TABLE III

COMPARISON OF ENTROPY PARAMETERS EVALUATED FROM PRECIPITATION TEMPERATURE AND INTRINSIC VISCOSITY

Polymer	Solvent	Θ (°K.)	ψ_1 Precipitation	ψ_1 Viscosity	Ratio
Polymethyl methacrylate[23]	Di-*n*-propyl ketone	305	0.61	0.23	2.6
Polyisobutylene[11]	Benzene	297.5	.65	.15	4.3
Polyisobutylene[12]	Ethylbenzene–phenyl ether (3:1)	300	.85	.17	5.0
Polydimethylsiloxane[24]	Butanone	298.2	.43	.08	5.4
Polystyrene[19]	Cyclohexane	307.2	1.06	.13	8.1
Natural rubber[25]	Heptanone-4	287.5	.80	.08^5	9.4

$[\eta]$ measurements, furnishes further evidence that this ratio is by no means constant for different polymer–solvent pairs.

The disagreement between values for the entropy parameter deduced from osmotic pressure and precipitation temperature is even more disturbing from a theoretical standpoint. The procedure involving A_2 would appear to have a slight theoretical advantage, inasmuch as the evaluation is carried out precisely at $T = \Theta$. Since the molecular expansion factor is unity at this temperature, any influence of the configuration of the polymer molecules upon the chemical potential of the solvent must vanish under these circumstances. On the other hand, Shultz and Flory have pointed out that the magnitude of the entropy parameter obtained by precipitation for polystyrene–cyclohexane stands in good agreement with that for the analogous small molecule system toluene–cyclohexane.[26] Until these differences can be resolved theoretically in favor of one of the procedures, there is no assurance that the ψ_1 values for different polymer–solvent pairs obtained by any one of these methods are even correct relative to one another.

In summary, the dilute solution treatment clearly represents a notable advance over the earlier treatments of polymer solutions. The factor $F(X)$ introduced thereby into the expression for the second virial coefficient allows, for the first time, a semi-quantitative description of the observed dependencies of A_2 on molecular weight and molecular weight heterogeneity. Although these successes are encouraging, nevertheless the accurate osmotic pressure measurements reported in this series of papers indicate the necessity for further improvement on the theory. In particular, the magnitude of the important entropy parameter, ψ_1, remains in doubt pending further refinement of the existing theories.

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(23) T. G. Fox, private communication.

(24) P. J. Flory, L. Mandelkern, J. B. Kinsinger and A. R. Shultz, THIS JOURNAL, **74**, 3364 (1952).

(25) H. L. Wagner and P. J. Flory, *ibid.*, **74**, 195 (1952).

(26) A. R. Shultz and P. J. Flory, abstracts of papers presented at the 124th meeting of the American Chemical Society, Chicago, Ill., Sept. 6-11 (1953).

DURHAM, N. C.

[CONTRIBUTION FROM THE GIBBS LABORATORY, DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

Macro-ions. III. The Solution Behavior of a Polymeric Ampholyte[1]

BY GERT EHRLICH[2] AND PAUL DOTY

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In order to explore the effects of electrostatic attractions between acidic and basic groups upon the configuration and solution behavior of a non-rigid macro-ion, copolymers of methacrylic acid and 2-dimethylaminoethyl methacrylate, ranging in composition from 23 to 57 mole per cent. methacrylic acid, have been prepared and characterized. It has been found that ionization of the monomers exerts a marked influence on the propagation reactions in methanol, invalidating the application of the two-component copolymerization equation. A light-scattering and viscometric study of one of these copolymers (53.7% methacrylic acid), which was water soluble over the whole *p*H range, was carried out. The most interesting behavior was that at the isoionic point in the absence of salt. Here it was found that the polyampholyte molecule was contracted relative to that at higher ionic strength, that the second virial coefficient was negative and that the intensity of scattered light increased with increasing angle. This information together with theoretical considerations, shows that the extension of the molecules is decreased by the net attraction of the oppositely charged segments, and that there is a pronounced intermolecular attraction, due to the existence of both positively and negatively charged molecules at the isoionic point. The intermolecular attraction produces a distinctive type of clustering in which there is probably little interpenetration in contrast to the association of uncharged polymer molecules near the point of phase separation. Changing the *p*H away from the isoionic point destroys this type of clustering and replaces it by an ordering of the solute molecules due to mutual repulsion of like charges and characterized by large positive second virial coefficients. The behavior of similar solutions, 0.1 *M* in NaCl, was close to that of uncharged polymers but it appeared that even here there was evidence of residual electrostatic attractions between oppositely charged segments.

Polymeric ampholytes (polyampholytes) differ from polymeric acids and bases in that they possess both positively and negatively ionized groups. The unique characteristic of polyelectrolytes carrying only one type of charge is the large contribution to the molecular free energy arising from electrostatic repulsions; this is exhibited most directly by the dependence of the molecular size and the degree of ordering in solution on the charge and ionic strength. In polyampholytes, however, the electrostatic interactions can be both repulsive and attractive, the extent depending on the *p*H of the

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(1) Presented in part at the 120th Meeting of the American Chemical Society, New York, N. Y., September 6, 1951.

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solution, since this controls the relative number of positive and negative charges. In strongly acidic or basic solutions polyampholyte molecules will have a large net charge and will reside within a cloud of gegenions; under these conditions their behavior should duplicate that already found for ionized or neutralized polyacids and polybases. In contrast, a quite different situation exists at the isoelectric point, that is, the *p*H at which the polyion is electrically neutral. Here there are no gegenions required and indeed all small ions can be removed. It would be expected that in this situation chain configurations which permit a degree of alternation in the spatial arrangement of the oppositely charged segments would be preferred and that these would lead to a net attraction among the segments and hence a contraction of the polyion.

Our interest lies in exploring the intermolecular and intersegmental interactions in a typical polyampholyte, particularly under the conditions just described. To this end we have employed viscometric, potentiometric and light-scattering techniques. The two previous publications in this series were concerned with the elucidation of the contribution of electrostatic repulsions to the solution properties of macro-ions. In the one case these macro-ions were rigid protein molecules where intra-molecular effects were absent[3] and in the other, dealing with polymethacrylic acid,[4] both intra- and intermolecular effects were substantial. Light-scattering techniques proved the most useful in these earlier studies but were nevertheless limited because the scattering from only three angles was measured. In the present work we have extended measurements of the intensity of scattering to the region from 30–135° and have sought to interpret the scattering envelopes observed.

Preparation of Polyampholyte

For this investigation a polyampholyte with the following properties was deemed desirable: (1) A high density of ionizable groups, sufficiently weak to yield only one type of charge at the extremes of *p*H but sufficiently strong to be ionized in the isoionic region. (2) A molecular weight of a few hundred thousand in order to give optimal response to the techniques employed in studying the solution behavior. (3) Solubility in water over the entire *p*H range of interest.

Of the various polyampholytes described in the literature,[5–13] the copolymers of 2-dialkylaminoethyl methacrylate and methacrylic acid of Alfrey and co-workers appeared most promising; unfortunately, samples supplied to us by him did not become molecularly dispersed even in salt solution. Consequently we undertook a more extensive study of the preparation of such a copolymer, concentrating particularly on that of 2-dimethylaminoethyl methacrylate and methacrylic acid in the hope of overcoming this difficulty and meeting the requirements listed above.

Experimental

Materials.—Glacial methacrylic acid, (M_2), obtained from Monomer-Polymer, Inc., was distilled under nitrogen immediately before use; 2-dimethylaminoethyl methacrylate (M_1), was prepared from methyl methacrylate and 2-dimethylaminoethanol following the procedure by Graves[9] and Harmon,[10] and fractionated at reduced pressure under nitrogen; b.p. 49–51° at 3 mm., $n^{25}D$ 1.436. In all polymerizations 2,2-azo-bis-isobutyronitrile was used as initiator and *n*-butyl mercaptan as chain transfer agent. Commercial absolute methanol was used as solvent; other materials were of reagent grade.

Small Scale Experiments.—Initiator was placed in a Pyrex tube having a constricted-neck and equipped with a ground glass joint. Methanol solutions of the monomers were mixed, cooled and added to the tube along with *n*-butyl mercaptan if a chain transfer agent was employed. After degassing, the tube was sealed and immersed in a water-bath at 60.5°. Polymerization was stopped by immersion in liquid nitrogen. The product was purified by repeated precipitation, usually from aqueous solution with excess acetone. Polymers 8 and 9, however, were dissolved in 0.5% methanolic hydroquinone and precipitated by addition of ethyl ether; polymer 10 was precipitated from aqueous hydroquinone solution with acetone. Polymers 8, 9 and 10 were dialyzed against distilled water, electrodialyzed and freeze-dried.

Large Scale Experiments.—These polymerizations were carried out in a special five-necked flask provided with reflux condenser, mercury sealed stirrer, thermometer, capillary for admission of nitrogen, a calibrated capillary for determination of the viscosity of the reaction mixture and a breakable initiator reservoir. The methanol solution of the monomers and mercaptan in the reaction flask was freed of oxygen with a stream of nitrogen and after bringing the mixture to 60.5° the reaction was initiated. The polymerization was interrupted when the conversion had reached the desired point as estimated by the flow rate through the capillary, and the formed polymer was precipitated with acetone. After two more precipitations, between which the polymer was filtered, it was dialyzed and freeze-dried. Inasmuch as some thermally initiated polymerization had occurred in preparing polymer 6 (as judged by the increase in viscosity on being brought to the reaction temperature), a crude fractionation was carried out using acetone as the precipitant of a 1.3% solution. The second and third of five fractions were combined and refractionated into three functions, the central one of which comprised half of the original sample. This was used for most of the investigations, following electrodialysis and freeze-drying. It was designated as P-7.

Copolymer Characteristics and an Anomaly in the Copolymerization Reaction.—The conditions of polymerization and the physical constants of the copolymers prepared in an effort to achieve the requirements set forth above are listed in Table I. The titration curves, one of which is shown in Fig. 1, indicate clearly that these materials are typical ampholytes in that they combine with both acid and base. The titration curves also permit a check of the structure of the polymers since in each case the total weight of the polymer can be accounted for, within the limits of error of the titrations, by the number of ionizable groups of each type and the molecular weights of the monomers involved.

An analysis of the data assembled in Table I should permit the calculation of the reactivity ratios r_1 and r_2, which are of interest not only for the

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(3) P. Doty and R. F. Steiner, *J. Chem. Phys.*, **20**, 85 (1952).

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

(5) T. Alfrey, H. Morawetz, E. B. Fitzgerald and R. M. Fuoss, THIS JOURNAL, **72**, 1864 (1950).

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

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

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

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(10) J. Harmon, U. S. Patent 2,139,762 (Nov. 29, 1938).

(11) G. D. Jones, British Patent 631,738 (Nov. 9, 1949).

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(13) M. Frankel and M. E. Reichmann, *J. Chem. Soc.*, 289 (1952).

TABLE I

COPOLYMERIZATION OF METHACRYLIC ACID (M_2) AND 2-DIMETHYLAMINOETHYLMETHACRYLATE (M_1) IN METHANOL AT 60.5°

Sample no.	3	9	8	5	6	7	11	4	10
Polymerization feed (in gram moles)									
M_1	0.0728	0.0729	0.0512	0.0365	0.0261	...	3.14	0.0292	0.0291
M_2/M_1	0.395	0.401	0.993	1.80	2.50	...	2.50	2.51	2.50
Initiator	0.00056	0.00056	0.00056	0.00056	0.00017	...	0.0204	0.00056	0.00056
Methanol	0.298	0.298	0.298	0.298	0.266	...	31.9	0.298	0.298
n-Butyl mercaptan	.....	....	....	0.000097	0.00128	...	0.0146	....	....
Duration of polymerization (minutes)	35	41	29.5	29	17	...	15	15	19.5
Wt. of isolated polymer	1.4	2.18	2.13	3.15	14.2	6.5	132.4	1.78	1.86
Conversion, %	10.1	15.6	17.0	27.7	14.6	...	12	16.4	17.2
Composition of polymer									
% N by Kjeldahl[a]	7.69	7.60	7.14	5.97	....	5.45	.....	5.23	5.16
% N by titration	.....	7.4	6.8	....	....	...	.....	....	5.0
Fraction titrated[b]	.....	0.95	0.99	....	....	...	.....	....	0.95
dM_2/dM_1	0.290 ± 0.02	0.32 ± 0.02	0.45 ± 0.03	0.90 ± 0.04	1.08 ± 0.05	1.16 ± 0.05	.....	1.29 ± 0.05	1.33 ± 0.05
Isoionic point[c]	.....	8.60	8.28	7.3	....	5.62	.....	5.60	5.62
Molecular weight[d]	.....	....	....	300000	330000	270000	.....	....	....

[a] Determined at the M.I.T. Microchemical Laboratory by the micro Kjeldahl method. [b] Fraction titrated = $\frac{\text{Carboxyl groups titrated} \times 86.1 + \text{amino groups titrated} \times 157.2}{\text{Total weight of copolymer in solution}}$. [c] Isoionic point: *p*H of a 0.2% salt-free solution at 25°. [d] Molecular weights: from light scattering measurements by the dissymmetry method.

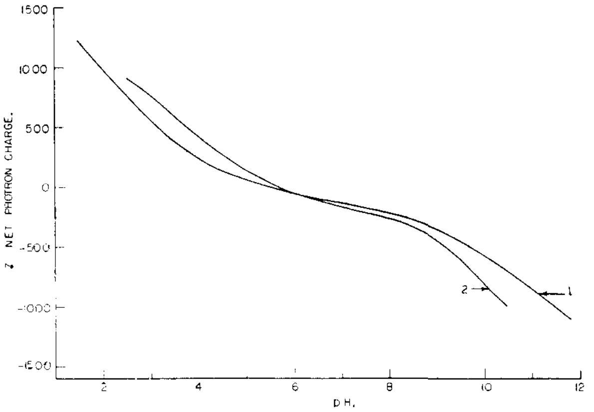

Fig. 1.—Average net proton charge *z* per molecule of P-7 as a function of *p*H: Curve 1, $c = 3.66 \times 10^{-3}$ g./cc., salt free; curve 2, $c = 3.30 \times 10^{-3}$ g./cc., 0.106 *M* NaCl.

prediction of the composition of the polymers formed from any arbitrary feed, but also for the detailed arrangement of monomer units in the polymer chain. Ordinarily these can be obtained from the slope and intercept of the straight line which results when the feed and polymer composition at low conversion are plotted in the manner shown in Fig. 2.[14] In this case, however, one does not obtain a straight line.[15] The deviation is clearly outside the limit of error of the analyses or that due to possible changes in monomer composition by ester interchange during the synthesis. The evolution of heat noticed on mixing solutions of the monomers in methanol suggested that in the polymerization feed the monomers were ionized, and this is substantiated by the results of resistance measurements on solutions of monomers. On addition of the basic monomer to a solution of methacrylic acid in methanol, the specific conductance increased approximately 2000-fold, and a change of the same order was found upon reversing the procedure and introducing acid into a solution of 2-dimethylaminoethyl methacrylate. From this it is apparent that in discussing the course of the copolymerization both ionized and un-ionized monomers must be taken into account.

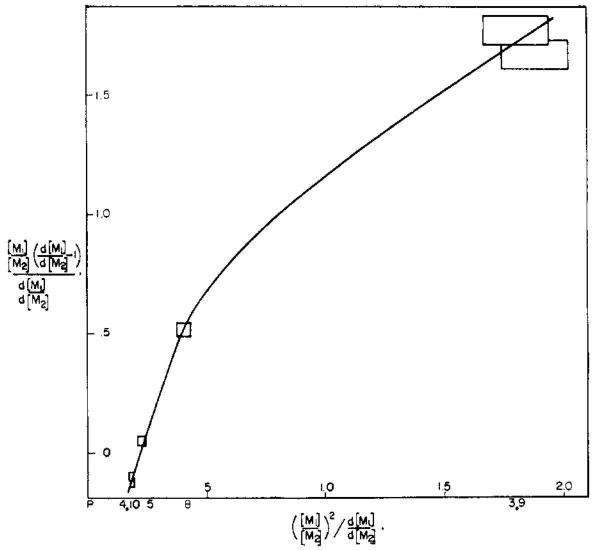

Fig. 2.—Copolymerization plot for methacrylic acid (M_2) and 2-dimethylaminoethyl methacrylate (M_1) in methanol.

The effects of ionization of monomers have been observed in the polymerization of methacrylic acid in aqueous solution by Katchalsky,[16,17] Melville,[18] and Pinner[19]; the first reported that the velocity of polymerization of methacrylic acid dropped significantly on increasing the *p*H, and approached zero in the vicinity of *p*H 6, where charged species predominate. Katchalsky has suggested that the polymerization of ionized monomers could be treated as a special case of copolymerization. If we apply this idea to our system, however, sixteen separate propagation steps must be considered, involving ionized and un-ionized species as distinct

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(14) M. Fineman and S. D. Ross, *J. Polymer Sci.*, **5**, 259 (1950).

(15) Such a discrepancy has also been observed by T. Alfrey, C. G. Overberger and S. H. Pinner, THIS JOURNAL, **75**, 4221 (1953).

(16) A. Katchalsky, "Proceedings of the International Colloquium on Macromolecules, Amsterdam," 1949, Centen, Amsterdam, p. 374.

(17) A. Katchalsky and G. Blauer, *Trans. Faraday Soc.*, **47**, 1360 (1951).

(18) H. W. Melville, ref. 16, p. 376.

(19) S. H. Pinner, *J. Polymer Sci.*, **9**, 282 (1952).

entities. The analytical data presented above do not, of course, permit such a quantitative treatment; however, a qualitative discussion of the course of copolymerization is possible.

Examination of Fig. 2 reveals that the reactivity ratios are themselves functions of the monomer feed and as the ratio of base to acid increases both r_1 and r_2 decrease, that is, less 2-dimethylaminoethyl methacrylate (M_1) enters the copolymer at high concentration of methacrylic acid (M_2) than expected on the basis of the behavior found at low concentrations of the acid. In a polymerization mixture with an excess of acid monomer, we can expect a significant fraction of the basic monomer to be ionized; similarly, for a run with an excess of 2-dimethylaminoethyl methacrylate the methacrylic acid will be substantially dissociated. The increase in alternation on increasing the ratio of base to acid in the feed thus implies that whereas the ionization of 2-dimethylaminoethyl methacrylate does not significantly affect its reactivity toward radicals, the ionization of methacrylic acid does indeed exert a very marked effect on its reactions, resulting in a preferential combination with a radical formed from the basic monomer. The data do not permit an assignment of the cause of this alternating effect, nor of the greater reactivity of 2-dimethylaminoethyl methacrylate in general. In their study of the polymerization of methacrylic acid in aqueous solution, Katchalsky and Blauer[17] found that ionized monomer did not react with dissociated or undissociated radicals, and that the addition of salt did *not* bring about an increase in the rate of polymerization. The inactivation accompanying ionization was therefore ascribed to the transition to the more symmetrical carboxylate structure rather than to repulsion between charges on the radical and monomer. Price and Walsh[20] have recently pointed out that according to the calculations of Kirkwood and Westheimer[21] electrostatic effects should be significantly influenced by the macroscopic dielectric constant only for the interaction of two charges, and then only when the separation between monomer and radical is large compared with that between monomer and solvent, so that coulomb interactions may still be responsible for the decreased reactivity of the methacrylate ion despite the apparent absence of an ionic strength effect. In any case, the absence of a significant effect of ionization on the reaction of 2-dimethylaminoethyl methacrylate can be attributed to the greater separation between the reactive site and the charged group. Whatever the mechanism of interaction between the charged group and the double bond may be, it is this effect which is responsible for the failure of the simple two-component copolymerization equation when applied to the polymerization of methacrylic acid and 2-dimethylaminoethyl methacrylate. Inspection of Fig. 2 does reveal, however, that for monomer feeds with a preponderance of acid (P8-10), the copolymerization plot is linear, presumably because of the small concentration of methacrylate ion existing over this composition range. Assuming the validity of the two-component equation for this region, the reactivity ratios are found to be $r_1 = 3.0$, $r_2 = 0.67$. From this and the relations

$$\begin{aligned}\bar{m}_1 &= r_1 \frac{[M_1]}{[M_2]} + 1 \\ \bar{m}_2 &= 1 + r_2 \frac{[M_2]}{[M_1]}\end{aligned} \tag{1}$$

one obtains for the average monomer sequence lengths $\bar{m}_1$ and $\bar{m}_2$ the values shown in Table II. It appears that the positive and negative charges on the polymer investigated (P-7) tend to occur in groups of two or three rather than in strict alternation or in a completely random fashion. Although the copolymerization reaction cannot be discussed quantitatively, the empirical correlation between monomer feed and polymer composition and molecular weight did result in establishing the conditions for obtaining a copolymer, P-7, which meets the requirements stated previously.

TABLE II

AVERAGE LENGTHS OF MONOMER SEQUENCES

P	8	5	7-10
n_2/n_1	0.45	0.90	1.16-1.33
$\bar{m}_2$	1.67	2.20	2.68
$\bar{m}_1$	4.03	3.33	2.20

Solution Properties of the Polyampholyte

Experimental Methods

Light Scattering.—Measurements of the reduced intensity of scattered light, R_θ, were made at 25° with a modified Brice-Speiser light-scattering photometer using unpolarized light of wave length 4360 Å. The calibration of this instrument[22,23] and of the Erlenmeyer shaped cell[24] have been previously described. Solutions were prepared from polyampholyte P-7, dried to constant weight in an evacuated desiccator at room temperature, and made up to the desired volume in calibrated volumetric flasks with redistilled water. Solvent and solution were centrifuged at about 100,000 *g* for at least one hour in a Spinco preparative ultracentrifuge using cellulose nitrate tubes provided with specially constructed, plastic coated caps. Measurements were made on solutions obtained by adding a weighed amount of 0.5% polyampholyte solution to a known volume of clean solvent having the desired *p*H and contained in the Erlenmeyer-type cell. The contents were stirred magnetically after each addition. The concentration of at least one solution in the cell was determined from dry weight or refractive index increment measurements.

Refractive Index Increment.—The refractive index increment, dn/dc, which is required for the evaluation of the constant $K = 2\pi^2 n_0^2 (dn/dc)^2/(N_0\lambda^4)$ (n_0 being the refractive index of the solvent, λ the wave length of the incident light, N_0 Avogadro's number) was determined on a Brice-Speiser differential refractometer.[25] This was calibrated before each determination with a standard KCl solution, c = 10.8691 g./kg. H_2O, for which the increment was taken as 0.0015204 on the basis of an extrapolation of data given by Kruis.[26] The solutions of P-7 were made up from uncentrifuged stock solutions employed in light scattering. The technique of drying the polymer at room temperature for preparing solutions of known concentration was established as reliable to within 5 parts per thousand by comparing the concentrations so obtained with those calculated from the weight of the polymer upon drying under reduced pressure at 85°. The results are listed in Table III.

Viscosity.—Viscosity measurements were made in a modified Ubbelohde viscometer, provided with two bulbs which

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[20] C. C. Price and J. G. Walsh, *J. Polymer Sci.*, **6**, 239 (1951).

[21] J. G. Kirkwood and A. Westheimer, *J. Chem. Phys.*, **6**, 506 (1938).

[22] B. A. Brice, M. Halwer and R. Speiser, *J. Opt. Soc. Am.*, **40**, 768 (1950).

[23] P. Doty and R. F. Steiner, *J. Chem. Phys.*, **18**, 1211 (1950).

[24] P. Doty and B. H. Bunce, THIS JOURNAL, **74**, 5029 (1952).

[25] B. A. Brice and M. Halwer, *J. Opt. Soc. Am.*, **41**, 1033 (1951).

[26] A. Kruis, *Z. physik. Chem.*, **34B**, 13 (1936).

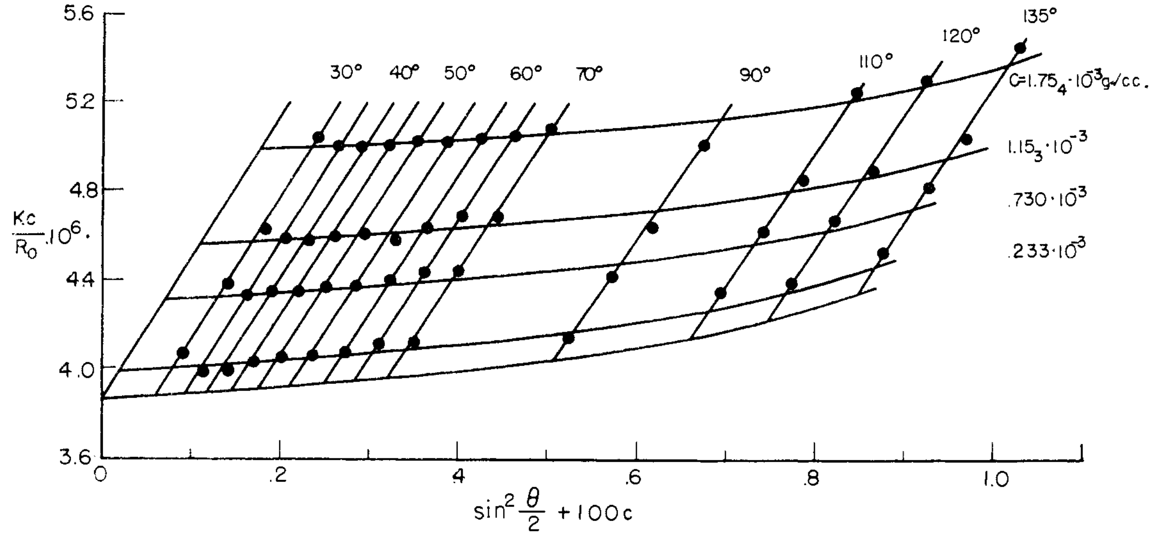

Fig. 3.—Reciprocal reduced intensity plot for isoionic P-7 in 0.10 *M* NaCl.

TABLE III

REFRACTIVE INDEX INCREMENTS OF P-7 AT 25°

λ = 4360 Å.

Concn. of P-7, g./cc. × 10³	Concn. NaCl g./cc. × 10³	*p*H	d*n*/d*c*	*K* × 10⁷
9.39	5.86	5.48	0.186	
4.57	5.86	. .	.193	
1.71	5.86	5.48	.189	5.72
4.57	0	5.61	.186	
3.55	0	5.60	.187	5.72
3.55	0	4.02	.188	5.78

permitted measurements at an average driving pressure of 23.3 and 9.33 g./cm.². At the isoionic point solutions of P-7 showed no gradient dependence. Because of a small dependence at other *p*H's the reduced viscosities were obtained from an extrapolation to zero driving pressure.[27] The efflux time for water (2 cc.) was 86.7 and 216.2 sec. at average velocity gradients of 1250 and 502 sec.⁻¹, respectively.

Titration.—The average net charge per polyampholyte molecule was calculated from *p*H measurements on isoionic solutions to which increasing amounts of either acid or base had been added.[28] All determinations were made at 25° in an atmosphere of nitrogen, using a Beckman *p*H meter (model G), equipped with external electrodes. Type E glass electrodes were used in the alkaline range.

Results

Isoionic Polyampholyte in 0.1 *M* NaCl Solution.—At this ionic strength the charges on the polyampholyte molecule should be sufficiently shielded locally to prevent significant electrostatic interactions. Consequently the solution behavior would be expected to approach that of a nonelectrolyte polymer. The light scattering results are shown in the form of a Zimm plot in Fig. 3. The data are seen to form the conventional grid with the extrapolated curves of zero angle and zero concentration having a common intercept on the ordinate corresponding to the reciprocal of the weight average molecular weight. From this intercept the molecular weight is found to be 258,000. On the basis of the previously given analytical data this indicates that the weight average chain consists of 2170 monomeric units (1.16 times as many acidic as basic) and if unbranched would have a contour length of 5470 Å. From the zero-angle line we find the second virial coefficient, *B*, to be 3.2×10^{-4} cc. moles/g.². From the zero concentration line one can calculate the radius of gyration and this can be translated into the characteristic dimensions of particular geometric forms, such as the root mean square end-to-end length for a Gaussian coil, $\sqrt{\overline{r^2}}$, given in Table IV.

TABLE IV

CHARACTERISTIC DIMENSIONS OF ISOIONIC P-7 IN 0.1 *M* NaCl

	Light scattering			Viscosity		
Model	Radius of gyration	$\sqrt{\overline{r^2}}$ coil	Radius sphere	r_F Flory-Fox	r_E Einstein	r Hard sphere
Dimension (Å.)	120	300	155	410	130	125

The viscosity measurements are shown in Fig. 4 from which the intrinsic viscosity is found to be 0.67, the *k*′ of Huggins' equation, 0.40. On the assumption that the hydrodynamic behavior of polymer molecules can be approximated by an equivalent sphere, the radius of which is proportional to the root mean square end-to-end length, $\sqrt{\overline{r^2}}$, which characterizes the Gaussian distribution of chain segments, Flory and Fox[29] have shown that the end-to-end length is related to the molecular weight and the intrinsic viscosity by the equation

$$(\overline{r^2})^{3/2} = [\eta]M/\Phi \qquad (2)$$

where Φ is a constant to which the value 2.1×10^{21} is assigned. Both the values of $\sqrt{\overline{r^2}}$ and Φ depend on the polydispersity. The dimension given in Table IV has been corrected for this as shown in appendix II. The radius of the equivalent Einstein spheres (r_E) corresponding to the observed viscosity also has been tabulated.

Still another estimate of the effective radius can be made if one assumes that there is no net inter-

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[27] H. T. Hall and R. M. Fuoss, THIS JOURNAL, **74**, 5029 (1952).

[28] J. T. Edsall in "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, Chap. 20.

[29] P. J. Flory and T. G. Fox, THIS JOURNAL, **73**, 1904 (1951).

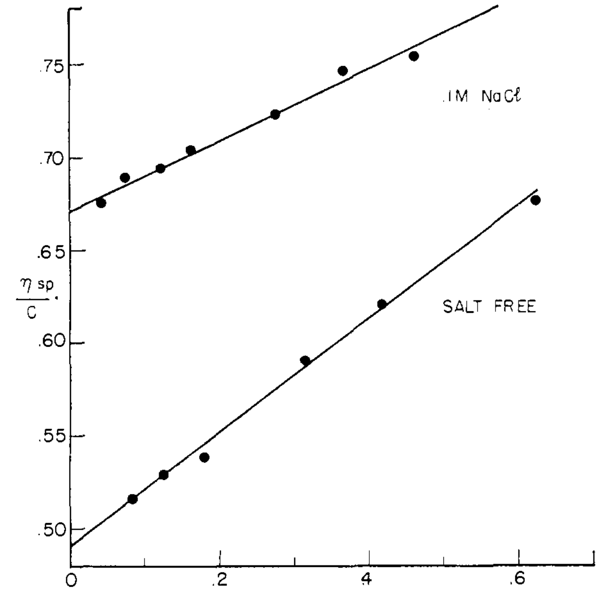

Fig. 4.—Reduced viscosity plot for isoionic P-7 without added electrolyte and in 0.10 M NaCl.

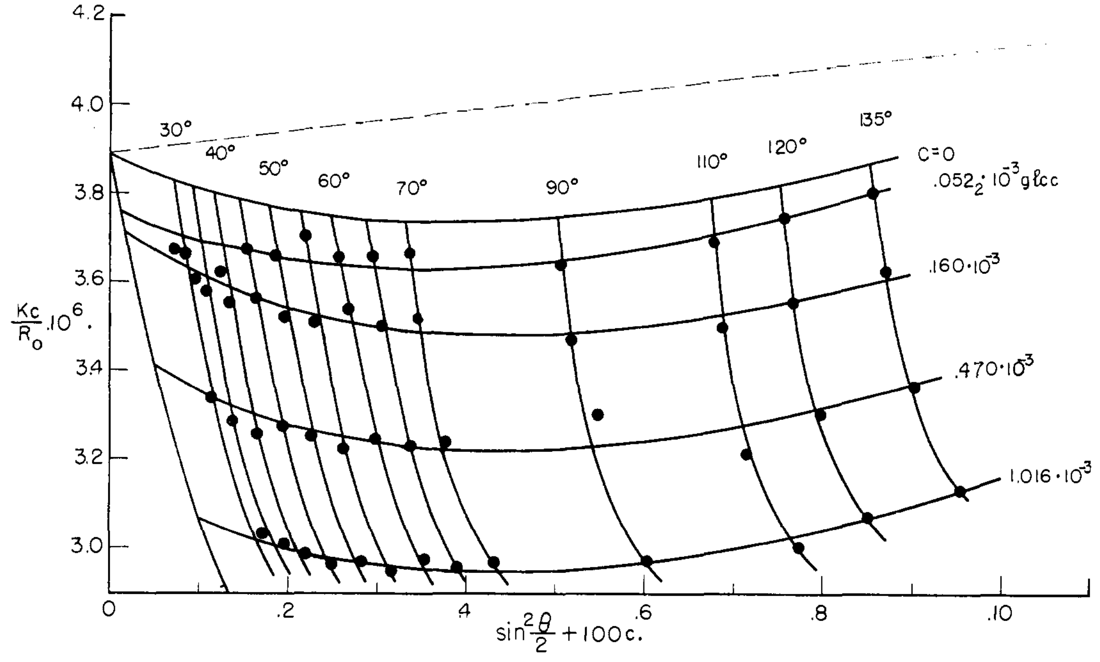

Fig. 5.—Reciprocal reduced intensity plot for isoionic P-7 in the absence of added electrolyte.

molecular attraction or repulsion. With this hard sphere approximation, the effective diameter is related to the second virial coefficient by means of $B = 2\pi/3(D^3N_0/M^2)$. With $B = 3.2 \times 10^{-4}$ a radius of 125 Å. is obtained.

We are unable to explain the upward curvature of the constant concentration curves (Fig. 3); even spheres of the required size would only account for half that observed.

Isoionic Polyampholytes in Water.—Upon removal of the screening electrolyte, the light-scattering behavior of the isoionic polyampholyte undergoes a remarkable change as can be seen from the Zimm plot shown in Fig. 5. The change is twofold. The second virial coefficient has become negative, -4.9×10^{-4}, and the reciprocal scattering envelopes, including the best extrapolation to zero concentration, show a negative limiting slope followed by an upward curvature. With this unusual result, it is obvious that the limiting slope cannot be related to the molecular dimension. The viscosity data (Fig. 4) show that the intrinsic viscosity has become still smaller and the value of k' has become considerably larger. This behavior, which indicates that the molecule has contracted and that the intermolecular attractions have increased, is considered in more detail below. The viscosity estimate of the size shows that the end-to-end length has decreased 10%.

Although the negative slopes of the zero concentration and zero angle extrapolations were invariably observed under these conditions, their absolute values were found to vary significantly from one experiment to another. An analogous phenomenon has been observed in the measurement of the osmotic pressure of isoionic protein solutions in the absence of salt[30] and was ascribed to contamination of the solution by contact with the atmosphere and glass containers.

***p*H and Ionic Strength Effects on Polyampholyte Behavior.**—Because of the poor reproducibility of the foregoing measurements, the effects of very small changes of *p*H in the absence of added salt were examined. Some results are shown in Fig. 6 where observations on single concentrations are plotted. The reciprocal envelopes marked 1 and 2 differ only in that for the first 28 moles of HCl was present per mole of polymer whereas 2 was isoionic. Similarly 3 and 4 show the effect when only 9 moles of HCl is added to the isoionic solution. Since there are about a thousand proton binding sites per poly-

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[30] G. Scatchard, A. C. Batchelder and A. Brown, THIS JOURNAL, **68**, 2320 (1946).

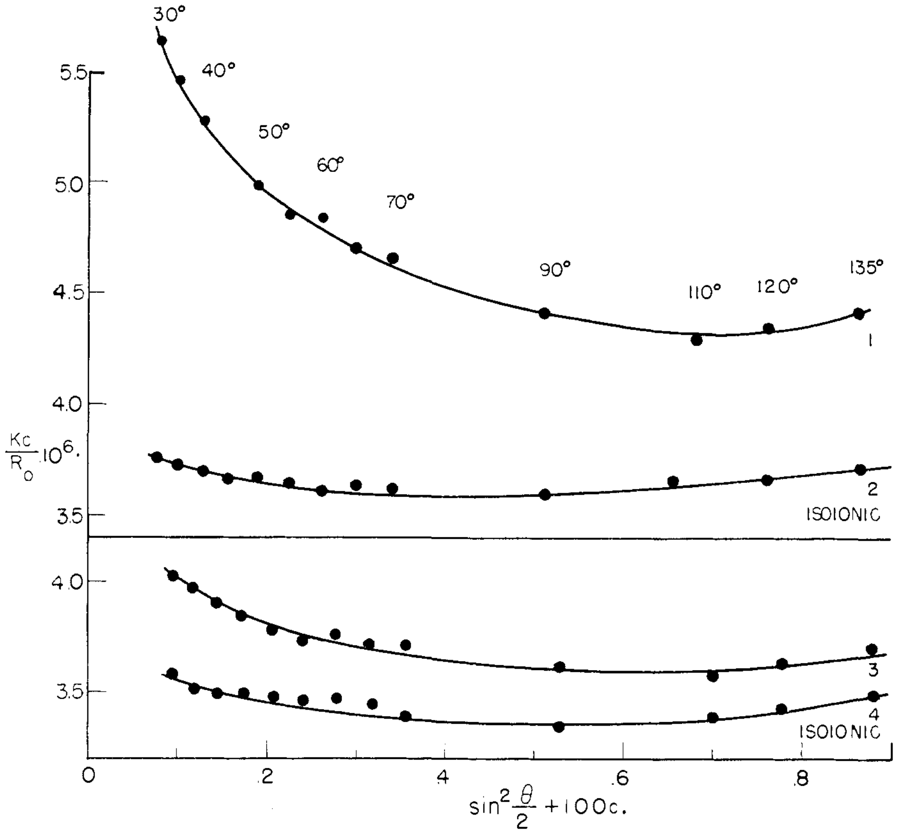

Fig. 6.—Reciprocal reduced intensity of the polyampholyte (P-7) solution as a function of added acid: curve 1, $c = 0.0766 \times 10^{-3}$ g./cc., 27.6 moles HCl/mole P-7; curve 2, $c = 0.0766 \times 10^{-3}$ g./cc., isoionic P-7; curve 3, $c = 0.245 \times 10^{-3}$ g./cc. 8.2 moles HCl/mole P-7; curve 4, $c = 0.245 \times 10^{-3}$ g./cc., isoionic P-7.

mer molecule, it is seen that extremely small shifts of the charge away from zero lead to pronounced changes in which the initial slope becomes even more negative. The over-all diminution in scattering upon charging is not great and is that expected for small repulsive interactions. Increasingly negative slopes and lower intensities occur upon the addition of more acid as shown in Fig. 7. Comparable changes are found (Fig. 8) upon the addition of base. It appears that maxima may exist in the reciprocal scattering envelopes at low angles; these may arise from intramolecular interference, but the low level of scattering in this region has so far prevented further examination.

The effect of adding electrolyte to the aqueous isoionic solution was much less marked than that brought about by acid or base. Typical results are shown in Fig. 9. The decrease in scattering corresponds to an increase in the second virial coefficient. The envelope at 0.027 *M* NaCl is the same as that found at 0.1 *M* showing that when the ionic strength has reached this level no further intermolecular or intramolecular changes occur, except possibly at very high ionic strength where salting out may set in.

Returning to the behavior upon the addition of acid to aqueous solutions, we can examine the extent to which the viscosity behavior is like that for polyacids such as polymethacrylic acid. The concentration dependence of the specific viscosity when 250 moles of HCl per mole of polyampholyte are present is plotted in Fig. 10 in the manner suggested by Fuoss. For comparison, data for polymethacrylic acid having the same charge but about half the molecular weight are also shown.[4] The behavior in the two cases is very similar. Assuming that the linear extrapolation is valid and that the Flory–Fox equation can be applied, one finds that the intrinsic viscosity of 28.5 for the polyampholyte corresponds to $\sqrt{\overline{r^2}} = 1530$ Å., that is a fourfold expansion. This is very similar to polymethacrylic acid where the expansion at the same charge is fivefold. The decrease in scattering shown in Fig. 6 is actually considerably greater than in the corresponding case for polymethacrylic acid but is comparable with current results on sodium carboxymethylcellulose.[31]

Discussion

At the isoionic point, solutions of the polyampholyte examined present a unique situation; although on the average electrically neutral, the individual molecules carry a large number of positive and negative charges,[32] and we can expect that interactions between these will significantly affect the solution behavior of the polyampholyte molecules. The polymer segments, constrained to a small volume, will seek to minimize the free energy by taking up positions in which segments of unlike charge alternate: this will be opposed by the Brownian motion of the chain and the point of equilibrium will be decided by the ionic strength due to added electrolyte. Another consequence of electrostatic attractions may be an ordering of the polymer molecules themselves. The maximum deviations from the interactions and the average configuration of a non-electrolyte polymer should, of course, occur when no added electrolyte is present, but it will be of interest to examine the results found in 0.1 *M* NaCl, as well as for the charged polyampholyte, with this question in mind. In the following we will discuss separately the effects of electrostatic attractions as they manifest themselves in (1) the configuration of the isoionic polymer, (2) the interaction of the isoionic polymer, and

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(31) N. Schneider, private communication.

(32) Estimates of the total charge at the isoionic point have been made by G. Ehrlich, THIS JOURNAL, in press.

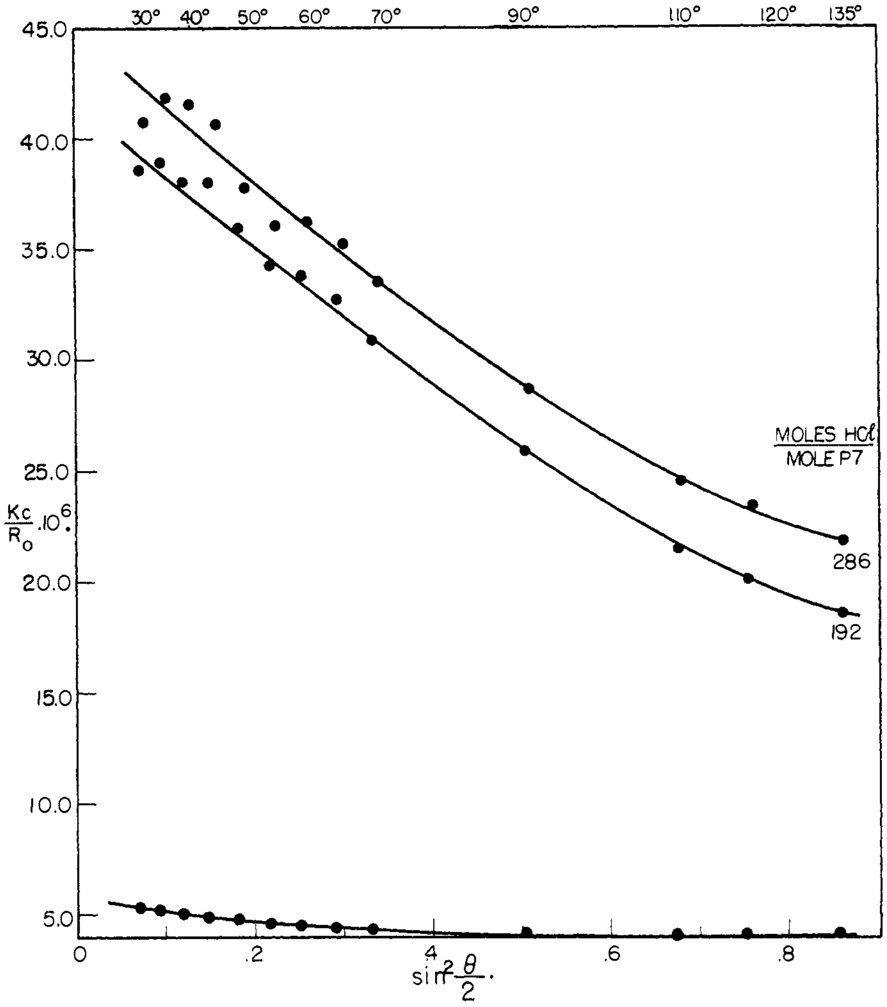

Fig. 7.—Reciprocal reduced intensity of isoionic P-7 as a function of the concentration of added HCl; $c = 0.199 \times 10^{-3}$ g./cc.

(3) the solution behavior of the charged polymer. Inasmuch as our conclusions are based primarily upon scattering data, some particular aspects of the theory of light scattering from non-ideal solutions, essential in the discussion, are developed in Appendix 1.

Molecular Size and Intramolecular Interactions of Isoionic Polyampholyte.—Consideration of the dimensions in Table IV shows that the polyampholyte in 0.1 M NaCl is somewhat *smaller* than would be expected if electrostatic effects were absent. Its effective bond length, $\sqrt{\overline{r^2}}/N$, assuming $M_z/M_w = 1.5$ is 5.3 Å. This is about 30% less than that of polystyrene in toluene where the second virial coefficient is the same, or in sodium polymethacrylate at high ionic strength. The difference is not as great if the value of $\sqrt{\overline{r^2}}$ calculated from viscosity is used and in either case the contribution which could be due to electrostatic attractions is small. It should be noted, however, that the discrepancy between the dimension obtained from light scattering and the Flory–Fox relation is, as shown in Appendix II, opposite to that to be expected on the basis of polydispersity.

The other measurements of size given in Table IV are useful in estimating the extent of interpenetration of these polyions. The radius of the equivalent hard sphere is seen to be almost equal to the radius of gyration instead of being about 60% thereof as found for polystyrene under comparable conditions.[33] Moreover, the value of k' is that associated with a thermodynamically good solvent. Hence it appears that there is no net attraction between the poly-ions under these conditions and consequently the interpenetration in dilute solution is negligible. Indeed the intrinsic viscosity is just that expected of impenetrable spheres of the same radius as found for the radius of gyration of the equivalent hard spheres.

The effects of electrostatic attractions, marginal in 0.1 M NaCl, would be expected to assume dominant importance in the absence of added electrolyte. The change in the viscosity (Fig. 4) is in the direction expected—smaller intrinsic viscosity and larger k'—but is less than overwhelming. Indeed, the question immediately arises as to why the contraction was not greater than the 10% (in linear dimension) observed, especially when one notes that the radius would be reduced to about 25 Å. if the polymer coiled up on itself completely. We suggest that the reason that further contraction does not occur is the net charges on the polyampholyte ions. Even though the average charge at the isoionic point is negligible (one electron per polymer), a substantial distribution of charge about this average value would be expected on the one hand due to fluctuations of charge and on the other to the heterogeneity of composition. Fortunately, the deviations from the average net charge arising from both of these factors can be estimated from the titration curve of the polyampholyte using the relation[29]

$$\left(\frac{\partial z}{\partial\, pH}\right)_I = -2.30\,(\overline{\Delta z^2}) \qquad (3)$$

in which z is the net proton charge per molecule, I the ionic strength, and $(\overline{\Delta z^2})$ the standard deviation of the net charge. From the titration curves in Fig. 1 this standard deviation is found to be 40 in the salt free solution and 75 in 0.1 M NaCl. This means, for example, that if the distribution of

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(33) P. Doty and R. F. Steiner, *J. Polymer Sci.*, **5**, 383 (1950).

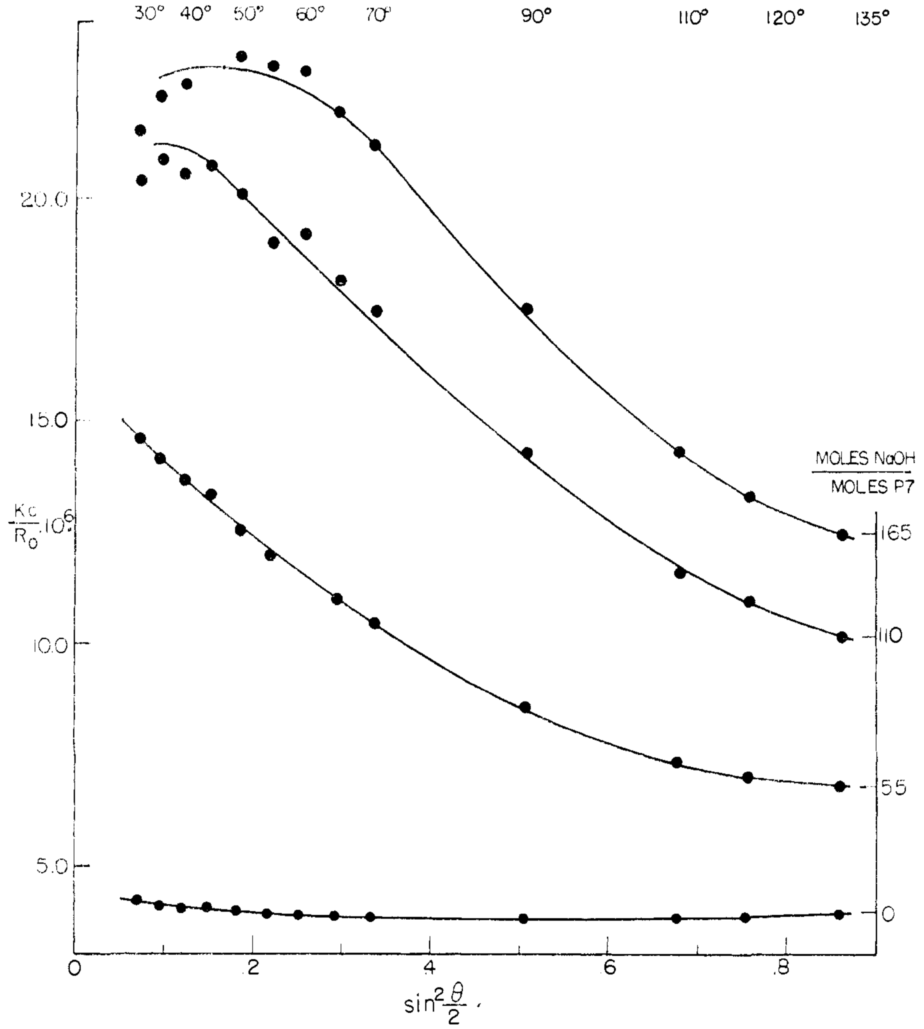

Fig. 8.—Reciprocal reduced intensity of isoionic P-7 as a function of the concentration of added NaOH; $c = 0.128 \times 10^{-3}$ g./cc.

charges over the individual polyions is Gaussian, 5% of the molecules at any one time have a net charge greater than 12 (actually -1 ± 12) and 32% have a charge greater than 6. From this it is clear that each polyion spends most of its time with a significant net charge which will cause an expansion that should partially compensate the contraction expected upon lowering the ionic strength. Furthermore, the polyions may become more highly charged in the course of the experiment since, despite considerable care, the pH of these unbuffered solutions may drift slightly from the isoionic point. These then appear to be sufficient reasons for the isoionic polyion not assuming a smaller size in salt-free solution.

It is interesting to examine the extent to which electrostatic interactions due to the presence of positively and negatively charged groups manifest themselves in the electrochemical behavior of the isoionic polyampholyte. In the absence of interactions between ionizing groups the isoionic points in Table I, together with the composition ratios, n_2/n_1, permit an evaluation of the pK of the dimethylamino group in the polymer. The relation employed is

$$\frac{k_1}{[\mathrm{H}^+] + k_1} + \frac{(n_2/n_1)k_2}{[\mathrm{H}^+] + k_2} = 1 \quad (4)$$

where k_2 is taken to be 1.4×10^{-5}. The best fit appears to be obtained for $k_1 = 6.0 \times 10^{-9}$ ($pK_1 = 8.22$) as shown in Fig. 11; four of the experimental points fall on the curve and two deviate by about 0.2 pH unit. It is surprising that the amine pK is more than one pH unit lower than that of 2-diethylaminoethyl methacrylate which is reported to be 10.04[7]; the replacement of ethyl by methyl would be expected to lower this only about 0.5 pH unit on the basis of the comparison of diethyl and dimethylbenzyl amine. Also, the discrepancy between calculated and observed results appears to exceed the probable error for the acidic polymers, in which, as was shown above, the methacrylic acid residues appear in groups. Since inductive effects are ruled out by the similarity of the ester and carboxyl groups, it appears that although the average net charge is essentially zero the average electric field in the isoionic polyampholyte acting on a proton may *not* be zero.

Intermolecular Interaction of Isoionic Polyampholyte Molecules.—It is clear from the previous section that, despite the instantaneous charges of both signs carried by the polyampholyte molecules in 0.1 *M* NaCl, the shielding of the polymer charges by the electrolyte is apparently sufficient to produce normal behavior with respect to solute interactions. In the absence of added electrolyte, on the other hand, the consequences of electrostatic attractions should be very marked and we wish to see if this can account for the two principal observations under these conditions: the negative second virial coefficient (more properly, the negative initial slope of Kc/R_0 with concentration) and the unusual shape of the reciprocal scattering envelopes (Fig. 5). The latter is a striking indication that the mutual interaction of the solute species, leading to ordering, persists even at the lowest concentrations at which measurements were performed, thereby preventing a determination of the configuration of the polymer through scattering experiments. From the viscosity data, however,

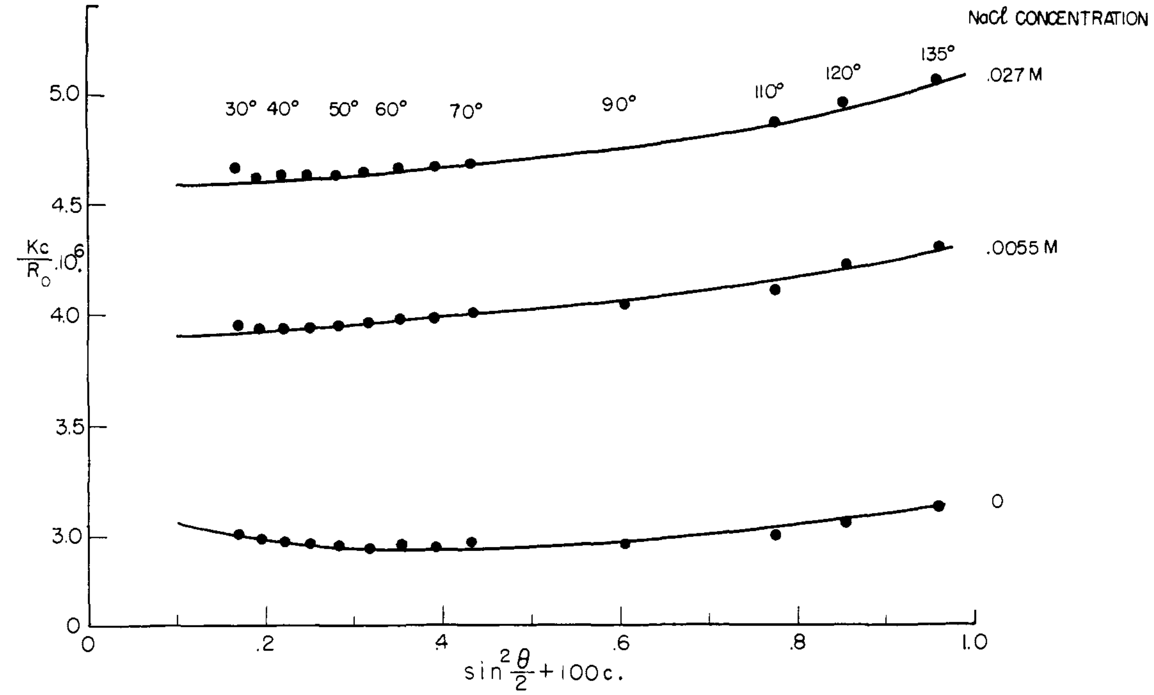

Fig. 9.—Reciprocal reduced intensity of isoionic P-7 as a function of the concentration of added NaCl; $c = 0.101 \times 10^{-3}$ g./cc.

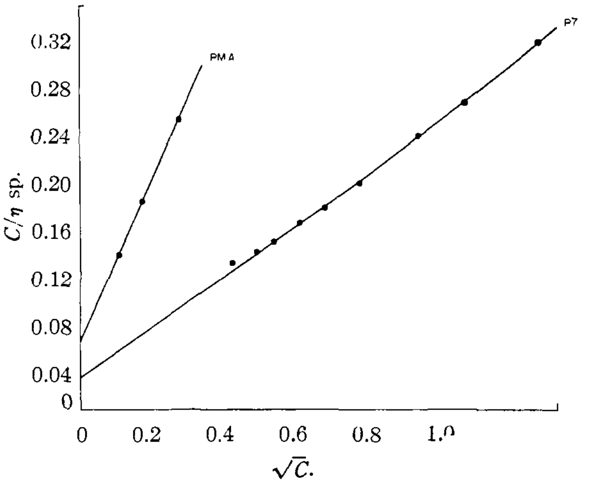

Fig. 10.—Reciprocal reduced viscosity plot for polymethacrylic acid[8] and P-7 at net charge of 250; PMA, $[\eta] = 18.5$; P-7, $[\eta] = 38.5$; concentration in g./100 cc.

we deduce, on the assumption of a Gaussian polymer chain, an end-to-end length of 270 Å., which would lead, in the limit of infinite dilution, to a plot of Kc/R linear in $\sin^2 \theta/2$ and having a positive slope, as indicated by the dashed line in Fig. 5. Hence the curved Kc/R_θ lines must be considered to arise from the superposition of two effects: the variation with angle of the reciprocal scattering factor due to molecular size (the dashed line) and of the external interference due to pronounced local ordering of the solute molecules. A quantitative resolution of these effects is not yet feasible, but because the polyampholyte molecules are relatively small, a qualitative discussion is possible.

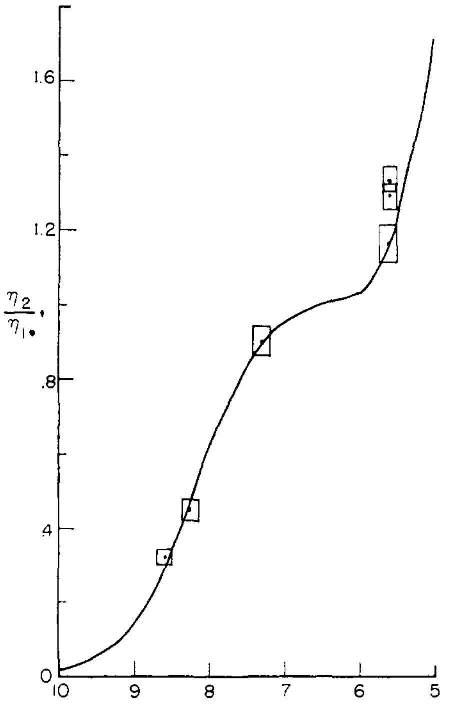

Fig. 11.—Mole ratio of acidic to basic constituents as a function of the pH of the isoionic point.

It follows from equation A7 that to satisfy the requirement of a negative virial coefficient it is necessary that

$$\int_0^\infty [g(R) - 1]R^2\, dR > 0$$

and consequently $g(R)$ must be larger than unity at distances of close approach. In addition the initially negative slope of the reciprocal scattering envelopes requires by virtue of equation A8 that

$$\int_0^\infty [g(R) - 1]R^4\,\mathrm{d}R < 0$$

This condition can only be simply met if $g(R)$ has a minimum at distances of close approach. Thus the simplest form of $g(R)$ that can meet these two conditions is one which, beginning at low values of R, passes first through a maximum and then decends to a pronounced minimum from which it rises to its limiting value of unity at large distances. Although this distribution resembles that for hard spheres, it must, in order to satisfy the first condition, have a maximum that is more pronounced. A distribution function of this form corresponds to a considerable degree of order in the arrangement of solute molecules and may be interpreted to mean that loose clusters of polyampholyte ions have a transient existence. We can imagine that aggregates form and dissociate constantly but, unlike the association of non-electrolyte polymers in poor solvents (negative second virial coefficients), where almost complete interpenetration occurs in the associating molecules, we appear in this case to have only clustering without interpenetration. Inasmuch as at the isoionic point both positively and negatively charged molecules have a transitory existence, we surmise that the clustering observed is a manifestation of the tendency of polyions of one charge to be surrounded by those of the opposite charge in much the same fashion as it occurs in an ordinary electrolyte solution. In general, ordering of the macromolecules in a solution occurs because of intermolecular repulsions, which may or may not be electrostatic in origin; it now appears that the same general effect can be produced by a net attraction when polyions of both charge types are present.

The forces between isoionic protein molecules have been discussed recently by Kirkwood and Schumaker,[34] who developed the point of view that fluctuations in the charge and charge distribution of protein molecules at the isoionic point may give rise to intermolecular attraction. For their purposes, they characterize the proteins as hard spheres, interacting beyond the region of overlap of the excluded volumes only through electrostatic forces, which are taken into account by a potential of average force of the form

$$W(R) = -\frac{(\overline{\Delta z^2})^2 e^4}{2\epsilon^2 kT}\,\frac{\exp[-2\kappa(R - D)]}{R^2(1 + \kappa D)^2} \tag{5}$$

Here ϵ is the dielectric constant, e the charge on the electron, and D the hard sphere diameter which enters through the assumption that the potential between spherical particles of diameter D is modified, in the presence of small ions, by a screening factor

$$\exp[-2\kappa(R - D)]/(1 + \kappa D)^2 \tag{6}$$

κ being the familiar reciprocal distance of Debye–Hückel theory. Since the distribution function is given by $g(R) = \exp(-W/kT)$, we can evaluate the scattering from a system of particles subject to this potential from equation A1. Although integration in closed form is not possible, series expansion leads to the scattering equation

$$K\frac{c}{R_\theta} = \frac{1}{MP(\theta)}\left\{ 1 + 2c\left[\frac{B'}{M} + \frac{\pi A N_0}{M\kappa(1 + \kappa D)^2} - \frac{\mu^2}{2}\left(\frac{B'}{2}\,\frac{D^5}{5} + \frac{\pi A N_0}{3M\kappa(1 + \kappa D)^2}\left\{D^2 + \frac{2(\kappa D + 1)}{2\kappa^2}\right\}\right)\right] + \ldots\right\} \tag{7}$$

in which

$$A = \frac{-(\overline{\Delta z^2})^2 e^4}{2\epsilon^2 k^2 T^2} \qquad B' = \frac{2}{3}\pi D^3 N_0$$

This expression correctly predicts a negative second virial coefficient, but upon evaluation it turns out to be -700×10^{-4}, a hundred times that observed. This discrepancy may arise from two sources: one is that the polymer molecules differ greatly from the system of hard spheres for which the equation was derived; in particular the flexibility of the polymer molecule may attenuate the attraction by local rearrangements near the points of intermolecular contact. Second, contamination of the isoionic solution by trace amounts of electrolytes may screen electrostatic attractions. Moreover, inspection of equation 7 shows that if the second term inside the square brackets is sufficiently negative to produce a negative second virial coefficient then the coefficient of μ^2 will be negative as well with the result that the plot of Kc/R_θ *versus* $\sin^2\theta/2$ will have a positive slope. Hence we do *not* find here any explanation of the observed angular dependence of scattering; the only prior indication of this type of scattering behavior has been in salt-free solutions of highly charged macro-ions[3,4] where only dissymmetry measurements were made. In these cases the behavior (dissymmetries less than unity) was shown to be due to the ordering caused by the mutual repulsion of the charged macro-ions, serum albumin and sodium polymethacrylate.

The calculation of the alteration of the angular dependence of the intensity of scattering due to external interference requires the specification of a radial distribution function for the scattering centers; Fournet[35] has implemented the Born–Green theory to calculate the angular dependence of the scattered intensity for the case of dense systems of hard spheres, with the aim of explaining the low angle X-ray scattering from gaseous and liquid inert gases. One specific case treated by him corresponds to a concentration of 0.24 volume per cent. and, since the constant concentration lines in Fig. 5 do not alter greatly with concentration, this can be used for a direct comparison with the data for our highest concentration. If a line with the slope of the dashed curve of Fig. 5 (the reciprocal scattering factor assumed for the particle at infinite dilution) is drawn through the zero angle end of the lowest constant concentration curve and the difference between these two multiplied by M, the data can be plotted as KcM/R_θ against $\mu^2 D^2$, and compared directly with Fournet's calculation. Quite good agreement is obtained if D, the hard

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[34] J. G. Kirkwood and J. S. Schumaker, *Proc. Natl. Acad. Sci. U. S.*, **38**, 863 (1952).

[35] G. Fournet, *Compt. rend.*, **228**, 1421 (1949); *J. phys. radium*, **11**, 516 (1950).

sphere diameter, is set equal to 135 Å., that is, half the end-to-end distance. This is exactly the relation between the end-to-end length and the effective hard sphere diameter found for polystyrene in toluene[33] and the similarity to the present cases argues from a different point of view that the interpenetration here is slight, being comparable to that of a non-electrolyte polymer in a thermodynamically good solvent.

We are thus in a position, on the one hand, of having attributed the negative second virial coefficient to a net attraction among polyions bearing positive and negative charges and, on the other hand, of having shown that the angular dependence is the result of a non-random distribution similar to that which is exhibited by hard spheres, of about the same size and concentration as the polyampholyte molecules. We can see why *both* results were not explained by either point of view. In the Kirkwood–Schumaker theory the distribution curve assumed was that for hard spheres at infinite dilution (a simple step function) as modified by the attractive potential of equation 5, which causes the distribution function to decrease monotonically from the hard sphere diameter outward; this distribution does not satisfy the requirement of having a substantial minimum following the first maximum and cannot give the observed dependence upon scattering angle. Fournet's treatment, however, does make use of the proper hard sphere distribution function at each concentration, but it does not, of course, have superimposed the net electrostatic attraction which must considerably increase the first maximum and is responsible for the clustering of the polyampholyte molecules, that is, to the negative virial coefficient. There is little doubt that the alteration of the hard sphere distribution at finite concentrations so as to accentuate the first maximum and first minimum would, upon following through Fournet's treatment, lead to a concentration dependence as well as an angular dependence similar to that observed to give a quantitative account of the effects of intermolecular attractions at the isoionic point.

Charged Polyampholyte in Salt-free Solution.—Although the data of Figs. 7 and 8 are very limited, they represent the first determination of the angular dependence of the scattering from salt-free solutions of highly charged polymer molecules and it is of interest to see if this is in accord with expectation. We note that at a charge per molecule of about 200, the value of R_0 has become about one-tenth of that for the isoionic polymer, and Kc/R_θ decreases by a factor of two between 0 and 180°. Again the only guide is the hard sphere model calculation, which in this case is certain to yield the proper sign for the concentration dependence, since the intermolecular repulsion which is so dominant here will manifest itself as an increase in the effective hard sphere diameter, which we can treat as an adjustable parameter. Assigning to it a value of 1090 Å., one obtains a volume concentration of 15.4% for $c = 10^{-4}$ g./cc. (the approximate concentration used in the measurements), which corresponds to a ratio of the hard sphere diameter to that of the volume per molecule of $^2/_3$. In Fournet's terminology this is the ratio *k*. The curve calculated by Fournet for this case shows a 9-fold reduction in R_0 over the ideal solution. Although the value of the diameter is about equal to the end-to-end length (as estimated from the reduced specific viscosity at this concentration) rather than half of this value, the difference is a reasonable contribution to expect from the repulsion mentioned above. However, the angular dependence is much greater than that actually observed. The calculated value of R_θ drops to one-half of R_0 when θ is only 18°. Conversely if one attempts to fit the angular dependence, the value of R_0 is reduced by only 1.5 rather than a factor of 10. This discrepancy is in the direction of that expected for spheres that are soft rather than hard. The hard sphere model did not show this effect in the isoionic solution, since there the polymer molecules were relatively compact, but it is to be expected in the present case where the polymer molecules are so much more extended. Hence on the basis of limited angular data and comparison with a very oversimplified model for charged chain molecules, we conclude that such solutions scatter in the fashion expected for highly extended strongly repelling polymeric electrolyte molecules.

Acknowledgments.—The titration curves reported above were determined by Mr. Rostislav Didchenko on a Shell Research Assistantship; part of the expenses of this program was covered by grants from the Mallinckrodt Research Fund and the Research Corporation.

Appendix I

Light Scattering and the Distribution Function of Scattering Elements.—The intensity of the radiation scattered from a beam of unpolarized light incident upon a two-component system as a function of angle, θ, in excess of the solvent scattering, can be related to the concentration c (in grams per cc.) and the molecular weight M of the solute by the equation

$$R^{\theta} = KcMP(\theta)\left[1 + 4\pi\frac{N}{V}\int_0^{\infty}[g(R) - 1]R^2\,\frac{\sin \mu R}{\mu R}\,\mathrm{d}R\right] \tag{A1}$$

valid for scattering centers of spherical symmetry, in which the particle scattering factor $P(\theta)$ accounts for the destructive interference between the segments of the same molecule and the bracketed quantity gives the diminution in scattering intensity due to deviations from a random arrangement of solute molecules. The arrangement of the solute is characterized by the radial distribution function, $g(R)$, which defines the probability of finding two specified molecules a distance R apart. The quantity $\mu = (4\pi/\lambda')\sin(\theta/2)$, where λ' is the wave length of light in the medium, and N denotes the number of solute molecules in volume V.

In the case of ordinary polymers or polyelectrolytes at high ionic strength, long range forces are absent. This is the usual situation and it is generally treated in terms of the relation developed by Zimm,[36] using a single contact approximation, which permits the ready determination of the par-

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(36) B. H. Zimm, *J. Chem. Phys.*, **16**, 1099 (1948).

ticle scattering factor, $P(\theta)$. Theoretically this quantity is given by

$$P(\theta) = \int_0^\infty \rho(r)\frac{\sin \mu r}{\mu r}\,\mathrm{d}r = \frac{R_\theta}{[KcM]_0} \quad \text{(A2)}$$

in which $\rho(r)$ is the distribution function for the frequency of occurrence of the distance r between pairs of scattering elements in the same solute molecule. The particle scattering factor, $P(\theta)$, having been determined, can be used to obtain information about the distribution function $\rho(r)$ and hence about the size and shape of the solute molecule in two ways. One of these involves the evaluation of the Fourier transform of equation (A2) which gives $\rho(r)$ directly

$$\rho(r) = \frac{2r}{\pi}\int_0^\infty \mu\left[\frac{R_\theta}{KcM}\right]_0 \sin \mu r\,\mathrm{d}\mu \quad \text{(A3)}$$

However, the values of θ for which R_θ is required are experimentally accessible only when the radius of gyration of the scattering particle is comparable to the wave length λ'. For smaller particles, that is, in the range of ordinary polymer molecules, the best that can be done is to expand $(\sin \mu r)/\mu r$ in a power series obtaining

$$\frac{Kc}{R_\theta} = \frac{1}{M}\left[1 - \frac{\mu^2}{3!}\int_0^\infty r^2\,\rho(r)\,\mathrm{d}r + \frac{\mu^4}{5!}\int_0^\infty r^4(r)\,\mathrm{d}r \ldots\right]^{-1} \quad \text{(A4)}$$

From this it is seen that a plot of Kc/R_θ against μ^2 will produce a curve whose initial slope is simply related to the radius of gyration and hence the characteristic dimensions of particles of simple geometrical shape. The value of the last term in equation (A4) has recently been evaluated for several types of particles.[37] This summary of operations related to $P(\theta)$ is of interest in the present case because an analogous development related to $g(R)$ can be made and is useful in interpreting the scattering envelopes found in the foregoing experiments at low ionic strength.

When long range forces are operative the dependence of scattering intensity upon angle will be greatly affected by the interference arising between scattering from different molecules. *Let us assume that the scattering particles are small so that $P(\theta)$ is unity.* Then by performing a Fourier inversion upon equation (A1), we can express the radial distribution function of the solute molecules, which dictates the external interference, in terms of the intensity of the scattered radiation. In analogy with equation (A3)

$$g(R) = 1 + \frac{1}{2\pi^2 R}\frac{M}{cN_0}\int_0^\infty \mu\left[\frac{R_\theta}{KcM} - 1\right]\sin \mu R\,\mathrm{d}\mu \quad \text{(A5)}$$

$g(R)$ can be evaluated by numerical integration only if R_θ can be accurately extrapolated to $\mu = \infty$ from the experimental scattering data, that is only for solutions in which ordering persists over regions equal to the wave length of light. However, even when this condition is not satisfied, information concerning the form of the radial distribution function can still be obtained by a procedure similar to that already developed in the discussion of internal interference effects.

Expanding the term $(\sin \mu R)/\mu R$ in equation (A1), we obtain for dilute solutions the expression

$$Kc/R_\theta = 1/M\left\{1 - \frac{4\pi c N_0}{M}\int_0^\infty [g(R) - 1]R^2\mathrm{d}R + \frac{4\pi c N_0}{M}\frac{\mu^2}{3!}\int_0^\infty [g(R) - 1]R^4\mathrm{d}R \ldots\right\} \quad \text{(A6)}$$

As in the first case, the slope of Kc/R_θ against concentration in the limit of zero scattering angle and infinite dilution yields the second virial coefficient, B, which is related to the distribution of the solute molecules at $c = 0$, $g^0(R)$, through

$$B = \frac{-2\pi N_0}{M^2}\int_0^\infty [g^0(R) - 1]R^2\,\mathrm{d}R$$

The initial slope of the reduced scattering intensity as a function of $\sin^2 \theta/2$ is also dependent upon the arrangement of solute molecules, however, and thus establishes an additional condition upon the form of the radial distribution function. Thus

$$\left[\frac{\partial(Kc/R_\theta)}{\partial c}\right]_{\substack{\theta = 0\\ c = 0}} = \frac{-4\pi N_0}{M^2}\int_0^\infty [g(R) - 1]R^2\,\mathrm{d}R \quad \text{(A7)}$$

$$\left[\frac{\partial(Kc/R_\theta)}{\partial \sin^2 \frac{\theta}{2}}\right]_{\substack{\theta = 0\\ c = 0}} = \frac{4\pi N_0 c}{M^2}\left(\frac{8\pi^2}{3\lambda^2}\right)\int_0^\infty [g(R) - 1]R^4\,\mathrm{d}R \quad \text{(A8)}$$

Although these conditions do not yield the distribution function, they provide sufficient information to deduce its general form.

From a broader point of view, it is of interest to note that as a consequence of equations (A7) and (A8) the initial slope of Kc/R_θ against concentration may depend on θ. For this to occur the value of the derivative in equation (A8) would have to have non-zero values. This in turn would require long range forces to be operative, and hence the absence of this type of angular dependence in measurements on neutral polymers is understood.

Appendix II

Size Determination on Polydisperse Systems. Light Scattering.—For a very dilute solution of identical particles the particle scattering factor can be expanded to give

$$P(\theta) = 1 - C\rho^2 \sin^2\frac{\theta}{2} + \ldots \quad \text{(A9)}$$

where C is a constant dependent upon the wave length and refractive index and ρ^2 is the square of the radius of gyration. When dealing with a distribution of particle sizes characterized by the weight fraction distribution function, $f(M)\,\mathrm{d}M$, we have

$$P(\theta) = \frac{\int_0^\infty P_M(\theta)Mf(M)\,\mathrm{d}M}{\bar{M}_w} \quad \text{(A10)}$$

where $\bar{M}_w$ is the weight average molecular weight, $\int_0^\infty Mf(M)\,\mathrm{d}M$. Expanding each $P_M(\theta)$ as in (A9), one obtains

$$P(\theta) = 1 - C\sin^2\frac{\theta}{2}\frac{\int_0^\infty Mf(M)\rho^2\,\mathrm{d}M}{\bar{M}_w} \quad \text{(A11)}$$

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[37] H. Benoit and P. Doty, *J. Phys. Chem.*, **57**, 958 (1953).

A comparison of (A9) and (A11) shows that

$$\overline{\rho^2} = \frac{\int_0^\infty Mf(M)\rho^2\,\mathrm{d}M}{\bar{M}_\mathrm{w}} \tag{A12}$$

Thus the dependence of the experimentally determined mean square radius of gyration on the molecular weight is rather complicated since it involves the relation of ρ to M. Only in the case of a random coil do we have a simple result as earlier found by Zimm.[36] In this case ρ^2 is proportional to M and it is clear that the size is that of the molecular species having the *z*-average molecular weight, thus

$$\bar{M}_\rho = \frac{\int M^2f(M)\,\mathrm{d}M}{\bar{M}_\mathrm{w}} = M_z \tag{A13}$$

For spheres, $\rho^2 \sim M^{2/3}$ and we have

$$\bar{M}_\rho = \left[\frac{\int_0^\infty M^{5/3}f(M)\,\mathrm{d}M}{\bar{M}_\mathrm{w}}\right]^{3/2} < M_z \tag{A14}$$

For rods, $\rho^2 \sim M^2$ and

$$\bar{M}_\rho = \left[\frac{\int_0^\infty {}_0\, M^3f(M)\,\mathrm{d}M}{\bar{M}_\mathrm{w}}\right]^{1/2} > M_z \tag{A15}$$

The molecular species to which these values refer will be smaller than the *z*-average for spheres and larger than the *z*-average for rods. It is apparent from equation A12, however, that the dimension found in light scattering is obtained by the *same* averaging process regardless of the particle shape and is by definition equal to the square root of the *z*-average of the square of the dimension of the individual scattering centers.

Viscosity.—Writing the Flory–Fox relation in the form

$$\eta_\mathrm{sp} = \Phi(\overline{r^2})^{3/2}\frac{c}{M} \tag{A16}$$

it follows that, for a polydisperse system in which the fraction of molecules with molecular weights between $M + \mathrm{d}M$ is given by $h(M)\,\mathrm{d}M$

$$[\eta] = \frac{\eta_\mathrm{sp}}{\int_0^\infty Mh(M)\,\mathrm{d}M} = \frac{\Phi\int_0^\infty (\overline{r^2})^{3/2}\,h(M)\,\mathrm{d}M}{\int_0^\infty Mh(M)\,\mathrm{d}M} \tag{A17}$$

That is, if we introduce the number average molecular weight in the Flory–Fox relation, we obtain the number average of $(\overline{r^2})^{3/2}$. If, as is more usual, the molecular weight is determined by light scattering, then we obtain by the use of this relation the number average of $(\overline{r^2})^{3/2}$, multiplied by the ratio of $M_\mathrm{w}/M_\mathrm{n}$.

Thus the dimensions calculated from angular light scattering measurements and listed in Table IV represent $\sqrt{\overline{L_z^2}}$. Those from the viscosity were obtained on the assumption that the value of Φ given by Flory is only negligibly in error due to the polydispersity of the original polymer solutions employed in the estimation of this parameter.[38] The average dimension tabulated, $\sqrt[3]{\overline{(\overline{r^2})_n{}^{3/2}}}$, has been obtained by assuming $M_\mathrm{w}/M_\mathrm{n} = 1.2$.

[38] S. Newman, W. R. Krigbaum, C. Langier and P. J. Flory, Abstracts, A.C.S. Meeting 124, Chicago, September, 1953.

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[CONTRIBUTION FROM THE WESTERN UTILIZATION RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE]

Reaction-Inactivation of Polyphenoloxidase: Temperature Dependence[1]

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Measurements have been made at several temperatures of (a) the initial rates of the polyphenol-catalyzed aerobic oxidation of catechol and (b) the amounts of substrate oxidized when the reaction stops as a result of concurrent inactivation of the enzyme. Two energies of activation were calculated from these data: (a) *B* for the rate of the enzyme inactivation reaction and (b) *A* for the rate of the oxidation. The value for *A* is about the same order of magnitude as those obtained for other oxidative enzymes. The low value found for *B* suggests that reaction-inactivation is a fairly simple reaction and involves no extensive damage to the protein such as denaturation or dissociation. A mechanism for the reaction inactivation that is consistent with the known experimental facts is proposed. It is postulated that an enzyme–semiquinone complex is an intermediate which may decompose either to (a) reaction products and free enzyme or to (b) an inactive enzyme.

W. H. Miller, *et al.*,[2] have shown that the polyphenoloxidase-catalyzed oxidation of catechol can be followed by measuring the oxidation of ascorbic acid added to the reaction mixture. The semiquinone and orthoquinone formed in the reaction oxidize the ascorbic acid to dehydroascorbic acid and the semiquinone and orthoquinone are reduced back to catechol. In our studies of the prevention of browning of fruit we have been interested in the fact that in the system described above the rate of disappearance of ascorbic acid does not remain constant but gradually decreases until, after 5–10 minutes, the rate becomes zero and no more ascorbic acid is oxidized. This effect has been shown to be due to a disappearance of enzyme activity and has been named "reaction-inactivation,"[3] since it occurs only during the catalyzed oxidation of catechols.

Recent research[4] has shown that the rate of reaction-inactivation of polyphenoloxidase during the

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[1] Presented in part at the 123rd A.C.S. meeting, Los Angeles, Calif., March 15–19, 1953.

[2] W. H. Miller, M. F. Mallette, L. J. Roth and C. R. Dawson, THIS JOURNAL, **66**, 514 (1944).

[3] B. J. Ludwig and J. M. Nelson, *ibid.*, **61**, 2601 (1939).

[4] L. L. Ingraham, J. Corse and B. Makower, *ibid.*, **74**, 2623 (1952).