JOURNAL OF THE
AMERICAN CHEMICAL SOCIETY

(Registered in U. S. Patent Office) (Copyright, 1954, by the American Chemical Society)

VOLUME 76 NOVEMBER 8, 1954 NUMBER 21

[CONTRIBUTION FROM THE H. M. RANDALL LABORATORY OF PHYSICS, UNIVERSITY OF MICHIGAN]

Infrared Studies on Solutions of Polymeric Electrolytes. I. Synthetic Polyacids and Polyampholytes

BY GERT EHRLICH[1]

RECEIVED MAY 29, 1954

In order to isolate the possible contributions of ionizing groups to the infrared spectra of natural and synthetic polymeric electrolytes in aqueous solution, the absorption spectra of D_2O solutions of polymethacrylic acid, as well as of copolymers of methacrylic acid and 2-dimethylaminoethyl methacrylate, and of acrylic acid with 2-vinylpyridine, have been recorded in the region from 5–8 μ, accessible to transmission measurements. The spectra show strong bands due to the carboxyl group (at 5.85 μ) and the carboxylate ion (at 6.45 and 7.08 μ), and, for the copolymer of acrylic acid and 2-vinylpyridine, those characteristic of the pyridine ring; the addition of a deuteron to the basic nitrogen in DCl solution changes the spectrum, displacing the absorption bands of the pyridine ring to lower wave lengths. From measurements of the intensity of the carboxylate absorption bands it appears that at the isoionic point the synthetic polymeric ampholytes exist as dipolar ions in solution, but that in films they carry a much smaller charge. The insolubility of polyampholytes existing as dipolar ions at the isoionic point indicates that a minimum number of ionized groups must be present on a polymer molecule in order to ensure the stability of a binary solution in water.

Although the globular proteins, nucleic acids and many carbohydrates vital in biological processes are polymeric electrolytes, and are in nature found in aqueous solutions, examination of their infrared absorption spectra has so far been generally confined to mulls, films or single crystals and even the limited work on D_2O solutions of proteins has been primarily concerned with the elucidation of the structure and configuration of the polypeptide chains.[2] Charged groups in polymeric electrolytes of biological importance are of considerable significance in determining interactions, both between polypeptide chains within the molecule,[3a,b] as well as with other macromolecules[3c] and with small ions,[4a,b] so that isolation of the contribution of ionizable groups may be important not only for a proper interpretation of the vibrational spectrum but also in elucidating the course of biological reactions.

The effects of proton transfer upon the vibration spectra of acids and bases in aqueous solution have been studied in some detail for small molecules by means of Raman scattering,[5] but the application of the methods of infrared absorption spectroscopy to these problems has been delayed by the strong absorption of water which obscures the fundamental region in the vicinity of 3 and 6 μ. The substitution of D_2O for H_2O as a solvent, however, permits examination of absorption spectra in the region from 1 to 3.5 μ and from 4.5 to 8 μ,[6] yet the exchange of labile hydrogen atoms in the solute, attendant upon this substitution, does not result in major changes in the electrochemical behavior of electrolytes, so that the perturbation of certain vibrational frequencies through ionization can be readily followed, provided deuteration does not move the group frequency beyond the region accessible to measurement.

The effects of changes in the state of charge upon the thermodynamic and hydrodynamic properties are particularly striking in solutions of synthetic polymeric electrolytes, which are characterized by the flexibility of their backbone and the very large number of ionizable groups. This preponderance of ionizable groups, as well as the simplicity of the repeating units in the polymer chains, also make these materials ideal for isolating the effects of ionization upon the vibrational spectrum. Of ionizable groups, carboxyls, –COOH, occur most frequently in proteins, and to some extent also in

---

[1] Research Laboratory, General Electric Company, Schenectady, N. Y.

[2] G. B. B. M. Sutherland, *Advances in Protein Chem.*, **7**, 291 (1952).

[3] (a) G. Weber, *Faraday Soc. Disc.*, **13**, 33 (1953); (b) P. Doty and G. E. Meyers, *ibid.*, **13**, (1953); (c) P. Doty and R. F. Steiner, *J. Chem. Phys.*, **20**, 85 (1952).

[4] (a) G. Scatchard, I. H. Scheinberg and S. H. Armstrong, Jr., THIS JOURNAL, **72**, 535 (1950); (b) G. Scatchard, I. H. Scheinberg and S. H. Armstrong, Jr., *ibid.*, **72**, 540 (1950).

[5] J. T. Edsall, J. W. Otvos and A. Rich, *ibid.*, **72**, 474 (1950).

[6] R. C. Gore, R. B. Barnes and E. Petersen, *Anal. Chem.*, **21**, 382 (1949).

polysaccharides, and we have therefore investigated polymethacrylic acid, as well as a number of polymeric ampholytes, copolymers of methacrylic or acrylic acid and an amine base, with a view to establishing the utility of infrared absorption measurements in the study of charge effects in polymeric electrolytes in general.

Experimental

Spectroscopic Techniques.—All absorption spectra were measured on a Perkin–Elmer double beam recording infrared spectrophotometer, Model 21, equipped with a rock-salt prism and calibrated against the known absorption bands of polystyrene and atmospheric water vapor. Because of the high viscosity of the polymer solutions of interest, an easily dismountable cell was employed, consisting of two 1-mm. silver chloride sheets separated by a 0.025 mm. platinum spacer, and held in a suitable frame. Partial compensation for the absorption of the solvent was achieved by inserting a duplicate cell, filled with sufficient D_2O to give a somewhat undercompensated spectrum, into the reference beam. In observations on films, a compensating AgCl plate was used. Inasmuch as the planarity of the windows, as well as the efficiency of the spreading of the solution, varied from run to run, quantitative intensity measurements can only be attempted with such cells if a reference band which serves as an internal standard can be found in the spectrum. In comparing the absorption coefficients of different bands, intensities were obtained at the absorption peak rather than by integrating over the whole band.

The spectrum of D_2O (Stuart Oxygen Co., >99.5%) upon which that of the solute species is superimposed, is recorded in Fig. 1. The broad absorption in the vicinity of 6.5 μ may correspond to the Raman line at approximately 6.25 μ which has been attributed to a hindered rotation of deuterium bonded water molecules around the O–O axis.[7] The transmission is still sufficient to permit observation in the range from 5–8 μ, although only semiquantitative intensity measurements in the 6.5 μ region are feasible without resorting to variable thickness compensating cells. Inasmuch as deuteration shifts N–H stretching frequencies into the region of the D_2O absorption band at 4 μ,[8] which also partly obscures C–H stretching frequencies, the absorption spectra of solutions are reproduced from 5.25 μ to 7.85 μ only.

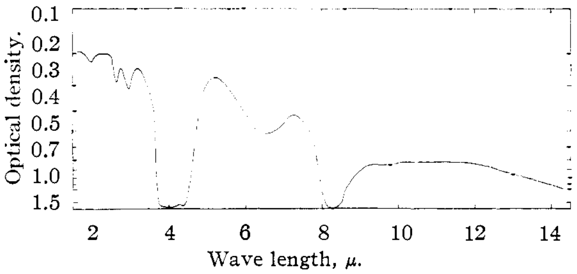


Fig. 1.—Absorption spectrum of D_2O (99.5%); path length 0.025 mm.

Materials.—Polymethacrylic acid (PMA) was prepared by Dr. Nathan Schneider at Harvard University by the polymerization of methacrylic acid in aqueous solution, using the H_2O_2–$FeSO_4$ oxidation–reduction system to initiate the reaction.[9] The polymer was precipitated from solution by addition of hydrochloric acid, redissolved in water and then dialyzed against distilled water until free of chloride ions. Final isolation was accomplished by freeze drying.

The preparation and physical properties of two polymeric ampholytes containing different amounts of methacrylic acid and 2-dimethylaminoethyl methacrylate and designated as P-7 and P-9, have been previously described.[10] The copolymer of acrylic acid and 2-vinylpyridine (W-L) was very kindly furnished by Professor F. A. Long of Cornell University in the form of the chloride.[11] This material was dialyzed against redistilled water until the *p*H of the supernatant liquid reached 5.2, and the gel which separated was isolated by freeze drying. The isoionic points, *p*I as well as the composition of these polyampholytes expressed as the ratio of the number of acidic groups n_2 to the number of basic groups n_1 are listed in Table I. Measurements on 2-methylpyridine (α-picoline) the model compound employed in studying the effects of *p*H changes on copolymer W-L were carried out on a carefully fractionated commercial sample, b.p. 125.5–126° (746 mm.), $n^{26}\text{D}$ 1.4970. 2 *N* solutions of NaOD or DCl in deuterium oxide were used to adjust the *p*D of the polymer solutions. NaOD was prepared by dissolving the requisite amount of freshly cut metallic sodium in D_2O (of >99.5% purity, furnished by the Stuart Oxygen Co.). DCl, generated by the reaction of 97% D_2SO_4 (Merck and Co., Inc.) with dried sodium chloride, was absorbed in D_2O to yield an approximately 2 *N* solution. Polymer solutions were made up from a stock solution prepared by allowing a weighed quantity of the material, dried in a vacuum desiccator over NaOH, to dissolve in D_2O for at least 10 hours. Sufficient D_2O, NaOD or DCl was then added to a part of the original stock solution to achieve the desired concentration, chosen so as to give a spectrum with well resolved absorption bands in the region of interest. These were: PMA, 6.4%; P-7, 8.4%; P-9, 8.2%; W-L, 8.7%. The record of isoionic W-L copolymer was obtained by filling the absorption cell with the gel obtained by adding D_2O to the dry dialyzed material. Solutions of 2-methylpyridine for absorption measurements were made up by volume and contained 25% solute. The preparation as well as transfer of solutions was accomplished in an atmosphere of nitrogen.

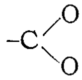
TABLE I

PHYSICAL CONSTANTS OF POLYMERIC AMPHOLYTES

Polymer	*p*I	n_2/n_1
P-7	5.62	1.16
P-9	8.60	0.315
W-L	4.7	.773

All films were prepared by evaporating the solvent from a 1% polymer solution spread on a silver chloride plate and exposed to the atmosphere; sufficient water was thus retained by the polymer to give a smooth, coherent film.

Infrared Spectra of Synthetic Polyelectrolytes

Polymethacrylic Acid.—Based on the study of the Raman scattering of aqueous solutions of carboxylic acids and their sodium salts, Edsall has assigned the bands near 5.85 μ (1710 cm.$^{-1}$) to a C=O stretching mode and those near 7.15 μ (1400 cm.$^{-1}$) and 6.33 μ (1580 cm.$^{-1}$) to the symmetric and antisymmetric valence vibrations respectively of  $-C\langle^{O}_{O^-}$ [substitution of deuterium for hydrogen in the carboxyl group does not significantly affect the location of these frequencies],[12,13] and it is apparent that these are the bands in the absorption spectrum of polymethacrylic acid most prominently affected by changes in the state of ionization (Fig. 2). In D_2O solution, the band at 5.90 μ (1695 cm.$^{-1}$) is most intense; on addition of NaOD however, it vanishes, to be replaced by an even stronger band at 6.45 μ (1550 cm.$^{-1}$) and a very much weaker one at 7.08 μ (1412 cm.$^{-1}$). The intensity relation between the latter two ($\kappa_{6.4}/\kappa_{7.1} = 7.6$) is the reverse of that observed in the Raman spectrum, in which the polarized 7.1 μ band

---

[7] P. C. Cross, J. Burnham and P. A. Leighton, THIS JOURNAL, **59**, 1134 (1937).

[8] J. T. Edsall and I. H. Scheinberg, *J. Chem. Phys.*, **8**, 520 (1940).

[9] R. Arnold and J. T. G. Overbeek, *Rec. trav. chim.*, **69**, 192 (1590).

[10] G. Ehrlich and P. Doty, THIS JOURNAL, **76**, 3764 (1954).

[11] H. L. Wagner and F. A. Long, *J. Phys. Colloid Chem.*, **55**, 1512 (1951).

[12] J. T. Edsall, *J. Chem. Phys.*, **5**, 508 (1937).

[13] R. C. Herman and R. Hofstadter, *ibid.*, **6**, 534 (1938).

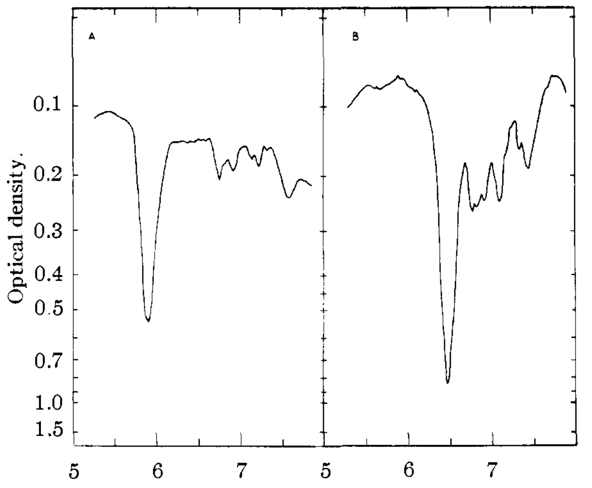


Fig. 2.—Absorption spectrum of polymethacrylic acid: A, in D_2O solution; B, in 0.4 *N* NaOD solution.

is much more prominent than the depolarized line at 6.4 μ, which appears with moderate intensity in some, but not all carboxylic acids investigated.[12] This provides additional support for the assignment of these lines. The carboxylate ion forms a bent triatomic group of type XY_2 with symmetry C_{2v},[14] and each of the three normal vibrations should be active in both the Raman effect and in the infrared; it is well known, however, that in the infrared the frequencies ν_2 and ν_3[15] are most intense, whereas in the Raman spectrum the symmetric stretching mode ν_1 dominates. Indeed, this reversal of intensities, characteristic of bent triatomic XY_2 molecules, explains the feasibility of observing Raman frequencies of solute molecules in aqueous solution near 1650 cm.$^{-1}$, a region of very pronounced absorption for water in the infrared.

The appearance of the band at 7.08 μ is accompanied by a general change in the frequency distribution in this region; ionization of the carboxyl groups brings about a shift of the absorption at 7.57 μ (1320 cm.$^{-1}$) found in PMA, to lower wave lengths, resulting in a doublet with peaks at 7.31 μ (1370 cm.$^{-1}$) and 7.41 μ (1350 cm.$^{-1}$). Edsall has associated the band at 7.46 μ (1340 cm.$^{-1}$) with the ionized carboxyl group,[12] but inasmuch as this is the region of the C–H deformation modes, which may be perturbed by the presence of an electric charge, a decision concerning such an assignment cannot readily be made.

Copolymers of Methacrylic Acid and 2-Dimethylaminoethyl Methacrylate.—Although the solution behavior of the polymeric ampholytes P-7 and P-9 obtained by the copolymerization of methacrylic acid and 2-dimethylaminoethyl methacrylate differs markedly from that of polymethacrylic acid,[16] the infrared spectra are very much alike. The carbonyl absorption bands of the un-ionized carboxyl groups overlap with those of the ester linkages of dialkylaminoethyl methacrylate residues, resulting in a band centered around 5.85 μ (1710 cm.$^{-1}$).

The intensity of this band relative to that at 6.45 μ decreases slightly on adding base to the isoionic polymer (Fig. 3A, C); addition of DCl, however, completely eliminates the 6.45 μ band, without significantly affecting the location of the absorption peaks of the carbonyl frequency. The differences in the spectra of the polyampholyte in DCl and NaOD are precisely those already observed between polymethacrylic acid in D_2O and NaOD. In base, the strong band at 6.45 μ and its weak companion at 7.08 μ, characteristic of the carboxylate ion, appear, together with a peak at 7.40 μ. All significant changes can thus be associated with the increase in the number of ionized carboxyl groups on the polyampholyte. Although such a change must be accompanied by a decrease in the number of R_3ND^+ groups, there is no indication of this in the spectrum, as N–D, as well as N–D^+ stretching or deformation bands, even if sufficiently intense for observation are probably obscured by the D_2O absorption at 4 and 8 μ.[8]

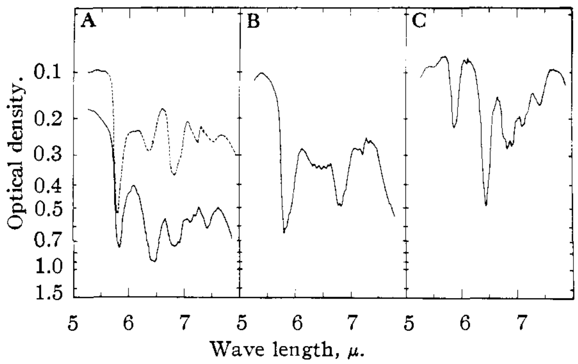


Fig. 3.—Absorption spectrum of P-7: A, in D_2O solution (—); film cast from H_2O (---); B, in 0.4 *N* DCl solution; C, in 0.4 *N* NaOD solution.

The spectrum of a film (Fig. 3A) strongly resembles that of a solution in DCl.[17] The intensity of the 6.45 μ band has decreased, the 7.08 μ band has vanished, and the absorption at 7.40 μ has moved to longer wave lengths. As is evident from Fig. 4, the change is even more startling for P-9, a polymeric ampholyte with a greater preponderance of basic groups than P-7. Here, upon drying, the absorption bands associated with the presence of carboxylate ions *disappear*, indicating that in the film the polyampholyte carries only an insignificant number of charged groups (estimated from the extinction coefficient to be less than 5% of the total number of acid groups).

The bands at 6.75–6.95 μ (1428–1440 cm.$^{-1}$) which persist in polymethacrylic acid, as well as in the polyampholytes, under all experimental conditions, are to be attributed to deformation modes of CH_2 and CH_3 groups in the polymer chain.[18]

Copolymer of Acrylic Acid and 2-Vinylpyridine.—Examination of Fig. 5A clearly shows that the bands characteristic of carboxyl groups, ionized as well as un-ionized, cannot account for the changes in the absorption spectrum of copolymer W-L as a

---

(14) W. H. Zachariasen, This Journal, **62**, 1011 (1940).

(15) G. Herzberg, "Molecular Spectra and Molecular Structure," Vol. II, D. Van Nostrand and Co., Inc., New York, N. Y., 1945, Ch. II.

(16) A. Oth and P. Doty, *J. Phys. Chem.*, **56**, 43 (1952).

(17) Films of deuterated material do not significantly differ from those cast from H_2O.

(18) N. Sheppard and D. M. Simpson, *Quart. Revs.* (*London*), **6**, 1 (1952).

TABLE II

ABSORPTION BANDS OF PMA AND P-7

PMA λ, μ		P-7 λ, μ			Tentative assignment
D_2O	NaOD	DCl	NaOD	D_2O	
5.90		5.82	5.86	5.83	$\nu(C{=}O)$
	6.45		6.43	6.45	$\nu_3(COO^-)$
6.75–6.90	6.75–6.90	6.75–6.90	6.75–6.90	6.75–6.90	δCH
	7.08		7.08	7.08	$\nu_1(COO^-)$
7.20		7.20	?	7.20	δCH
	7.31			?	
	7.41		7.40	7.40	δCH_3
7.55					
		7.70			

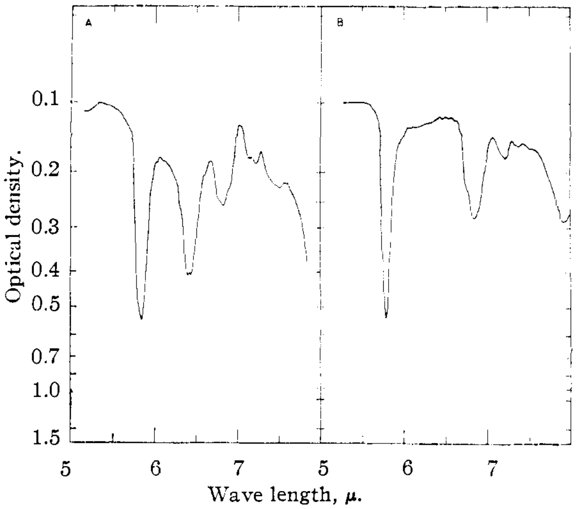


Fig. 4.—Absorption spectrum of P-9; A, in D_2O solution; B, film, cast from H_2O.

function of the *p*D of the solution, and that the contributions of the pyridine ring vibrations must be taken into consideration. The spectra of 2-methylpyridine in D_2O, DCl and NaOD are represented in Fig. 6. In D_2O and NaOD solution, the bands are in good agreement with those observed for liquid pyridine,[19,20] listed in Table III. In DCl, however, the absorption bands corresponding to ring vibrations appear to be shifted to lower wave lengths, and also suffer significant changes in intensity. Most striking is the appearance of bands at 6.13 and 6.61 μ, attended by a diminution in absorption at 6.36 and 6.73 μ. Such a displacement of absorption bands can be explained in terms of a valence bond description of the bonding in pyridine. The contribution of ionic forms is greater in the pyridinium ion than in pyridine, inasmuch as the addition of the proton increases the effective electronegativity of the nitrogen,[21] resulting in an increase of the resonance energy, which also affects the force constants governing ring vibrations. A shift of the Raman lines in going from solutions of pyridine to those of pyridinium chloride has actually been reported.[22,23] Not all of the permitted ring frequencies of the pyridine system were observed in these investigations, however, making comparison impossible.

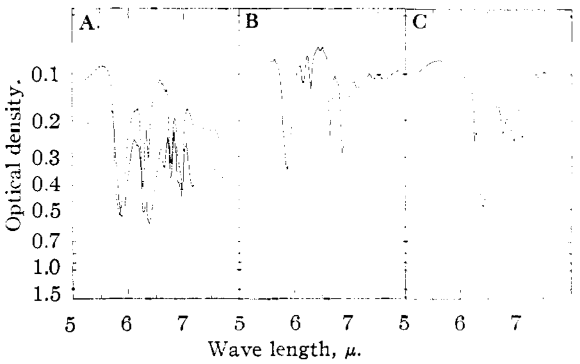


Fig. 5.—Absorption spectrum of copolymer W-L: A, gel in D_2O (—); film, cast from D_2O (---); B, in 0.6 *N* DCl; C, in 0.6 *N* NaOD.

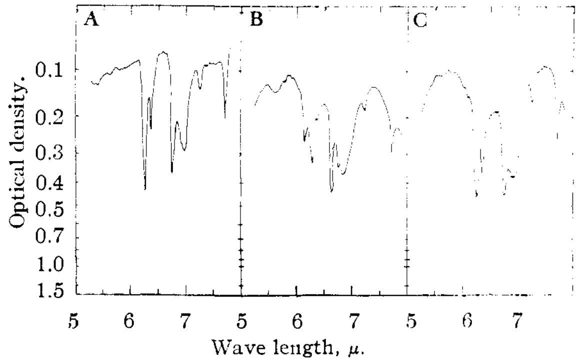


Fig. 6.—Absorption spectrum of 2-methylpyridine: A, in D_2O solution; B, in 1.5 *N* DCl; C, in 1.5 *N* NaOD.

The locations of the absorption bands of the W-L copolymer in D_2O, DCl and NaOD are given in Table III. In acid, the spectrum shows the bands characteristic of COOH and pyridinium ions, in base, those of carboxylate ions and pyridine rings, although there is some overlapping of bands for the latter two groups. The spectrum of the isoionic gel (Fig. 5A) contains contributions from both ionized and un-ionized carboxyl and pyridine groups; upon drying, however, only the absorption of un-ionized groups can be detected, just as found for P-9.

---

(19) L. Corrsin, B. J. Fax and R. C. Lord, *J. Chem. Phys.*, **21**, 1170 (1953).

(20) A. Langseth and R. C. Lord, *Kgl. Danske Videnskab Selskab, Math.-fys. Medd.*, **16**, No. 6 (1938).

(21) L. Pauling, "The Nature of the Chemical Bond," Cornell Univ. Press, Ithaca, N. Y., 1945, p. 65.

(22) P. Krishamurti, *Indian J. Phys.*, **6**, 401 (1931).

(23) H. J. Bernstein and W. H. Martin, *Trans. Roy. Soc. Can. (III)*, **31**, 95 (1937).

TABLE III

ABSORPTION BANDS OF PYRIDINE, 2-METHYLPYRIDINE AND COPOLYMER W-L

Pyridine[20] λ, μ	2-Methylpyridine λ, μ		Copolymer W-L λ, μ				Tentative[a] assignment
	D_2O	DCl	NaOD	DCl	D_2O	Film	
				5.85	5.87	5.85	ν(C=O)
		6.13		6.15			
6.33	6.25		6.25		6.25	6.25	8a
		6.27		6.28			
6.36	6.36	6.36				6.35	8b
			6.40		6.37		ν_3(COO⁻)
		6.61		6.62	6.64		
6.74	6.73	6.73	6.75		6.75	6.75	19a
		6.82		6.85	6.87		
6.95	6.91–6.95	6.96	6.91		6.95	6.95	19b
			7.12	7.13	7.13		
7.28	7.23	7.21					14
7.67	7.70	7.70		7.67	7.67		2 × 6b

[a] The designation of pyridine ring vibrations is that given in reference 20.

It is interesting to observe that the W-L copolymer, which does not contain methyl groups, does not show a band at 7.40 μ in the presence of NaOD, as observed for PMA and P-7, suggesting that this band may be due to a frequency of the methyl groups, adjacent to the ionized carboxyls.

The State of Ionization of Synthetic Polymeric Ampholytes at the Isoionic Point

The data presented here clearly show that infrared bands characteristic of the ionizable groups on synthetic polymeric electrolytes can be used to determine the state of ionization of these macromolecules, knowledge of which is essential for an understanding of their solution properties. Particularly suited for this purpose are the 6.4 μ COO⁻ and the 5.8 μ C=O bands.

Assuming that the absorption coefficient for the carbonyl band at 5.85 μ is the same in the carboxyl as in the ester group, and using the data from Table I in conjunction with the intensities shown in Fig. 3C, we find that $\kappa_{6.4}/\kappa_{5.8} = 2.3$. The degree of ionization of the carboxyl groups at the isoionic point, calculated from the intensity of the 6.45 μ band relative to that at 5.85 μ was found to be 0.89 for P-7 and P-9 and 0.2 for copolymer W-L; the last value is particularly subject to error, because of the overlapping of a pyridine and a carboxylate vibration, and must be considered as a *lower limit only*. In films the extent of ionization is significantly lower.

The results for solutions of P-7 and P-9 are in agreement with 1st-order calculations based on the dissociation constants of the basic and acidic monomers ($pK_1 = 10.01$,[24] $pK_2 = 4.85$[9]). However, from measurements of titration curves, viscosity and solubility of copolymers of acrylic (or methacrylic) acid and 2-vinylpyridine, both Wagner and Long[11] as well as Alfrey and Morawetz[25] have concluded that at the isoionic point these polyampholytes do not exist as dipolar ions.[26] It may be objected that our conclusions concerning copolymer W-L are invalidated because fluctuations in the number of groups ionized (even in an uncharged polymer) may contribute sufficiently to account for the observed spectra, and because differences in the ionization process of deutero-acids in D_2O from those of proto-acids in H_2O may negate conclusions concerning the state of ionization in H_2O drawn from work in D_2O. Both objections readily can be refuted.

Inasmuch as the time of observation of an absorption band is of the order of seconds, whereas the Langevin relaxation time for a carboxylate group is of the order of 10^{-8} second, only the *average* deviation from equilibrium need be considered. The mean square deviation from the average net proton charge, $(\Delta z^2)_{av}$, at the isoionic point, calculated from the titration curve using the relation[28]

$$-\frac{1}{2.3}\frac{dz}{dpH} = (\Delta z^2)_{av} \qquad (1)$$

is 47; this leads to an effective degree of ionization (due to fluctuations) of only 0.01, compared with 0.2, calculated from the intensity of the 6.4 μ band.

The lowering of the zero point energy which accompanies the substitution of deuterium for hydrogen results in a lowering of the equilibrium constant for deutero-acids in D_2O below that found for the corresponding proto-acid in H_2O[29]; the ratio of the constant for the proton system (K_p) to that of the deuteron system (K_d) depends upon the strength of the acid concerned, increasing with decreasing acidic dissociation. It is possible to make an approximate calculation of the dependence of the degree of ionization α of the carboxyl groups at the pI upon the ionization constants of the basic (K_1) and acid groups (K_2) if we assume that the constants correspond to those of 2-methylpyridinium ion ($K_1 = 1.14 \cdot 10^{-6}$) and isobutyric acid ($K_2 = 1.41 \cdot 10^{-5}$), respectively, and that electrostatic interactions can be neglected. It follows then that α in terms of K_1/K_2 and the number of basic (n_1) and acidic groups (n_2) is given by

$$\alpha^2 n_2 \left(1 - \frac{K_1}{K_2}\right) - \alpha(n_2 + n_1) + n_1 = 0 \qquad (2)$$

For a 10% decrease in K_1/K_2, (an upper limit for the change to be expected in going from a proton to a deuteron system) the decrease in α calculated from 2 is of the same order as the possible contribution from fluctuations (α = 0.03) and we can therefore safely conclude that in solutions of H_2O as well as D_2O, the isoionic W-L copolymer exists in the form of dipolar ions, with at least 20% of the carboxyl groups charged. This is in qualitative agreement with ultraviolet absorption measurements on a similar copolymer, carried out by Katchalsky and Miller,[30] which also indicated the presence of ionized groups at the pI. That the polymeric ampholyte is insoluble in spite of charged groups on the chain is not surprising; it will dissolve at the isoi-

---

(24) T. Alfrey, Jr., R. M. Fuoss, H. Morawetz and H. Pinner, THIS JOURNAL, **74**, 438 (1952).

(25) T. Alfrey, Jr., and H. Morawetz, *ibid.*, **74**, 436 (1952).

(26) This to some extent appears to be based on an inappropriate estimate of the intrinsic *pK* of the pyridinium ion in the copolymer. The value of 5.10 for 2-vinylpyridine cation is too low, a better approximation being that for 2-methylpyridinium ion, $pK_a = 5.94$.[27]

(27) E. F. G. Harington, *Faraday Soc. Disc.*, **9**, 26 (1950).

(28) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 462.

(29) R. W. Gurney, "Ionic Processes in Solution," McGraw-Hill Book Co., New York, N. Y., 1953, p. 150.

(30) A. Katchalsky and I. Miller, Abstracts, Am. Chem. Soc., 120th Meeting (New York, N. Y., Sept., 1951).

onic point only if it carries a sufficiently large number of solvating groups to outweigh the attraction both due to Coulomb as well as van der Waals forces between the individual macromolecules. This effect is quite strikingly shown by the studies on the solubility of copolymers of 2-diethylaminoethyl methacrylate and methacrylic acid as a function of composition,[24] in which it was found that copolymers containing less than 35 mole % or more than 79.6% basic groups were insoluble at the *p*I. Inasmuch as at the isoionic point the number of positive and negative charges must be equal,[31] the maximum total number of ionized groups in a two component copolymer cannot exceed $2(1 - X)$, where X is the mole fraction of the component present in excess. Thus the total number of charges per polymer molecule,[32] and therefore also the solvation of the polyampholyte, decreases as the composition is varied from the equimolar mark, resulting in precipitation of the copolymers at the extremes of the composition scale.

It is apparent that the determination of the state of ionization is particularly simple in polymethacrylic acid and the copolymers of methacrylic acid and 2-dimethylaminoethyl methacrylate because the carboxylate ions absorb in a region not otherwise obscured by extraneous bands. That this is *not* a prerequisite for the success of this technique, however, is shown by the measurements on the copolymer of acrylic acid and vinylpyridine, in which there is considerable overlapping of the frequencies characteristic of ionized groups. The more difficult problem of the determination of the ionization of proteins, in which bands due to peptide links dominate the spectrum in the region of interest, will be considered in the following communication.

I would like to acknowledge my indebtedness to Dr. G. B. B. M. Sutherland for his generous advice and for many stimulating discussions during the course of this study, which was supported by Contract N6 onr-232249 with the Office of Naval Research and Contract DA-44-109-QM-1037 with the Quartermaster Corps.

ANN ARBOR, MICHIGAN

---

[31] J. T. Edsall, H. Edelhoch, R. Lontie and P. R. Morrison, THIS JOURNAL, **72**, 4641 (1950).

[32] Inasmuch as the molecular weight in this series of copolymers was not held constant, and the dissociation constants of the monomers do not suffice to quantitatively describe the dissociation of the groups on the polymer, calculations of the actual number of charged groups have not been carried out.

[CONTRIBUTION FROM THE H. M. RANDALL LABORATORY OF PHYSICS, UNIVERSITY OF MICHIGAN]

Infrared Studies on Solutions of Polymeric Electrolytes. II. Proteins

BY GERT EHRLICH[1] AND G. B. B. M. SUTHERLAND

RECEIVED MAY 29, 1954

The possibility of using infrared methods to detect carboxylate groups in globular proteins at the isoionic point has been investigated. Using N-methylsuccinamic acid in D_2O solution as a model compound, it has been shown that spectral changes in the region between 5 and 8 μ associated with variations in *p*D can be attributed to changes in the number of COOD and COO^- groups, while the absorptions in this region due to the peptide link are essentially unaltered. Two proteins were investigated, *viz.*, bovine plasma albumin and β-lactoglobulin. Both were shown to contain COO^- groups, which in acid solution could be converted into COOD groups, thus providing direct evidence for the existence of these proteins as dipolar ions at the isoionic point. The absorption band which best characterizes the COO^- group lies at 6.4 μ, *i.e.*, very close to a peptide band at 6.45 μ. The latter, however, is shifted to 6.8 μ when the protein is deuterated, thus revealing the carboxylate group band. In undeuterated proteins the two bands overlap and this may affect deductions on protein structure made from observations on the 6.45 μ band in undeuterated proteins. The possibility of extending the infrared method to the detection of other ionic groups in proteins is discussed.

The numerous investigations of the electrochemical behavior of proteins, and of the dependence of their colligative properties on the state of charge,[2,3] provide an impressive body of evidence testifying to the importance of ionized groups in determining the physical chemistry of solutions of globular proteins. The majority of infrared studies on proteins have hitherto been aimed at elucidating the structure of the peptide linkages and the configuration of the individual polypeptide chains forming the protein skeleton, and with a few exceptions[4,5] have ignored the vital part played by ionized groups in determining protein structure and reactions. It has been shown in the preceding paper[6] that information on the state of ionization of certain synthetic polymeric electrolytes in solutions of heavy water can be obtained from infrared bands, characteristic of the carboxyl and carboxylate groups. The most numerous of the ionizable groups which have been identified in globular proteins are the carboxyls of glutamic and aspartic acid residues. If present in sufficiently large numbers, these should contribute to the infrared absorption spectra of proteins and the possibility arises of using infrared analysis for determining the state of ionization of protein molecules in solution. The feasibility of such a method depends mainly on the bands of the ionizable groups not being obscured either by (a) bands due to other parts of the protein molecule or (b) bands due to the solvent.

The infrared spectrum of a typical protein is

---

[1] Research Laboratory, General Electric Co., Schenectady, New York.

[2] W. Pauli and E. Valko, "Elektrochemie der Kolloide," Julius Springer, Wien, 1929, Chs. 42–52.

[3] E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943.

[4] I. M. Klotz, P. Griswold and D. M. Gruen, THIS JOURNAL, **71**, 1615 (1949).

[5] H. Lenormant and J. Chouteau, *J. physiol. et path. gén.*, **41**, 203A (1949).

[6] G. Ehrlich, THIS JOURNAL, **76**, 5263 (1954).