tra-cage effects. For the thermal decomposition reaction in boiling toluene,[5] small amounts of materials arising from radical disproportionation are formed. In the case of the present study at 25°, such products cannot perhaps be entirely ruled out, for, if the energy of activation of the thermal decomposition reaction,[3] *ca.* 30 kcal. mole^{-1}, is equated to D(RN_2–R), the species formed in step 15 may possess between them some 45 kcal. mole^{-1} in excess of their normal average kinetic energy, on account of the size of the activating quantum. On the other hand, there is the possibility that part of this excess energy might be dissipated as a result of the relatively frequent encounters with solvent molecules, by AZO* before dissociation, yielding a molecule still capable of dissociating, or by the radicals themselves, for example. Such encounters with radicals would be expected to compete favorably with radical–radical collisions. Benzene is also particularly effective in energy transfer processes.[17] The simple kinetics observed exclude the possibility of much disproportionation occurring, or, for that matter, other processes that would tend to cause f to change its value during the reaction, *e.g.*, the thermal decomposition of DKI. It should be emphasized that the claimed isosbestic wave length range,[16] 0.3 mμ, allows a total variation in f of 3%.

The quantum efficiency for AZO removal, 0.47 ± 0.02 mole einstein^{-1}, agrees only moderately well with that found previously,[2] 0.43. This must be considered satisfactory in view of the qualitative nature of the earlier study. The agreement between the two values of ϕ determined, respectively, from Fig. 2 and 3 is good, particularly on account of the fact that Fig. 2 involves initial rate data. The estimated data for f are also in good accord. As discussed above, f might vary slightly during a run, and the figure for f based upon the isosbestic wave length takes this possible change into account. However, the other set of data relies on the assumption that f is time invariant, the ratios $-\Delta D^{366}/\Delta D^{\lambda}$ for the three wave lengths being calculated on the supposition that $-\Delta D^{366}$ varies linearly with ΔD^{λ}. Consequently, these ratios may be more uncertain than their calculated statistical probable errors (which are trivially small). It is hardly likely that the values of f could be more than 1% underestimated on this account.

The fractional molar conversion of AZO into DKI reported here is somewhat larger than the approximate estimate[3a] for this quantity in the case of the thermal decomposition of AZO in the similar solvent toluene at 80.4°, namely, *ca.* 0.3. So far no conversion measurements have been made[18] for the thermal decomposition reaction at 25°. Such measurements would help settle whether the two ways of decomposition are exactly alike, apart from the obvious, formal difference in the primary activation steps producing AZO*, namely, the photolytic process 12 and the thermal reaction

$$\mathrm{AZO} + C_6H_6 \longrightarrow \mathrm{AZO}^* + C_6H_6 \qquad (16)$$

Acknowledgments.—This work was supported in part by grants from E. I. du Pont de Nemours and Company and the Research Corporation. For these we are most thankful. We are particularly indebted to Dr. George S. Hammond and his coworkers at Iowa State College for a small sample of DKI and for the opportunity of reading a manuscript before publication. The assistance of Mr. P. E. Miller and Mr. J. S. Vranka in performing the actinometry is gratefully acknowledged.

LAFAYETTE, INDIANA

(17) For example, K. J. Laidler, "The Chemical Kinetics of Excited States," Oxford University Press, London, 1955, Chapter VI. Similar arguments could be given if the primary products of step 15 were two R$^{\cdot}$ radicals and one N_2 molecule.

(18) P. Smith and S. Carbone, unpublished data, have found that the conversion of AZO into DKI at 60–80° for the thermal decomposition in benzene solution is not appreciably different from the photochemically derived results at 25° given in this present study. The DKI formed, of course, decomposes in its turn. The thermally obtained conversions show at the most a slight variation with temperature, suggesting that the photolytic and thermal processes at 25° may have no more than a small difference in conversion.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA]

A Study on the Absorption Spectra of Ion-pairs[1]

BY ALEXANDER I. POPOV AND RAY E. HUMPHREY[2]

RECEIVED NOVEMBER 8, 1958

Ultraviolet absorption spectra and electrical conductances of tetraphenylarsonium chloride were determined in water, acetonitrile, ethylene dichloride, 70% dioxane and 95% dioxane. Ion-pair dissociation constants were determined and were found to be 1.12×10^{-3}, 4.81×10^{-3} and 2.3×10^{-8} for the last three solvents, respectively, the dissociation being essentially complete in water and in acetonitrile. No changes in the position of absorption maxima were observed in different solvents, while relatively slight fluctuations in the intensity of absorbance could not be correlated with the ion-pair formation.

Introduction

The effect of ion-pair formation on the ultraviolet or visible absorption spectra of solutions has been a subject of controversy during the past few years. Several investigators[3–6] have postulated ion-pair formation in aqueous solutions of metal thiosulfates, nitrates and sulfates and have calcu-

(1) From the Ph.D. Thesis of Ray E. Humphrey, State University of Iowa, June, 1958. Presented at the 134th Meeting of the American Chemical Society, Chicago, Illinois, September 11, 1958.

(2) Eastman Kodak predoctoral fellow.

(3) F. G. R. Gimblett and C. B. Monk, *Trans. Faraday Soc.*, **51**, 793 (1955).

(4) G. O. Thomas and C. B. Monk, *ibid.*, **52**, 685 (1956).

(5) H. M. Hershenson, M. E. Smith and D. N. Hume, THIS JOURNAL, **75**, 507 (1953).

(6) E. W. Davies and C. B. Monk, *ibid.*, **80**, 5032 (1958).

lated the extent of association from the changes in the absorption spectra of the resulting solutions. On the other hand, studies on the absorption spectra of salts, primarily in organic solvents,[7-9] have shown that ion-pair association does not produce noticeable change in the absorption spectra of solutions, *i.e.*, the absorption due to dissociated ions is identical to that of ion-pairs.

In this work a study is made of the ion-pair formation and of absorption spectra of tetraphenylarsonium salts in water and in a number of organic solvents, in order to see whether there is any correlation between the two properties. Tetraphenylarsonium salts were selected for this study since the large size and the symmetry of the cation would tend to minimize any solvent effects.

Experimental Part

Solvents.—Commercial acetonitrile (Mathieson, Coleman and Bell) was purified by first shaking it twice with saturated potassium hydroxide solution to remove acidic impurities, twice with anhydrous sodium carbonate to remove gross amounts of water and finally with phosphorus pentoxide to remove the remaining water. It was then distilled from phosphorus pentoxide at a slow rate with the first and last fractions being discarded. The purified solvent came over at 81.0° at 750 mm. (lit val. 81.6° at 760 mm.).[10] The specific conductance of the purified solvent varied from 0.8 to 2.5 $\times 10^{-7}$ ohm^{-1} cm.$^{-1}$.

Ethylene dichloride (Mathieson, Coleman and Bell) was first shaken several times with concentrated sulfuric acid until the acid layer was no longer colored yellow and then shaken three times with distilled water. Gross amounts of water were removed as the azeotrope at 77° and the remainder taken up by refluxing over phosphorus pentoxide and distilling from this material. This solvent boiled at 83.0° at 750 mm. (lit val. 83.4° at 760 mm.).[11] The specific conductance was calculated to be less than 1 $\times 10^{-10}$ ohm^{-1} cm.$^{-1}$.

Dioxane (Eastman Kodak Company, White Label) was purified by refluxing over sodium metal and distilling until most of the glycol acetal impurity had been removed. This solvent was refluxed over sodium for a period of ten days to two weeks and then distilled. The middle fraction which was collected came over at 101.4° at 750 mm. (lit. val. 101.3° at 760 mm.).[12] The specific conductance was calculated to be less than 1 $\times 10^{-10}$ ohm^{-1} cm.$^{-1}$.

Conductivity water was obtained by passing distilled water through a mixed bed ion-exchange column containing "Amberlite" resins IR 120 cation-exchange resin and IRA 400 anion-exchange resin, until the specific conductance was of the order of 5 to 8 $\times 10^{-7}$ ohm^{-1} cm.$^{-1}$.

The dioxane-water mixtures were prepared by weight. The specific conductance of the 70% dioxane–30% water mixture ranged from 5 to 7 $\times 10^{-7}$ ohm^{-1} cm.$^{-1}$ while that of the 95% dioxane–5% water mixture was so low that it could not be measured.

Chloroform (Brothers Chemical Company, ACS reagent grade) was purified by shaking twice with concentrated sulfuric acid, twice with distilled water and distilling from calcium chloride. No attempt was made to measure the specific conductance since this solvent was used only for spectrophotometric measurements.

Compounds.—Tetraphenylarsonium chloride (Hach Chemical Company) was obtained as the hydrochloride. This compound was purified by the method of Blicke and Monroe which consisted of evaporating the neutralized aqueous solution to dryness, extracting the residue into absolute ethanol, evaporating to a small volume and precipitating by addition of absolute ether. The melting point of this purified compound was 261–263° as compared to the value of 256–257° reported by Blicke and Monroe.[13]

Tetraphenylarsonium tetraphenylborate was prepared by adding an ethanol solution of sodium tetraphenylborate slowly, with stirring, to an ethanol solution of tetraphenylarsonium chloride. This compound was recrystallized from acetonitrile and small needle-like crystals were obtained which melted at 293–295°, identical with the literature value.[14]

Tetraphenylarsonium picrate was prepared by adding an ethanol solution of picric acid slowly, with stirring, to an aqueous solution of tetraphenylarsonium chloride. The precipitated product was recrystallized from ethanol and had a melting point of 203-204°, identical with the literature value.[15]

Sodium tetraphenylborate (J. T. Baker Chemical Company, assay 100%) was purified by dissolving in a mixture of 60% ethyl ether and 40% cyclohexane and then warming to precipitate the compound. This procedure is similar to that reported by Wittig and Claus.[14] The purified material did not have the characteristic odor (attributed to biphenyl) of the commercial compound.

Absorption Spectra.—Absorption spectra were determined using a Cary Recording Spectrometer, Model 11, with silica cells having a path length of 1.00 ± 0.01. All of the measurements were made at a room temperature of 25 ± 2°.

Conductance Measurements.—A total of four conductance cells of the Jones–Bollinger type were used in this work in order to keep the resistance values in the range of 1,000 to 20,000 ohms. One of these cells, with a cell constant of 0.0012, was used to measure the conductance of some of the solvents. The other three cells had cell constants of 0.050, 0.1284 and 1.840. They were calibrated using potassium chloride which had been crystallized three times from conductivity water and fused in a platinum crucible. The conductance values for potassium chloride reported by Shedlovsky[16] were used in the calculation of the cell constant values. The cells exhibit a slight Parker[17] effect and were calibrated over a ten-fold range of concentration. Bright platinum electrodes were used.

Electrical conductance was measured with a Jones Conductance Bridge[18] manufactured by the Leeds and Northrup Company. An accuracy of ±0.02% in the resistance measurements is claimed for this instrument. A 30,000 ohm shunt arrangement was used for measuring very high resistances (solvents and the 95% dioxane solutions). A Hewlitt-Packard oscillator was employed in conjunction with a DuMont Model 208B oscilloscope with a five inch screen as a null-point indicator. The frequency of the signal employed was 2000 cycles per second.

A Fisher "Isotemp" bath filled with light hydrocarbon oil was used to maintain a constant temperature of 25°. The bath temperature was adjusted to 25 ± 0.02° with the use of a National Bureau of Standards certified thermometer and the variations in this temperature were observed with a Beckmann differential thermometer. The maximum variation in the temperature was ±0.01°.

Solutions.—All volumetric flasks and pipets were recalibrated. Pipets for the dilution of tetraphenylarsonium chloride solutions in water were coated with a silicone film (General Electric SC 87 Dry Film) in order to assure good drainage, since these solutions seem to stick to the sides of the pipets.

All of the solutions used in this investigation were found to be quite stable and did not show any changes in electrical conductance or in absorption with time.

Results

A. Conductance Studies.—Conductance of tetraphenylarsonium chloride solutions was determined in water. The phoreogram[19] was a straight line which was extrapolated to the limiting molar conductance of 95.6 as compared with 92.5 given

---

[7] H. V. Halban and B. Szigeti, *Helv. Chim. Acta*, **20**, 746 (1937).

[8] I. M. Kolthoff, D. Stocesoca and T. S. Lee, THIS JOURNAL, **75**, 1834 (1953).

[9] A. I. Popov and R. F. Swensen, *ibid.*, **77**, 3724 (1955).

[10] P. Walden, *Z. physik. Chem.*, **55**, 281 (1906).

[11] E. R. Smith and H. Matheson, *J. Research Natl. Bur. Standards*, **20**, 641 (1938).

[12] E. R. Smith and M. Wojciechowski, *ibid.*, **18**, 461 (1937).

[13] F. F. Blicke and E. Monroe, THIS JOURNAL, **57**, 720 (1935).

[14] G. Wittig and K. Claus, *Ann.*, **577**, 26 (1952).

[15] F. F. Blicke and E. L. Cataline, THIS JOURNAL, **60**, 423 (1938).

[16] T. Shedlovsky, *ibid.*, **54**, 1411 (1932).

[17] H. C. Parker, *ibid.*, **45**, 1366, 2017 (1923).

[18] P. H. Dike, *Rev. Sci. Inst.*, **2**, 379 (1931).

[19] Term proposed by R. M. Fuoss for Λ *vs.* $C^{1/2}$ curve. *J. Chem. Educ.*, **32**, 527 (1955).

by Blicke and Monroe.[13] The slope and the intercept were calculated by the least squares method. The deviation from the Onsager slope was slight, indicating an essentially complete dissociation of the electrolyte. The data obtained are summarized in Tables I and II.

TABLE I

CONDUCTANCE DATA ON TETRAPHENYLARSONIUM CHLORIDE

In water		In acetonitrile		In 70% dioxane	
$C \times 10^4$	Λ	$C \times 10^4$	Λ	$C \times 10^4$	Λ
1.871	94.6	0.770	149.2	0.963	37.8
3.169	93.9	2.626	147.1	1.925	36.1
4.844	93.4	3.852	146.3	3.851	39.9
7.923	92.8	7.703	144.3	8.776	33.6
9.354	92.5	15.41	141.3	9.626	32.6
15.85	91.8	17.51	140.5	19.25	31.1
23.39	90.6	25.68	138.2	38.51	29.4
46.77	89.6	51.36	132.1		

In 95% dioxane		In ethylene dichloride	
$C \times 10^4$	Λ	$C \times 10^4$	Λ
0.758	0.83	0.690	53.0
2.275	.52	0.863	52.5
3.033	.46	1.380	50.6
3.792	.33	2.070	48.6
7.584	.30	2.760	46.8
9.864	.29	3.450	45.5
12.33	.27	13.80	36.6
15.17	.25		
24.66			

TABLE II

DEVIATIONS FROM THE ONSAGER EQUATION AT 25°

Compound	Solvent	Onsager slope	Exper. slope	ΔS^a
TPA-Cl	H_2O	82.1	93.3	+14
TPA-Cl	CH_3CN	338	252	−26
TPA-TPB	CH_3CN	313	354	+13
Na-TPB	CH_3CN	364	293	−19
TPA-Pi	CH_3CN	326	338	+ 4

^{a} $\Delta S = \dfrac{100(S_{\text{expt}} - S_{\text{calcd}})}{S_{\text{calcd}}}$.

Studies of electrical conductances also were made in acetonitrile solutions. The compounds used were tetraphenylarsonium chloride, tetraphenylarsonium tetraphenylborate, tetraphenylarsonium picrate and sodium tetraphenylborate. The solubility of tetraphenylarsonium tetraphenylborate is somewhat limited in acetonitrile, and a saturated solution had a concentration of 1.6×10^{-3} *M*. Again here the behavior was compatible with essentially complete dissociation of the electrolytes. Attempts were made to determine the ion-pair dissociation constant by the Fuoss and Shedlovsky[20] method, but the plots were horizontal, again showing extremely large dissociation.

Somewhat different results were obtained on tetraphenylarsonium chloride solutions in 70% dioxane, ethylene dichloride and 95% dioxane, which have the dielectric constants 19,[21] 10.36[22] and 4,[21] respectively. In the first two cases, phoreograms showed a definite curvature indicating some association. The ion-pair dissociation constants were calculated by the Fuoss–Shedlovsky method and the results are listed in Table III. The limiting equivalent conductance could not be determined in 95% dioxane because of extreme curvature of the phoreogram (Fig. 1).

TABLE III

ION-PAIR DISSOCIATION CONSTANTS

Compound	Solvent	*D*	*K*
TPA-Cl	Water	78.5	V. large
TPA-Cl	Acetonitrile	36.5	V. large
TPA-Cl	70% Dioxane	19	4.81×10^{-3}
TPA-Cl	Ethylene dichloride	10.36	1.12×10^{-3}
TPA-Cl	95% Dioxane	4	2.30×10^{-8a}
TPA-Pi	Ethylene dichloride	10.36	1.37×10^{-3}

^{a} Approximate value.

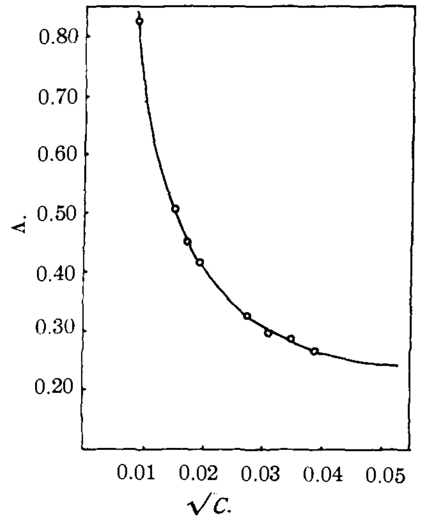

Fig. 1.—Molar conductance of TPA-Cl in 95% dioxane–5% water mixture.

As seen from Table IV the limiting conductance viscosity products for the tetraphenylarsonium salts in acetonitrile and in ethylene dichloride are relatively constant and therefore the limiting conductance of the salt in 95% dioxane was calculated from Walden's rule. The constant used was the average of the values listed in Table III, namely, 0.48, and the viscosity for the solution was taken as 1.2739 centipoises.[23] This yielded a value of 39 which, is of course, only an approximate value,

TABLE IV

LIMITING CONDUCTANCE–VISCOSITY PRODUCTS AT 25°

Compound	Solvent	Λ_0	η	$K = \Lambda_0 \times \eta$
TPA-Cl	CH_3CN	151.1	0.00344	0.52
TPA-Cl	$C_2H_4Cl_2$	61.6	.00787	.49
TPA-Pi	CH_3CN	136.0	.00344	.47
TPA-Pi	$C_2H_4Cl_2$	53.4	.00787	.42
				$K_{\text{av.}} = 0.48$

---

(20) R. M. Fuoss and T. Shedlovsky, THIS JOURNAL, **71**, 1496 (1949). It was unnecessary for the purpose of this investigation to use the more exact but more tedious method of calculation of Fuoss (*ibid.*, **79**, 3301 (1957)) since small variations in a large dissociation constant would not affect the absorption spectra of ion-pairs. It also should be noted that the more approximate method gives good results if $K << 1$.

(21) Interpolated from the data of Fuoss and Kraus, *ibid.*, **55**, 476 (1933).

(22) "Table of Dielectric Constants of Pure Liquids," NBS circular 514, 1951.

(23) B. B. Owen and G. W. Waters, THIS JOURNAL, **60**, 2371 (1938).

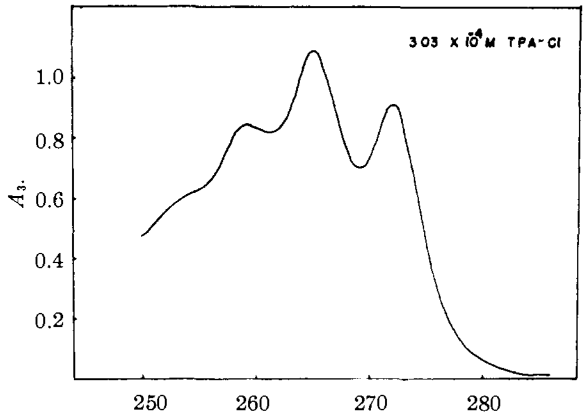

Fig. 2.—Absorbance of 3.30×10^{-4} M solution of TPA-Cl in 95% dioxane–5% water mixture.

but at least the order of magnitude of the ion-pair dissociation constant could be calculated from the approximate equation

$$K = \frac{C\alpha^2 f^2}{1 - \alpha}$$

where α is the degree of dissociation determined for each solution by the method described by Fuoss,[24] and then the corresponding K values were extrapolated to infinite dilution. The activity coefficients were calculated from the Debye–Huckel limiting law.

Table V summarizes the limiting molar conductances of the salts in this investigation.

TABLE V

LIMITING MOLAR CONDUCTANCES AT 25°

Compound	Solvent	Λ_0
TPA-Cl	Water	96.5
TPA-Cl	Acetonitrile	151.2
TPA-Cl	70% Dioxane	41.6
TPA-Cl	Ethylene dichloride	61.6
TPA-Cl	91% Dioxane	39
TPA-Pi	Acetonitrile	136.0
TPA-Pi	Ethylene dichloride	53.4
TPA-TPB	Acetonitrile	117.5
Na-TPB	Acetonitrile	134.2

It was possible to calculate the limiting ionic conductance of tetraphenylarsonium and of tetraphenylborate ions from the limiting molar conductance. The results are shown in Table VI.

TABLE VI

LIMITING IONIC CONDUCTANCES AT 25°

Ion	Compound	Solvent	λ^+
TPA$^+$	TPA-Cl	Acetonitrile	52.1[a]
TPA$^+$	TPA-Cl	Ethylene dichloride	22.5[b]
TPA$^+$	TPA-Pi	Ethylene dichloride	22.2[c]
TPB$^-$	Na-TPB	Acetonitrile	64.4[d]

[a] Based on the value of 49.1 for Cl$^-$, N. E. Skelly, Ph.D. Thesis, State University of Iowa, 1955. [b] Based on the value of 77.7 for Pi$^-$, P. Walden and E. J. Birr, *Z. physik. Chem.*, **144**, 269 (1929). [c] Based on the value of 31.2 for Pi$^-$, L. F. Gleysteen and C. A. Kraus, THIS JOURNAL, 69, 451 (1947). [d] Based on the value of 69.8 for Na$^+$, P. Walden and E. J. Birr, *Z. physik. Chem.*, **144**, 269 (1929).

B. Spectrophotometric Measurements.—The absorption spectra were determined for TPAC in water, acetonitrile, ethylene dichloride, chloroform, 70% dioxane and 95% dioxane. Several concentrations were run in these solvents and Beer's law was found to hold in each case. The positions of the absorption maxima were essentially constant in all the solvents, occurring at 258, 264 and 271 mμ in water and acetonitrile, and at 259, 265 and 272 mμ, in ethylene dichloride, dioxane and water mixtures and in chloroform. A typical curve is shown in Fig. 2. Results of the intensity measurements are given in Table VII.

TABLE VII

MOLAR ABSORPTIVITIES OF TPA-Cl IN VARIOUS SOLVENTS AND THE ION-PAIR DISSOCIATION CONSTANTS

Solvent	D	$a_{\mathrm{m}259}$ mμ	$a_{\mathrm{m}265}$ mμ	K
Water	78.5	2550	2780	....
Acetonitrile	36.5	2660	2830	....
70% Dioxane	19	2600	2850	4.81×10^{-3}
Ethylene dichloride	10.36	2950	3150	1.12×10^{-3}
95% Dioxane	4	2770	3040	2.30×10^{-8}

The ultraviolet spectrum of sodium tetraphenylborate in acetonitrile exhibits peaks at 266 and 275 mμ with the absorption rising sharply from 264 mμ toward shorter wave lengths. The ultraviolet spectrum of tetraphenylarsonium tetraphenylborate is similar to that for tetraphenylarsonium chloride except that the absorption continues to rise from 258 mμ toward shorter wave lengths. The molar absorptivity of tetraphenylarsonium tetraphenylborate at any wave length is exactly equivalent to the additive values of the molar absorptivities of the tetraphenylarsonium ion and the tetraphenylborate ion.

The spectrum of tetraphenylarsonium picrate resembles that of tetraphenylarsonium borate with peaks at 258, 264 and 271 mμ, with a sharp increase in absorption from 258 mμ toward shorter wave lengths. Measurement of the absorbance at several concentrations in acetonitrile showed that Beer's law is followed in this solvent while in ethylene dichloride there is a marked increase in molar absorptivity with decreasing concentration. No explanation for this behavior can be offered at this time.

Discussion

Table VII lists the changes in the molar absorptivity of the TPAC solutions as the solvent is changed. It is seen that there is no apparent correlation between the values of molar absorptivity and the ion-pair dissociation constant. It is quite likely that this variation comes from the changes in the refractive index of the medium.

It should be noted that all previous correlations of spectral changes on ion-pair dissociation or association have been concerned only with metal ions and various inorganic anions. That is, in all cases, the distinction between an ion-pair and a complex could not be made with any degree of certainty. In a recent publication Smithson and Williams[25] differentiate between a complex M–L

---

[24] R. M. Fuoss, THIS JOURNAL, **57**, 488 (1935).

[25] J. M. Smithson and R. J. P. Williams, *J. Chem. Soc.*, 457 (1958).

where the ligand L is in direct contact with the metal M, and an ion-pair M–S–L, where a molecule of solvent is interposed between the metal and the ligand. These authors argue that in the latter case one cannot expect any change of the visible spectrum but that the positions of the ultraviolet bands would be affected because of the "charge-transfer" nature of the ultraviolet spectra. It seems to the present authors, however, that if a "charge-transfer" process occurs between the metal ion and the ligand, we have no longer a simple ion-pair formation by purely coulombic interaction.

It also should be pointed out that in instances where ion-pair formations and the absorption spectra of resulting solutions were studied on nonmetallic systems,[7-9] in all cases the data indicated that ion-pair formation has very little influence on the absorption spectra. In this connection a recent series of papers by Kolthoff and Bruckenstein[26] on acid–base dissociations in glacial acetic acid should be mentioned also. These authors postulated that any dissociation in solvents of low dielectric constant occurs in two steps, $AX \rightarrow A^+X^- \rightarrow A^+ + X^-$, where the first step is ionization into ion-pairs and the second, the dissociation of the ion-pairs. They found that the second step does not influence the absorption spectra of solutions and, on this basis, were able to calculate dissociation constants of acids from spectrophotometric measurements which were in agreement with values obtained potentiometrically.

In conclusion, it seems that ion-pair dissociation or association does not seem to influence the absorption spectra of solutions to any significant degree, provided that the attraction is purely electrostatic. Where such changes have been observed, either an alternate explanation of a complex formation can be postulated, or, as Robinson and Stokes[27] suggest, we may have an interaction between the cation and an induced dipole in the anion. The argument may be rather semantic than factual and one may agree with the recent statement of Cohen[28] that the definition of an ion-pair is largely determined by the experimental method used in such studies.

IOWA CITY, IOWA

---

[26] I. M. Kolthoff and S. Bruckenstein, THIS JOURNAL, **78**, 1 10. 2974 (1956); **79**, 1 (1957).

[27] R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworths Scientific Publications, London, 1955, p. 413.

[28] S. R. Cohen, *J. Phys. Chem.*, **61**, 1670 (1957).

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

Reactivity of Sulfhydryl and Disulfide in Proteins. IV. Reaction between Disulfide and Sulfite in Bovine Serum Albumin Denatured in Guanidine Hydrochloride and Urea Solutions

BY I. M. KOLTHOFF, ADA ANASTASI[1] AND B. H. TAN

RECEIVED OCTOBER 8, 1958

Four molar guanidine hydrochloride and 8 *M* urea have approximately the same denaturing properties toward bovine serum albumin as far as reduced viscosity, optical rotation and reactivity toward sulfite are concerned. The equilibrium concentration of reacted disulfide in 0.05 *M* and some other concentrations of sodium sulfite has been determined in these two denaturing media in the *p*H range between 2 and 9. A flat maximum is found between *p*H 4 and 6; at larger and smaller *p*H, the reacted disulfide decreases. The general appearance of the reacted disulfide *p*H curves is the same in both denaturing media. In 4 *M* GHCl 14 to 14.5 and in 8 *M* urea 16 to 16.5 react with 0.05 *M* sulfite at *p*H 5, the total number of disulfide groups in BSA being equal to 17. The value of 14.2 in 4 *M* GHCl does not change when the sulfite concentration is increased from 0.02 to 0.2 *M*. Experiments in solutions of denaturing agents over a wide range of concentrations revealed that the reactivity of disulfide groups does not increase when the GHCl concentration is made greater than 3 *N* or the urea concentration greater than 6 *N*. The reactivity of disulfide groups is already considerable at low concentrations of denaturant, where the extent of denaturation is small.

In a previous paper,[2] procedures have been given for the determination of the equilibrium concentrations of disulfide and sulfhydryl groups in bovine serum albumin (BSA) in its reaction with sulfite at *p*H 6 to 6.5. In the absence of mercuric chloride, no disulfide groups were found to react in the native state; but when denatured in 4 *M* guanidine hydrochloride (GHCl), the maximum value of the number of disulfide groups reacted was eleven. In the present paper, equilibrium values of reacted disulfide have been determined in BSA denatured in 4 *M* GHCl and 8 *M* urea in the *p*H range between 2 and 9. The reactivity of disulfide was also determined in more dilute and more concentrated solutions of the denaturing agents.

Experimental

Materials.—The same materials were used as in the previous papers.[2] Urea was a Mallinckrodt product which was purified as previously described.[3]

Methods.—Air-free solutions of known composition were allowed to react with sodium sulfite at the specified *p*H. For convenience the sulfite concentration is expressed as molarity of total sulfite, irrespective of whether it was present mainly as sulfite, bisulfite or sulfurous acid. After given periods of reaction time, the *p*H was adjusted to 2 and the sulfhydryl groups titrated with mercuric chloride using the rotated mercury pool (RMPE) as indicator electrode.[2] When the concentration of the albumin was 1% in the denaturation mixture, it was diluted to about 0.1% with a solution of the denaturing agent of the same concentration as used in the reaction mixture. Quite generally the dilution was made by running the reaction mixture into an acid solution of the denaturing agent, keeping the *p*H during the dilution equal to 2. When the original BSA concentration was 0.1% in the reaction mixture, the dilution was kept at a

---

[1] On leave from S. A. Farmitalia, Milano, Italy.

[2] I. M. Kolthoff, A. Anastasi and B. H. Tan, THIS JOURNAL, **80**, 3235 (1958).

[3] I. M. Kolthoff and A. Anastasi, *ibid.*, **80**, 4248 (1958).