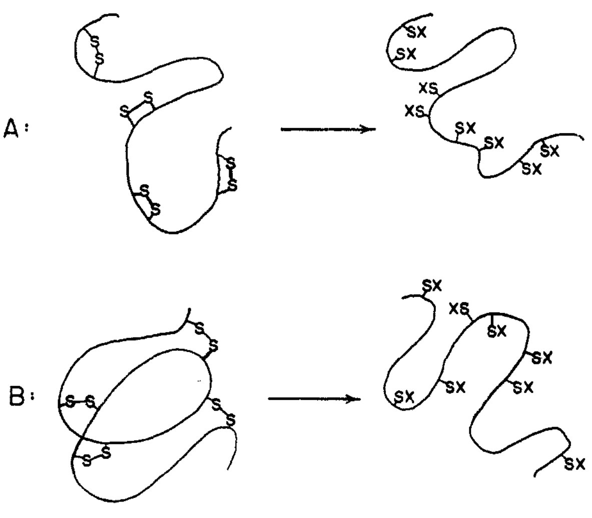

Fig. 8.—Effect of arrangement of cystine residues on shape of unfolded serum albumin molecule: A, two halves of each cystine residue are close neighbors along polypeptide chain. Rupture of —S—S— has slight effect on coiling. B, two halves of each cystine are far apart along chain. Rupture of —S—S— permits a more extended coil.

much when the disulfide links were ruptured (Fig. 8).

It is worth pointing out that the intramolecular disulfide cross links in serum albumin may be responsible for the ease with which the denaturation of serum albumin may be reversed. The cross links may restrict the uncoiling of the denatured molecule to such an extent that on removal of the denaturing agent the molecule is able to find its way back to a folded state similar to that in the native molecule. The well-known irreversibility of the denaturation of ovalbumin would then be accounted for by the absence of such a partially uncoiled state because there are so few SS links in ovalbumin. Furthermore, the denaturation of serum albumin should become irreversible when the intramolecular disulfide links are broken. Elsewhere we shall show that this is indeed probably the case.

The disulfide cross links may also be responsible for the comparative ease with which serum albumin is denatured because these relatively abundant cross links might place constraints on the polypeptide chains making it impossible for them to fold into a structure having the same high degree of stability as would otherwise be attainable. Ovalbumin, with only two disulfide cross links, is not subject to this constraint and might therefore be able to fold in a more stable configuration which can better resist the action of urea.

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[CONTRIBUTION NO. 1176 FROM THE STERLING CHEMISTRY LABORATORY OF YALE UNIVERSITY]

Electrolyte–Solvent Interaction. III. Tetrabutylammonium Bromide in Methanol–Methyl Ethyl Ketone Mixtures[1]

BY F. MURIEL SACKS[2] AND RAYMOND M. FUOSS

RECEIVED JULY 8, 1953

Tetrabutylammonium bromide shows slight ionic association (K = 0.04) in methanol, and considerably more (K = 0.00159) in methyl ethyl ketone. In mixtures of these solvents, the salt shows negligible association ($K > 1$). As the ketone content of the mixtures increases, the viscosity decreases, but the equivalent conductance of the salt decreases also, so that the Walden product $\Lambda_0\eta$ initially decreases. A minimum then appears, and in the ketone-rich mixtures, conductance increases much more rapidly than the viscosity decreases. Both the change of association and mobility with composition of the solvent can be accounted for by assuming (1) that methanol is depolymerized by addition of ketone and (2) that specific interaction ("solvation") then occurs between monomeric methanol and the solute.

Introduction

Recent work[3,4] on the conductance of electrolytes in mixed solvents has given experimental confirmation of the expectation that the conventional spheres-in-continuum model for the system electrolyte–solvent would fail in cases where specific interaction between solute and solvent occurs. The ultimate goal of the investigation is to clarify the rather vague concept of solvation. In a general sort of way, we may expect to find all gradations from stoichiometric complexes to loose aggregates in which an ion simply drags with itself an average (not necessarily integral) number of solvent molecules by ion–dipole attraction. The size of the sphere which is hydrodynamically equivalent to a given ion would then vary from solvent to solvent, depending on the nature of the bonding and the effective thickness of the solvate shell; the diagnostic symptom will obviously be a variation of the Walden product $\Lambda_0\eta$ with solvent. Likewise, the size of the electrostatically equivalent sphere (as calculated from the association constant) should vary from solvent to solvent if solvation is selective. Furthermore, the hydrodynamic and electrostatic radii need not agree numerically, and certainly should differ if solvate molecules are expelled on the formation of ion pairs.

Rather than merely comparing results in different solvents, studies of mixed solvents are preferred, because two additional pieces of information then become available: (1) any abrupt change in electrolyte properties on adding solvent A to B or *vice versa* will indicate a preferential interaction of cation or anion with A or B, and (2) interaction between the solvents themselves will appear as a

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(1) Office of Naval Research Project NR 051-002, Paper No. 39.

(2) Results presented in this paper are abstracted from a dissertation presented by F. Muriel Sacks to the Graduate School of Yale University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, June, 1953.

(3) H. Sadek and R. M. Fuoss, THIS JOURNAL, **72**, 301 (1950); correction, *ibid.*, **72**, 5803 (1950).

(4) R. C. Miller and R. M. Fuoss, *ibid.*, **75**, 3076 (1953).

change in electrolyte behavior in the mixture as contrasted with that in either solvent. Mixtures of other solvents with methanol demonstrate the latter effect in a striking fashion: quaternary bromides are slightly associated in pure methanol, but become ideal strong electrolytes if nitrobenzene, nitromethane or benzene is added. These observations confirm the hypothesis[5] that the added solvent depolymerizes methanol if we assume that the monomeric methanol released then solvates the anions. The conductance is not changed much by the addition of the nitro compounds but is strongly depressed by the addition of benzene, while the addition of a little methanol to nitrobenzene decreases mobility sharply; association is, however, decreased in all three cases. These isolated facts do indeed show that specific interactions occur; it will, however, be necessary to investigate a number of systems before regularities begin to appear in these inconsistencies with the simple model. We present here results for the system tetrabutylammonium bromide–methanol–methyl ethyl ketone (Bu_4NBr–MeOH–MEK). The latter was chosen because it is intermediate in size between nitromethane and nitrobenzene, and has a fairly strong dipole moment. It also inhibits ion association, but unlike the nitrocompounds, causes a marked decrease in mobility.

Experimental

Materials.—Tetrabutylammonium bromide[3] was prepared by Dr. Francis E. Crane, Jr., to whom grateful acknowledgment is made. Methanol was distilled from aluminum methoxide[6]; the conductance varied from 0.03 to 0.3×10^{-6}. Methyl ethyl ketone was purified in two ways; fractional distillation after 3–4 hr. refluxing (a) from activated alumina and (b) from alkaline permanganate.[7] No significant difference in the different lots was observed. The specific conductance was less than 10^{-8}. Mixtures of MeOH and MEK were made up by weight; volume concentrations of electrolyte were calculated from the solvent densities, which were determined in a 20-ml. pycnometer. Viscosities were determined in a modified Ostwald viscometer.[8] Dielectric constants were measured in a platinum cell,[9] using a General Radio type 716C (unguarded) bridge at 200 kc. Appropriate corrections for lead capacitance were made. The value[10] of $D = 32.63$ for the dielectric constant of methanol and unity for air were used as the calibrating data. Properties of the solvents are summarized in Table I, where w_2 is weight % MEK, x_2 is mole fraction MEK, ρ is density, $100\ \eta$ is viscosity in centipoises, D is dielectric constant and κ_0 is solvent conductance. For compactness in presentation, some of the figures in the table are interpolated; the original data are shown in Fig. 1.

TABLE I

PROPERTIES OF MeOH–MEK MIXTURES AT 25.00°

No.	w_2	x_2	ρ	$100\ \eta$	D	$10^6\ \kappa_0$
1	0.00	0.0000	0.7865	0.546	32.63	0.284
2	5.10	.0233	.7912	.535	32.0	.109
3	8.27	.0385	.7925	.523	31.4	.080
4	18.24	.0902	.7960	.501	30.2	.091
5	49.62	.3044	.8017	.446	25.6	.119
6	54.26	.3452	.8020	.439	24.6	.208
7	78.23	.6150	.8031	.400	21.1	.082
8	87.89	.7636	.8019	.390	19.8	.049
9	100.00	1.0000	.8011	.377	18.4	.005

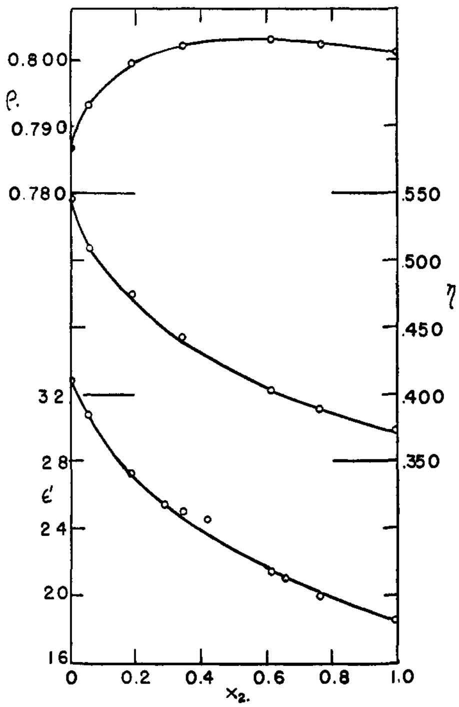

Fig. 1.—Properties of MeOH–MEK mixtures: top curve, density, ordinates left; middle curve, viscosity, ordinates right; bottom curve, dielectric constant, ordinates left.

Apparatus and method were essentially as described in the preceding papers.[3,4] The cells had constants equal to 2.516, 1.113, 0.0529, 0.0542 and 0.0649. Equivalent conductances Λ were calculated after subtracting the solvent conductance: the largest correction (4%) was, of course, in the most dilute solution in methanol.

Results

An excerpt of the conductance data is given in Table II, where c is concentration of salt in equivalents per liter, Λ is equivalent conductance at 25° and the various solvent mixtures are identified by w_2, the weight % of methyl ethyl ketone.

Discussion

Over our working range of concentration (approximately 0.05 to $1.0 \times 10^{-3}\ N$), the conductance data of Table II give very nearly linear Λ–$c^{1/2}$ plots. It is, however, not permissible to conclude immediately that these systems are all unassociated electrolytes: while they conform to the empirical Kohlrausch relationship, they do not all have the slope S calculated by the Debye–Hückel–Onsager theory. This is best demonstrated by plotting S'/Λ_0 against the mole fraction of MEK in the solvent, as shown in Fig. 2. In the middle and methanol-rich range, the empirical slope S' agrees quite well with the theoretical value; the somewhat lower empirical values are in all likelihood due to the effect of terms in c and $c \log c$. The empirical slope in methanol[3] is, however, distinctly greater than the theoretical value; the difference is due to the small but unmistakable association ($K = 0.038$) which occurs in methanol. In the ketone-rich end of the

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(5) R. Mecke, *Discussions Faraday Soc.*, **9**, 161 (1950).

(6) H. Hartley and H. R. Raikes, *J. Chem. Soc.*, **127**, 524 (1925).

(7) J. B. Conant and W. R. Kirner, THIS JOURNAL, **46**, 245 (1924).

(8) E. B. FitzGerald and R. M. Fuoss, *Ind. Eng. Chem.*, **42**, 1603 (1950).

(9) D. J. Mead and R. M. Fuoss, THIS JOURNAL, **61**, 204 (1939).

(10) A. A. Maryott and E. R. Smith, National Bureau of Standards Circular No. 514, p. 1 (1951).

TABLE II

CONDUCTANCE OF TETRABUTYLAMMONIUM BROMIDE IN METHANOL–METHYL ETHYL KETONE MIXTURES

$10^4 c$	Λ	$10^4 c$	Λ	$10^4 c$	Λ
5.10		49.62		87.89	
0.000	(95.0)	0.000	(102.5)	0.000	(118.4)
0.872	92.8	0.637	99.9	0.924	112.1
1.450	92.2	1.589	98.3	1.841	109.5
2.713	91.3	2.610	97.2	3.026	107.5
4.974	89.9	4.717	95.3	4.975	104.6
8.881	87.9	54.26		7.971	101.4
		0.000	(104.2)		
8.27		0.670	101.1	100.0	
0.000	(94.8)	1.229	100.0	0.000	(125.9)
0.894	92.1	2.055	98.9	0.702	116.5
1.715	91.4	2.974	97.7	1.614	112.0
3.482	90.1	4.472	96.5	2.187	108.6
6.301	88.1	5.946	95.3	4.239	101.3
10.991	86.4	78.23		5.925	96.9
18.24		0.000	(112.7)	7.073	94.4
0.000	(95.5)	0.519	109.9	8.907	90.9
0.536	93.6	1.058	108.5	11.633	86.9
0.994	92.9	1.798	107.0		
1.883	92.2	3.085	105.3		
3.477	90.9	4.148	103.7		
6.222	89.2	6.124	102.0		

curve, a marked positive deviation from theoretical is observed in the empirical slope. Calculation of reciprocal association constants by Shedlovsky's method[11] gives $K = 0.0093$ for the mixture with $x_2 = 0.764$ and $K = 0.00159$ for pure MEK as the solvent. The small change in dielectric constant between solvent 7 (D 21.1) and solvent 8 (D 19.8) hardly seems sufficient to account for the onset of association, especially when we recall that no association is detectable over the range $32.0 \geqslant D \geqslant 21.1$. We must therefore conclude that initial addition of a little MEK to methanol changes Bu_4NBr from a slightly associated electrolyte to a non-associated one and that a similar but more pronounced effect occurs when methanol is added to MEK. If we assume that methanol is depolymerized in solution in MEK or by the addition of the ketone, and that at least one ionic species (presumably the bromide ion) is solvated by monomeric methanol, we have a qualitative explanation of the observations. On the methanol end, there is only slight ion association due to the relatively high dielectric constant of the methanol; when the average ion size is increased by the assumed solvation, the electrostatic potential energy then no longer suffices to stabilize an ion pair. On the MEK end, association is greater than in methanol (for a given ion size), due to the lower dielectric constant. When methanol is added, two effects combine to decrease association sharply: increase in ion size due to the postulated solvation, and increase in dielectric constant.

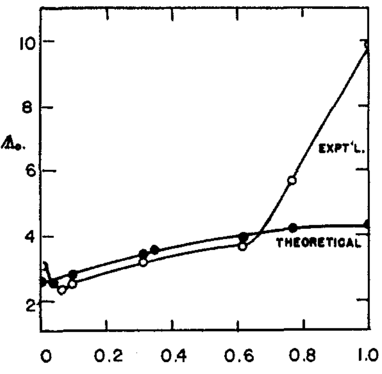

Fig. 2.—Comparison of empirical (○) and theoretical (●) slopes of (Λ/Λ_0)–$c^{1/2}$ curves.

Similar conclusions can be drawn from the dependence of limiting equivalent conductance on solvent composition. If the ionic size were independent of solvent, the Walden product $\Lambda_0\eta$ would be a constant. The values of Λ_0 are given in parentheses in Table II at zero concentration: for the first six systems, they were obtained by empirical extrapolation (where S' has very nearly the theoretical value); for the last two they were obtained simultaneously with the K-values by the Shedlovsky extrapolation. As shown in the lower curve of Fig. 3, the Walden product goes through a minimum as the solvent is changed from MeOH to MEK. This is, of course, evidence that the radius of the equivalent hydrodynamic sphere changes with composition: in other words, the continuum model is inadequate. Solvation by monomeric methanol would again account for the observed result. The further possibility of solvation of cation by MEK cannot be ruled out; the fact that the $\Lambda_0\eta$ product is smaller in MEK than in MeOH indeed argues that such solvation is occurring, because we would expect the equivalent conductance of $(Bu_4N^+{\cdot}MEK)\ Br'$ to be less than that of $Bu_4N^+(Br'{\cdot}MeOH)$.

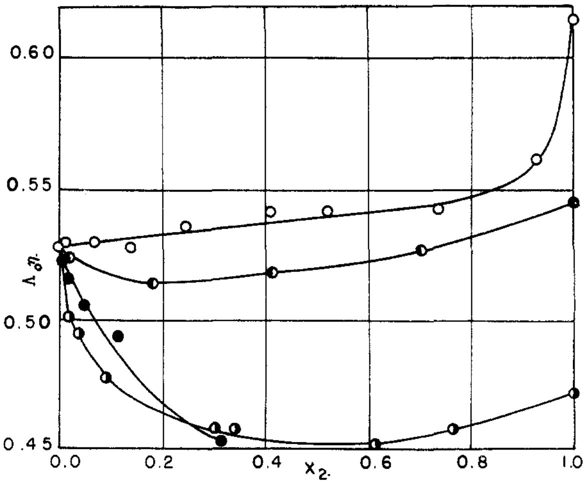

Fig. 3.—Walden products for Bu_4NBr in mixtures of methanol with nitrobenzene (open circles), nitromethane (left-black), benzene (solid) and methyl ethyl ketone (right-black).

In Fig. 3, we have summarized the four solvent mixtures which have recently been studied. A rough generalization can be made: addition of other solvents to methanol usually causes ions in solution to move more slowly, and to associate less. Both of these effects can be described by saying that the effective ion size (hydrodynamic and elec-

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[11] T. Shedlovsky, *J. Franklin Inst.*, **225**, 735 (1938).

trostatic) is increased by the addition of other solvents to methanol. These results support the hypotheses that methanol is depolymerized by addition of other solvents and that selective solvation of ions can occur.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE DEPARTMENT OF INDUSTRIAL CHEMISTRY, FACULTY OF ENGINEERING, KYOTO UNIVERSITY]

Kinetics of the Diazotization of Anilines

BY MASAYA OKANO AND YOSHIRO OGATA

RECEIVED JANUARY 26, 1953

The rates of diazotization of aniline and substituted anilines in aqueous sulfuric acid at 0° have been estimated by coupling of the diazonium salts with β-naphthol. From *p*H 0.6 to 3.3 the rate of diazotization was first-order with respect to aniline concentration and second-order with respect to nitrous acid. The relation between *p*H and the rate constant indicated that $HN_2O_3^+$ is the attacking agent. Substituted anilines were studied in competitive diazotizations at *p*H 0.6 with sulfanilic acid as a standard, and the relative rates satisfied a Hammett equation with a ρ-value of −1.96.

The rate of reaction of amines with nitrous acid has generally been expressed as k[amine][nitrous acid]^2.[1] In a recent study of the reaction of methylamine or ammonia with nitrous acid, Dusenbury and Powell[2] have suggested that the rate is first-order with nitrous acid, implying an attack by NO^+ ion. Early studies on aromatic diazotization[3] led to a rate law k[amine][nitrous acid], but Schmid[1d,4] and Ingold and his collaborators[5] have found the rate to be second-order with respect to nitrous acid over a comparatively wide *p*H range. This means that either the formation of, or the attack by, N_2O_3 or $HN_2O_3^+$ is rate-determining, but it is not clear which is the more probable. Moreover, although a rough comparison of the apparent rates of diazotization of substituted anilines has been made,[6] no decisive data on the effect of substituents on the rate are available. The present study was undertaken in order to supply this information.

I. Kinetics of the Diazotization of Aniline

Experimental

Materials.—Commercial aniline and β-naphthol were purified by duplicate vacuum distillations. Sodium nitrite, sulfuric acid and sodium sulfate were of the best grade available.

General Procedure for Rate Measurements.—A mixture of 150 cc. of 0.02 *N* aniline sulfate solution, 150 cc. of 0.2 *N* sulfuric acid–sodium sulfate buffer[7] and 240 cc. of distilled water was placed in a 1-l. three-necked flask equipped with thermometer and stirrer, and thermostated at 0° by immersion in an ice-bath. Now 60 cc. of 0.05 *N* sodium nitrite solution, previously cooled to 0°, was added. Five 100-cc. aliquots were withdrawn by means of a calibrated pipet and the time of each withdrawal was noted. Each aliquot was added to about 150 cc. of water, which contained 0.000625 mole (at least 25% excess) of β-naphthol, about 1 g. of sodium acetate and a quantity of sodium hydroxide sufficient to leave a slight excess after neutralization of the sulfuric acid present.[8] The azo compound was immediately formed. The mixture was allowed to stand overnight, heated, cooled and filtered through a sintered glass funnel. The collected azo compound was dried at 80° for 3 hours and weighed. This method of estimation is believed to be nearly quantitative because after complete consumption of the nitrous acid the yields of azo compound obtained always exceeded 98% of the theoretical amount.

Experimental Results and Calculations.—Table Ia summarizes the effect on the rate of the molar ratio and of the concentration of the reactants, and the *p*H effect is shown in Table Ib. The *p*H was determined at 15° by means of a glass electrode. The rate constants were calculated from (2) which arises on integration of (1), where a and b are the initial concentrations of aniline and nitrous acid, respectively, and x is the concentration of the diazonium salt present after t seconds.

$$dx/dt = k(a - x)(b - x)^2 \qquad (1)$$

If $a = b$

$$k = \frac{1}{2t}\left\{\frac{1}{(a - x)^2} - \frac{1}{a^2}\right\}$$

if $a \neq b$

$$k = \frac{1}{t(a - b)}\left\{\frac{1}{b - x} - \frac{1}{b} + \frac{1}{a - b}\ln\frac{a(b - x)}{b(a - x)}\right\} \qquad (2)$$

Discussion of Results

The order with which the reactants appear in the rate equation lead us to assume a rate-determining step involving an electrophilic attack by

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(1) (a) The reaction of ammonia with nitrous acid: E. Abel, H. Schmid and J. Schafranik, *Z. physik. Chem.*, *Bodenstein Festband*, 510 (1931); (b) the reaction of aliphatic amines with nitrous acid: T. W. J. Taylor, *J. Chem. Soc.*, 1099, 1897 (1928); T. W. J. Taylor and L. S. Price, *ibid.*, 2052 (1929); (c) the reaction of amino acid with nitrous acid: E. Abel and H. Schmid, *Z. Elektrochem.*, **39**, 863 (1933); (d) the reaction of aromatic amine with nitrous acid: H. Schmid and G. Muhr, *Ber.*, **70**, 421 (1937).

(2) J. H. Dusenbury and R. E. Powell, THIS JOURNAL, **73**, 3266, 3269 (1951). And also A. T. Austin, E. D. Hughes, C. K. Ingold and J. H. Ridd, *ibid.*, **74**, 555 (1952). Recently Abel suggested another mechanism which involves an attack of NO on ammonium or aminium ion in this case [E. Abel, *Monatsh.*, **83**, 1103 (1952)], but this seems less probable in view of present electronic theory.

(3) A. Hantzch and H. Schümann, *Ber.*, **32**, 1691 (1899); E. Tassilly, *Bull. soc. chim.*, **27**, 19 (1920); J. Böeseken, W. F. Brandsma and H. A. J. Schoutissen, *C. A.*, **15**, 1023 (1921).

(4) H. Schmid and A. Wappmann, *Monatsh.*, **83**, 346 (1952).

(5) E. D. Hughes, C. K. Ingold and J. H. Ridd, *Nature*, **166**, 642 (1950).

(6) S. Ueno and T. Suzuki, *J. Soc. Chem. Ind., Japan*, **36**, 615B (1933).

(7) No effect of ionic strength on the rate was observed on addition of sodium sulfate. In weakly acidic media, an increase of *p*H was recognized during the reaction; therefore, we employed sulfuric acid–sodium sulfate solution with constant ionic strength as a buffer. Rate measurements in other buffers (for example, biphthalate-hydrochloric acid or citric acid–citrate buffer) were also carried out at *p*H *ca.* 3; the data, however, did not agree with those in sulfuric acid–sulfate buffer. Perhaps this is due to a catalytic action of the anions as observed in the case of chloride ion in the diazotization in hydrochloric acid.[1d]

(8) Although coupling is faster in the presence of a substantial excess alkali, the danger of losses due to solubility of the product argued against the use of higher *p*H. Too low a *p*H leads to uncertain data because the rate of coupling falls below that of diazotization.