corresponding amines and alkyl halides: tetramethylammonium bromide, recrystallized from 3:1 $H_2O-EtOH$ (% Br found: 51.76, 51.81; theoretical 51.87); methylpyridinium iodide, from ethanol (% I found 57.33; theoretical 57.41); N,N-dimethylpiperidinium iodide, from ethanol (% I found 52.43, 52.31; theoretical 52.63).

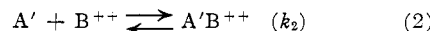
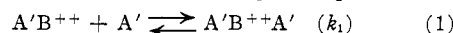
Method.—Methanol and ethanol were dried over aluminum amalgam; the conductance was usually about 1.5×10^{-8} . Cells^{6,7} and electrical equipment⁸ have already been described. Both concentration and dilution runs were made; they agreed in the overlapping range.

Results

The results are summarized in Tables I-V, where $\Lambda = 1000 \kappa/c$, c is concentration in equivalents per liter, and κ is solution conductance corrected for solvent conductance.

Discussion

The conductance data for the bisquaternary salts were analyzed by the method of Sacks and Fuoss,⁹ in order to obtain limiting conductances and the constants k_1 and k_2 describing the equilibria



The dielectric constant of ethanol is sufficiently low to cause considerable binary association and it is therefore necessary to obtain k_1 by successive approximations before k_2 and Λ_0 can be calculated. The simpler $\Lambda' - x$ curves¹⁰ which can be used when

TABLE I
CONDUCTANCES OF $I'[Me_3N^+(CH_2)_nN^+Me_3]I'$ IN ETHANOL

$n = 3$		$n = 4$		$n = 5$	
10 ⁴ c	Λ	10 ⁴ c	Λ	10 ⁴ c	Λ
0.2131	44.97	0.1342	50.56	0.0899	53.67
.2506	43.26	.2224	46.76	.2167	48.59
.4237	39.46	.2744	45.48	.3355	45.33
.5469	37.16	.4065	42.10	.4110	43.85
.7681	34.74	.6307	38.75	.5741	41.09
1.310	30.77	1.178	33.76	.7898	38.63
2.660	26.17	3.152	27.24	1.276	34.77
4.632	23.20	4.557	25.03	3.704	27.57
6.191	21.72	8.657	21.85	7.320	23.84
8.280	20.38	14.509	19.51	11.606	21.79
18.52	16.70				
20.55	16.36				

TABLE II
CONDUCTANCE OF 1-1 SALTS IN ETHANOL

10 ⁴ c	Λ	10 ⁴ c	Λ	10 ⁴ c	Λ
Me_3NI		N-Methyl- pyridinium iodide		N,N-Dimethyl- piperidinium iodide	
1.1057	53.95	0.7352	55.52	0.3102	54.53
1.435	53.42	1.084	54.93	.5844	54.03
2.438	52.18	1.416	54.56	.6005	53.97
3.747	50.82	1.928	53.87	1.122	53.13
4.276	50.39	4.826	51.15	1.768	52.21
7.571	47.99	14.73	45.73	3.111	50.74
				3.733	50.18

(2) R. M. Fuoss and V. F. H. Chu, *THIS JOURNAL*, **73**, 949 (1951).

(3) A. Hantzsch, *Ber.*, **52**, 1544 (1919).

(4) E. D. Bergmann, F. E. Crane, Jr., and R. M. Fuoss, *THIS JOURNAL*, **74**, 5979 (1952).

(5) I. Kolthoff and E. B. Sandell, "Methods of Quantitative Analysis," 3rd edition, the Macmillan Co., New York, N. Y., 1952, p. 581.

(6) J. C. Nichol and R. M. Fuoss, *J. Phys. Chem.*, **58**, 696 (1954).

(7) O. V. Brody and R. M. Fuoss, *ibid.*, **60**, 177 (1956).

(8) H. Eisenberg and R. M. Fuoss, *THIS JOURNAL*, **75**, 2914 (1953).

(9) F. M. Sacks and R. M. Fuoss, *J. Electrochem. Soc.*, **99**, 483 (1952).

(10) R. M. Fuoss and D. Edelson, *THIS JOURNAL*, **73**, 269 (1951).

TABLE III

CONDUCTANCE OF 1-1 SALTS IN METHANOL			
10 ⁴ c	Λ	10 ⁴ c	Λ
N,N-Dimethylpiperidinium iodide			
Me ₄ NI			
0.4682	128.65	0.1859	123.0
.8111	127.95	.3690	122.6
1.061	127.5	.4268	122.5
1.333	127.15	.6345	122.2
1.907	126.5	1.174	121.3
		2.189	120.2
		2.664	119.9
N-Methylpyridinium iodide			
Me ₄ NBr			
0.4124	123.15	0.1173	127.7
.8128	122.9	.2267	127.4
1.634	121.5	.3483	127.2
3.738	119.25	.4617	127.05
		.9085	126.25
		1.747	125.1
		2.735	124.1

TABLE IV

CONDUCTANCE OF PY ₂ CH ₂ (NO ₃) ₂ IN METHANOL	
10 ⁴ c	Λ
0.2168	132.4
.4139	130.3
.7162	127.55
.8357	126.2
1.492	122.2
1.676	120.7
2.645	116.1
2.919	114.5
4.705	108.4

TABLE V

CONDUCTANCE OF N,N-DIMETHYLPIPERIDINIUM IODIDE IN WATER	
10 ⁴ c	Λ
2.149	111.2
4.730	110.45
8.244	109.9
13.26	109.2
15.77	108.9
34.04	107.1
37.89	106.7
117.50	102.3

binary association is negligible become double valued, as was shown for the case of hexamethonium bromide in ethanol.¹¹

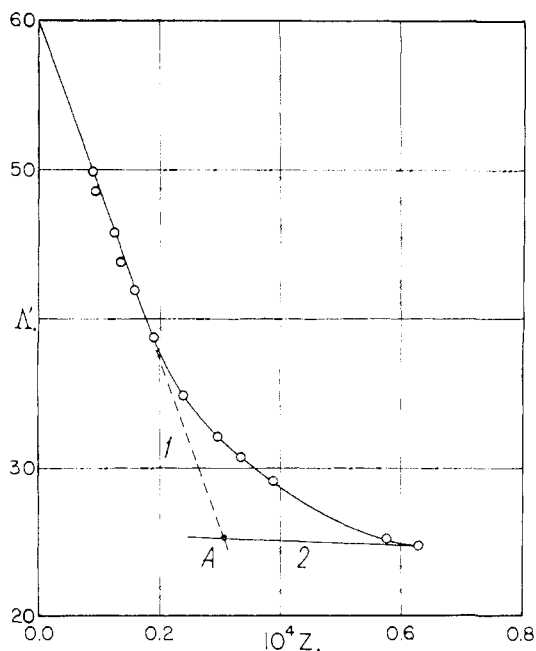


Fig. 1.—Determination of k_1 for trimethonium iodide in ethanol.

A simpler method of obtaining k_1 has been devised, as shown in Fig. 1 for trimethonium iodide in ethanol, where Λ' is plotted against Z , using $k_1 = 0.00109$; the latter preliminary estimate was obtained by the previous method of considering the salt as a 1-1 electrolyte in the intermediate range of concentrations. Then a series of k_1 values in this order of magnitude (0.00109–0.0022) were used to compute values of Λ' and Z for the point at the highest concentration, and the resulting values were plotted (line 2) on the same $\Lambda' - Z$ scale. At low concentrations, Λ' becomes insensitive to k_1 and

(11) O. V. Brody and R. M. Fuoss, *J. Phys. Chem.*, **60**, 156 (1956).

the $\Lambda' - Z$ curve approaches linearity; the linear portion of the $\Lambda' - Z$ curve is therefore extrapolated (line 1) until it intersects line 2, at point A. This determines value which Z should have for the point at highest concentration in order to bring it onto the linear prolongation of the $\Lambda' - Z$ curve from low concentrations. In order to obtain the corresponding value of k_1 , k_1 is plotted against $Z(k_1)$ and the desired value of k_1 is found by interpolation at Z_A . In Fig. 2 are shown the $\Lambda' - Z$ plots for salts I–III; all are linear as required.

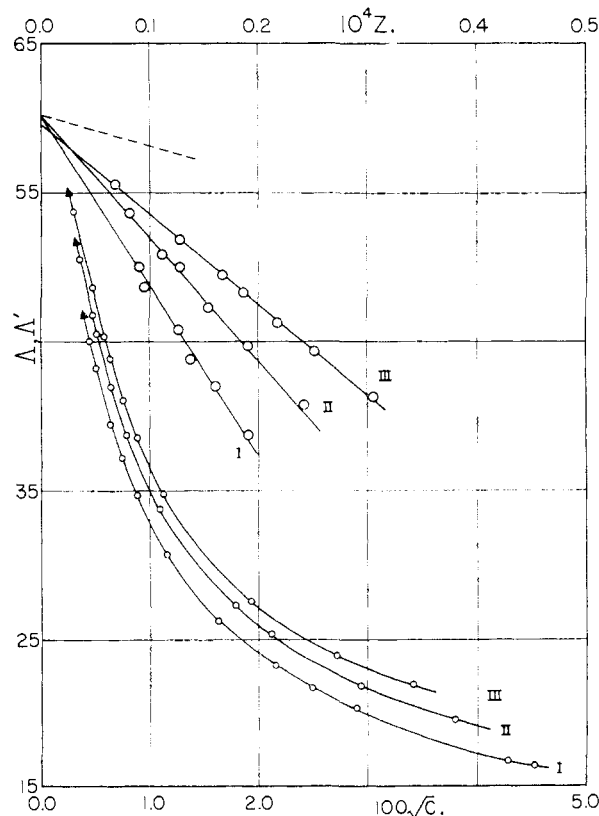


Fig. 2.—Conductance curves (bottom) and extrapolation functions (top) for salts I, II and III in ethanol.

The values of the derived constants for the bis-quaternary salts are given in Table VI; salt H is the hexamethonium iodide for which conductance data were previously reported.¹⁰ The limiting conductances change by about the same ratio in

TABLE VI

CONSTANTS FOR BISQUATERNARY SALTS							
No.	Solvent	Λ_0	λ_0	10^3k_1	10^3k_2	R	Λ_{07}
I	EtOH	60.0	32.1	1.46	2.6	4.74	0.6468
II	EtOH	60.2	32.3	2.00	3.7	5.10	.6490
III	EtOH	59.6	31.7	2.44	4.9	5.44	.6425
H	EtOH	57.2	31.4	9.5	7.5	6.56	.6166
IV	MeOH	136.3	77.2	∞	100	6.46	.7422

methanol and ethanol, as might be expected: the ratio of the Walden products $\lambda_0^{+\eta}$ (MeOH)/ $\lambda_0^{+\eta}$ (EtOH) is 1.264, 1.268 and 1.260 for the tri-, tetra- and pentamethonium ions, respectively. It will be noted that both k_1 and k_2 steadily increase as the number of methylenes joining the charged sites increases. This is, of course, a consequence of diminishing attraction of each cationic site for the anion which eventually associates with the other cationic site at the far end of the chain. The result is that the conductance curves at higher concentrations are in the reverse sequence of the limiting conductances, as shown in Fig. 2. The conductance curves are, of course, much steeper than the Onsager tangent (dashed line) due to ion association in the working range of concentrations.

The approximate charge-charge distance, calculated by Bjerrum's equation¹²

$$k_2 = (1/2)K \exp(-e^2/RDkT)$$

increases regularly in going from the trimethylene to the hexamethylene derivative. The value of K , the intrinsic constant, was obtained from our data on tetramethylammonium iodide, for which $K = 0.0425$. Numerically, they are somewhat smaller than the values obtained from the same salts (5.95, 6.49, 6.61, 7.30) in methanol; no physical significance should be ascribed to the difference, which almost certainly reflects the inadequacy of the simple sphere-in-continuum model.

The iodide and bromide of the dipyrindinium methane cation were both unstable in methanol; resistance changed so rapidly with time that no reliable data could be obtained. The nitrate was much more stable, so that an estimate of $k_2 = 0.0010$ could be obtained. This value is about the same as that obtained¹³ for $\text{Py}^+(\text{CH}_2)_2\text{Py}^+$ dibromide, so the compound presumably has the structure assigned to it.

The data for the 1-1 salts were treated by Shedlovsky's method¹⁴ in order to obtain limiting conductances and reciprocal association constants; the results are summarized in Table VII. Our value

(12) N. Bjerrum, *Z. physik. Chem.*, **106**, 219 (1923).

(13) J. C. Nichol and R. M. Fuoss, *This Journal*, **77**, 198 (1955).

(14) T. Shedlovsky, *J. Franklin Inst.*, **225**, 739 (1938).

TABLE VII

CONSTANTS FOR 1-1 SALTS					
Salt	Solvent	Λ_0	λ_0^+	10^3K	Λ_{07}
Me_4NI	MeOH	130.6	67.9	42.5	0.711
Me_4NBr	MeOH	125.3	68.8	32.8	.682
MePyI	MeOH	129.0	68.0	54.6	.702
Me_2PpI	MeOH	124.3	63.3	110	.677
Me_4NI	EtOH	56.6	28.7	4.91	.611
MePyI	EtOH	57.6	29.7	5.69	.621
Me_2PpI	EtOH	55.8	27.9	5.14	.601
Me_2PpI	H_2O	112.5	35.7	..	1.007

of Λ_0 for Me_4NBr agrees exactly with that of Hartley¹⁵ while our value for the iodide is 1.2 units lower. If Gordon's values of single ion conductances ($\lambda_0[\text{Br}^-] = 56.6$, $\lambda_0[\text{I}^-] = 62.8$) are used, Hartley's data give for the conductance of the tetramethylammonium ion 68.7 from bromide and 69.0 from iodide, while our data give 67.8 and 68.7, respectively. The discrepancy is puzzling, because our iodide analyzed within 0.03% of theoretical, while Hartley's iodide (no analysis reported) was prepared from bromide which analyzed 0.25% low.

The limiting conductances and association constants follow the sequences which one might expect from the structures of the salts, with the exception of dimethylpiperidinium iodide in methanol. The phoreogram is so near the limiting tangent, however, that the K -value in the table probably means little, because the method of calculation forces into the numerical value of K all the approximations of the method of calculation, which become especially poor when K begins to approach unity. The curve for this salt was determined in water to see whether it might lie above the limiting tangent, in view of its low degree of association in methanol. The phoreogram is catatic¹⁶; since all long range effects due to ionic atmospheres can only give conductance curves which lie *above* the Onsager tangent,¹⁷ we must conclude that some ion association must occur in this salt in water. Since both ions are large, association as the consequence of Coulomb attraction seems quite unlikely and some other mechanism must be acting. Since both ions are polarizable (especially the iodide ion), one might consider as the source of the binding energy of the ion pairs attraction between the dipoles which the charge of each ion induces in the other.

NEW HAVEN, CONNECTICUT

(15) T. H. Mead, O. L. Hughes and H. Hartley, *J. Chem. Soc.*, **135**, 1207 (1933).

(16) R. M. Fuoss, *J. Chem. Education*, **32**, 527 (1955).

(17) R. M. Fuoss and L. Onsager, *Proc. Natl. Acad. Sci.*, **41**, 274, 1010 (1955).