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PHYSICO-CHEMICAL STUDIES OF POLY-D-GLUTAMIC ACID FROM *BACILLUS ANTHRACIS* GROWN *IN VITRO*

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CONTENTS

The behaviour of polyglutamic acid and of its alkali salts in aqueous electrolyte solutions of varying ionic strengths has been examined by light scattering and in the ultracentrifuge, and parallel diffusion and viscosity studies have been made. Both behave in solution as flexible chain molecules of effective volume large for their weight. The effective volume per mole is much greater for the ionized form than for the largely unionized form (free acid), and the effective volume of the alkali salts increases markedly with reduction in the ionic strength of the solvent as would be expected from their polyelectrolyte nature. Sedimentation studies indicate a substantial degree of ion-pair formation between the polyglutamate ion and the cation of the solvent electrolyte. Despite this, the alkali polyglutamates show a 'secondary charge effect' of considerable magnitude, much greater than that predicted from the measured electrophoretic mobility of the polyglutamate ion. In contrast to the free acid, both the sedimentation and diffusion coefficients are markedly concentration-dependent. These coefficients have been extrapolated to infinite dilution, the sedimentation coefficients on the assumption that their inverse varies linearly with concentration and the diffusion coefficients by the method of Mandelkern & Flory (1951), using a value of the second virial coefficient derived from light-scattering data. Combination of these extrapolated values leads to a molecular weight for the free acid of 172000 and for sodium polyglutamate of 200000 ± 5000 after allowance for the effects of selective solvation. Detailed analysis of the sedimentation data for sodium polyglutamate in salt solutions of varying density indicates that the molecule is selectively solvated with not more than its own weight of water, a more probable figure being approximately 60 %. Detailed light-scattering studies of sodium polyglutamate have been made at ionic strengths 0·2 and 1·1. The mean of a number of determinations gives a weight-average molecular weight of 238000 and a number-average of 88000, the latter figure being in good agreement with a value obtained by assay of free amino groups. Values of the root-mean-square end-to-end distance of coils having the number- and *z*-average molecular weights are also given.

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Introduction

It has long been known that most capsulated strains of *Bacillus anthracis* are virulent, while all non-capsulated strains isolated have proved to be avirulent. Bruckner & Ivanovics (1935), Ivanovics & Erdös (1937) and Ivanovics & Bruckner (1937) isolated poly-D-glutamic acid as a major constituent of capsular material. Hanby & Rydon (1946) showed their material to consist largely, if not exclusively, of poly-D-glutamic acid. Several groups of workers (Tomczic & Ivanovics 1938; Watson, Cromartie, Bloom, Hickley, McGhee & Weissman 1947; Smith, Keppie, Ross & Stanley 1954) examined this material and found it to be non-toxic. Recent work by Smith & Zwartouw (1954) and Smith, Zwartouw & Gallop (1954) on material isolated from *in vivo* growth of the organism by very mild procedures has confirmed this lack of toxicity, though the material has been shown to have weak aggressive properties. It seems probable, therefore, that any effect that poly-glutamic acid has on virulence must lie in its power to protect the organism against the defence mechanisms of the host (see also Zwartouw & Smith 1956). Some of the unusual physico-chemical properties of this material are considered in the present work.

The experimental work will be recorded here under four main headings, namely, viscosity, light scattering, diffusion and sedimentation.

1. Materials

(*a*) *Sodium polyglutamate*

The Bruce White (1946) strain of *B. anthracis* was grown on agar containing casein hydrolysate and yeast extract (Gladstone & Fildes 1940) and the organisms reaped by suction. The polyglutamic acid was isolated as its sodium salt as described by Hanby & Rydon except that the initial acidification was carried out at $+2°$ C and the final dialysis was carried out at *ca.* pH 8 by the addition of a few drops of caustic soda solution to the distilled water outside the dialysis sac. The final material, after freeze-drying, was identical in all respects with the product described except that the sodium content was slightly higher. The work described here has been carried out on two preparations which amounted to 6 g (Na = 13·6 %) and 12 g (Na = 14·2 %) respectively.

This report is concerned primarily with the smaller, first sample, *PGA* 1, and in the absence of special notation all statements refer to this sample. Where experiments with the second sample are concerned the second sample will be described as *PGA* 2.

(*b*) *Polyglutamic acid*

Hanby & Rydon (1946) observed that the preparation of the free acid by acidification of the sodium salt with subsequent dialysis was followed by degradation of the peptide. In this work the acid was prepared without detectable degradation by use of an ion-exchange resin.

50 g of cross-linked polymethacrylic acid resin, Amberlite I.R.C. 50 (Rohm & Haas), in its H^+ form in a column 30 cm long and 18 mm in diameter was washed with distilled water till the pH of the eluate rose to 5·0. 300 mg of sodium polyglutamate was applied to the top of the column and elution of the column with distilled water initiated. The initial

efflux of the polyglutamic acid from the column was marked by a fall in pH of the eluate, and elution was continued until the pH of the eluate rose to 4·5. The collection of the eluate occupied 45 min and the eluate was immediately freeze-dried. The products from this and other similar preparations always contained approximately 0·2 % sodium; however, it was considered that a second treatment with the resin would have been inadvisable. The sample used in this work had a sodium content of 0·21 % and an equivalent (determined by electrometric titration) of 141. The apparent pK_a measured in 1 M-potassium chloride was 3·7, a value in good agreement with that determined by Waley (1955) on the same parent sample of salt (3·6).

The sodium salt prepared from the acid had the same viscosity, sedimentation and light-scattering characteristics as the parent salt (see 'Results').

The free acid is relatively stable in aqueous solution, no significant change being observed in the viscosity of a 0·2 % solution left overnight at 0° C. A 40 % reduction in the specific viscosity was, however, observed after storage at 20° C for 7 days.

2. Definition of symbols

Almost all determinations on sodium polyglutamate were carried out in solvent containing phosphate buffer pH 8 of ionic strength 0·1 (0·0326 M-Na_2HPO_4 + 0·0022 M-KH_2PO_4) in addition to the strength of alkali halide specified. This will be referred to as 'standard phosphate buffer' and departures from this procedure are indicated in the text.

The following commonly occurring symbols are used throughout. Less commonly occurring symbols are defined as used:

General

A_2	second virial coefficient
A_3	third virial coefficient
c (g/100 ml. or g/ml.)	concentration of macromolecular solute
f	frictional coefficient per mole for translation
f'	frictional coefficient per molecule
f_0	frictional coefficient per mole for an unsolvated impervious sphere of the same molecular weight
I	ionic strength
M	molecular weight
N	Avogadro's number
R	gas constant per mole
T	temperature, absolute
Π	osmotic pressure

Viscosity

η_0	viscosity of solvent
$\eta_{sp.} = \eta_{rel.} - 1$	specific viscosity
$\eta_{sp.}/c$ (c in g/100 ml.)	reduced viscosity
$(\eta_{sp.}/c)_{c \to 0} = [\eta]$	intrinsic viscosity

Light scattering

$M_{n,w,z}$	number-, weight-, z-average molecular weight
$N_{n,w,z}$	number-, weight-, z-average degree of polymerization
n	refractive index
n_s	refractive index of solution
n_0	refractive index of solvent
$P(\theta)$	particle scattering factor at angle θ
R_θ	reduced intensity at angle θ of a true Raleigh scatterer or of a non-ideal scatterer, fully corrected
$R_{\theta,u}$	observed reduced intensity of non-ideal scatterer using unpolarized light
$R_{\theta,v}$	observed reduced intensity of non-ideal scatterer using vertically polarized light
R'_θ	reduced intensity of a non-ideal scatterer corrected only for depolarization
$\overline{r^2}_{n,w,z}$	mean square end-to-end distance of a coil having the number-, weight-, z-average molecular weight
z	dissymmetry
$[z]$	intrinsic dissymmetry
λ	wavelength of light *in vacuo*
λ'	wavelength of light in solution
ρ	depolarization ratio
τ	turbidity

Diffusion

D	diffusion coefficient
$D_{20,w}$	diffusion coefficient corrected to water at 20° C
D_0	diffusion coefficient corrected to water at 20° C and extrapolated to zero concentration of solute

Sedimentation

s	sedimentation velocity
u	electrophoretic mobility
V	apparent partial specific volume
ρ_a	density of solvate in free solution
ρ_e	density of solvate subject to electrostriction
ρ_p	density of sedimenting unit
ρ_s	density of solvent

3. Viscosity

The viscosity behaviour of the free acid and of the sodium salt in water was kindly examined by Dr A. G. Ogston, F.R.S. (Department of Biochemistry, University of Oxford), in a Couette viscometer which showed that the viscosity of both substances was independent of rate of shear at rates between zero and 50 s^{-1}. Bateman (1953) has reported similar

behaviour for a different sample of sodium polyglutamate (molecular weight 100 000) at rates of shear between 700 and 3000 s^{-1}. These results indicate that the particles are not highly asymmetric. All viscosities were subsequently measured at 20° C in Ostwald-type viscometers either B.S.S. no. 1 (water time 270 s) or in a viscometer of similar design but of capacity 2 ml. and water time 120 s. Solutions were cleansed by centrifugation or, in the case of solutions of higher densities, by filtration through a small filter (covered to prevent concentration by evaporation) and their densities determined pyknometrically.

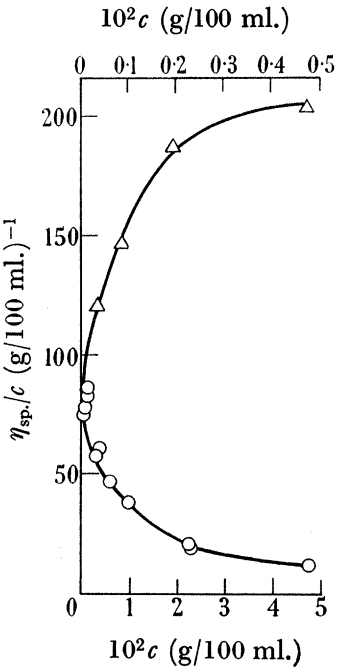

FIGURE 1. Variation of reduced viscosity with concentration. △, sodium polyglutamate in water (abscissa above); ○, polyglutamic acid in water (abscissa below).

The reduced viscosity of sodium polyglutamate in water increases from approximately 35 to 200 over the concentration range 0·5 to 0·01 %, and with further dilution below 0·002 % decreases to give an approximate intrinsic viscosity of 60 to 100. The results below a concentration of 0·005 % are shown in figure 1. Such anomalous behaviour is typical of flexible chain polyelectrolytes; the decrease of reduced viscosity as the concentration diminishes at very low values to zero has already been predicted (see, for example, Rosen, Kamath & Eirich 1951), and observed by Conway (1956).

The high value of the intrinsic viscosity indicates a very bulky particle of effective volume of the order 10^3 times that of a compact molecule (e.g. a globular protein) of the same molecular weight, assuming the Einstein–Sutherland relation to give the approximate order of magnitude.

In common with other flexible chain polyelectrolytes the anomaly in viscosity behaviour rapidly decreases with increasing ionic strength of solution. At ionic strength 0·1, the peak in the curve of reduced viscosity against concentration has completely disappeared, and at this and higher ionic strengths the relation is approximately linear. Values of the intrinsic viscosities of sodium polyglutamate at various ionic strengths are shown in table 1.

TABLE 1. RELATION BETWEEN THE INTRINSIC VISCOSITY, IONIC STRENGTH AND K FOR SAMPLES *PGA* 1 AND *PGA* 2

solvent	intrinsic viscosity $[\eta]$	K (100 ml./g)	$K/[\eta]$
	PGA 1		
0·1 M-NaCl	4·00	5·16	1·29
1·0 M-NaCl	2·20	2·66	1·21
5·0 M-NaCl	1·55	1·98	1·28
0·3 M-KCl	3·10	—	—
1·0 M-KCl	2·25	3·63	1·61
2·75 M-KCl	1·85	2·86	1·55
1·0 M-RbBr	2·35	2·94	1·25
1·0 M-CsCl	2·40	—	—
1·0 M-NaCl (D_2O)	2·20	2·70	1·23
	PGA 2		
0·1 M-NaCl	4·55	—	—
1·0 M-NaCl	2·12	—	—

The figures given in table 1 indicate clearly that the particles contract with increasing ionic strength; again, although the Einstein–Sutherland equation will not rigidly apply, it appears that in the presence of moderate amounts of salt the particles have an effective volume greater by a factor of 100 than impervious spheres of the same weight. A good linear relation exists between the intrinsic viscosity, $[\eta]$, and the reciprocal square root of the ionic strength (see, for example, Fuoss & Strauss 1949).

The reduced viscosity of polyglutamic acid in water is 2·4 at 0·5 % concentration and increases slowly as dilution to 0·1 % occurs. With further dilution the reduced viscosity increases rapidly due most probably to increasing ionization of the acid. The results at concentrations below 0·05 % are also shown in figure 1, and extrapolation suggests a value for the intrinsic viscosity which is close to that of the sodium salt.

The specific viscosities of solutions of sodium polyglutamate, *PGA* 1, at concentrations 0·394 and 0·101 g/100 ml., were 21·4 and 9·3 respectively, whilst the specific viscosities of solutions of sodium polyglutamate prepared from the free acid were, at the same concentrations, 21·9 and 9·7.

Values of the intrinsic viscosities of sample *PGA* 2 are also shown in table 1 and are in reasonable agreement with those of *PGA* 1.

4. LIGHT SCATTERING

(*a*) *Theoretical considerations*

It is now customary to determine the molecular weight, M, of a solute from light-scattering data with the aid of the equation (see Zimm 1948*a*)

$$\frac{Kc}{R_\theta} = \frac{1}{MP(\theta)} + 2A_2c, \tag{1}$$

where

$$K = \frac{2\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}c)^2}{N\lambda^4} \quad \text{or} \quad \frac{4\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}c)^2}{N\lambda^4}$$

for unpolarized or vertically polarized incident light respectively. A $(1+\cos^2\theta)$ term is also to be included in K if the incident light is unpolarized. A_2 is the second virial coefficient, R_θ is the reduced intensity of scattering at angle θ to the incident beam, and other

symbols have their customary significance. A further correction may be required for possible depolarization of the scattered light. $P(\theta)$, the particle scattering factor, may for simple models be ascertained from dissymmetry data and corrects the intensity of scattered light for the diminution due to intramolecular interference which takes place if the scattering molecule has a dimension exceeding one-tenth of the wavelength of light.

Benoit, Holtzer & Doty (1954) have shown that for polydisperse systems of random coils of root-mean-square end-to-end distance, $\sqrt{(\overline{r_z^2})}$, between 800 and 3000 Å, the relation between $(Kc/R_\theta)_{c=0}$ and $\sin^2\frac{1}{2}\theta$ is linear only for the unique case $M_z:M_w:M_n::3:2:1$. The intercept and limiting slope at $\theta = 0$ gives $1/M_w$ and a z-average dimension respectively in all cases. If the above plot is curved, the asymptote to the curve at high values of $\sin^2\frac{1}{2}\theta$ yields $1/2M_n$ as intercept at $\theta = 0$ and $\sqrt{(\overline{r_n^2})}$ can be obtained from the slope of this asymptote.

Supplies of sample *PGA* 1 (the sample with which other sections of this paper are principally concerned) sufficed only for experiments at ionic strength 1·1 and not for their repetition, and these experiments were carried out in the belief that determination of values of R_{90} would, with dissymmetry measurements, prove adequate. Further experiments with sample *PGA* 2 (which had properties almost identical with *PGA* 1; cf. also viscosity and diffusion), where the angular variation of R_θ was studied over a wide range, did not support this belief but indicated that interpretation of the results should be made as indicated above for distributions having a value $\sqrt{(\overline{r_z^2})} = 800$ to 3000 Å and polydispersity exceeding that corresponding to a weight-to-number average molecular weight of 2. The correct interpretation of the results obtained for *PGA* 1 can only be made if they are viewed in conjunction with those of *PGA* 2.

(*b*) *Experimental*

(i) *Refractive increment*

Materials were brought to constant weight *in vacuo* (0·05 mm Hg) at room temperature (18° C). Stock solutions were prepared by weight, their densities determined pyknometrically and from them series of solutions prepared by weight to cover suitable concentration ranges. Measurements on these solutions were made in a differential refractometer already described (Brice & Halwer 1951); in this instrument Δn, the refractive index difference between solution and solvent, is related to a measured image displacement, Δd, by the equation $\Delta n = k\Delta d$. A mean value of k from readings (using light of $\lambda = 589$ mμ) on eight solutions of sucrose of known refractive indices was $9{\cdot}42 \times 10^{-4}$, and an alternative value of k deduced from the dimensions of the instrument was $9{\cdot}43 \times 10^{-4}$.

All measurements were made at 20·0° C using light of wavelengths 436, 546 and 589 mμ.

(ii) *Preparation of solutions for scattering measurements*

The stock solutions (made up by weight and of known densities and nitrogen contents) were filtered, under positive pressure, through 5/3 sintered glass filters to clean them for light-scattering measurements. The filtrations were repeated (through the same filter, without intermediate cleaning of the pad) until the measured dissymmetries were constant and the solutions had a satisfactory appearance when viewed at low angles in a strong beam of light. Filtrations and all other manipulations were, where possible, carried out in a Perspex cabinet maintained at a slightly positive pressure by the supply of dust-free

compressed air. The concentrations of the filtered stock solutions were determined by measurement of Kjeldahl nitrogen concentrations, and solutions of appropriate strength for light-scattering measurements were prepared by progressive additions, by weight, of filtered stock to known weights of similarly cleansed solvent in the scattering cell.

(iii) *Light-scattering measurements*

All measurements were made in a B.S. light-scattering photometer (Phoenix Precision Instrument Co. Ltd). The calibration of this photometer to give values of R_{90} (the reduced intensity at 90° to the incident light beam) in absolute units has been described by Brice, Halwer & Speiser (1950).

The validity of this calibration was checked by scattering and transmission measurements of colloidal silica (Ludox) solutions (Mara & L'on 1954; Oster 1952; Tietze & Neurath 1952) at $\lambda = 546$ and 436 mμ. These independent experiments led to results in agreement within the limits expected (4 to 5 %).

TABLE 2. LIGHT-SCATTERING RESULTS FOR BOVINE PLASMA ALBUMIN

	experiment 1			experiment 2		
λ(mμ)	436	546	589	436	546	589
dn/dc	0·1927	0·1872	0·1850	0·1886	0·1828	0·1812
$[z]$	1·03	1·04	—	1·02	1·02	—
$(\rho_u)_{c=0}$	0·025	—	—	—	0·011	—
$10^4 A_2$	0·58	0·63	—	0·28	1·23	—
M (corr. for depolarization)	62700	63900	—	62300*	63400*	—

* Calculated using values of dn/dc from experiment 1.

As a further check a duplicate determination of the molecular weight of bovine serum albumin (Armour Laboratories, fraction V) was carried out in 1·0 M-sodium chloride (see table 2). The mean value obtained (63 100) is somewhat lower than those given by other workers using this technique, who have recorded values of 73 000 to 79 000 (Reichmann & Charlwood 1954; Edsall, Edelhoch, Lontie & Morrison 1950; Halwer, Nutting & Brice 1951; Doty & Steiner 1952). These values may be compared with 69 000 reported by Scatchard, Batchelder & Brown (1946) from osmotic pressure measurements and 65 000 to 67 000 indicated by sedimentation (Creeth 1952) and diffusion studies (Akely & Gosting 1953). Halwer *et al.* (1951) have shown that aggregation effects can cause the light-scattering molecular weight to increase markedly (up to 137 000) and that these aggregates are removed from solution by filtration. A marked decrease in concentration (*ca.* 25 %) was observed when preparing solutions for light scattering by filtration of stock solutions so that the solutions examined may have been unusually free from large-sized aggregates.

All measurements were recorded as the means of two sets of readings taken, respectively, before and after reversing the scattering cell on the photometer table. All measurements were made (unless otherwise stated) using unpolarized incident light and were corrected for the scattering due to the solvent alone.

Dissymmetry measurements were made at 45 and 135°; depolarization measurements were made at $\lambda = 436$ and 546 mμ, and correction was made for the dependence of photomultiplier tube sensitivity on the plane of vibration of the light falling upon it.

The cylindrical cell used for measurements at angles required additionally to 45, 90 and 135° has been described (see Wittnauer & Scheer 1952), and when tested with fluorescein showed scattering intensities which increased by approximately 1 % as θ, the angle between incident and viewed rays, increased from 30 to 135°.

(*c*) *Results*

Light-scattering measurements were made on the sodium salt of polyglutamic acid (*PGA* 1) in standard phosphate buffer $+ I = 1{\cdot}0$ sodium chloride, and on the sodium salt of polyglutamic acid *PGA* 2 in phosphate buffer

($I = 0{\cdot}1$ phosphate, $= 0{\cdot}0326$ M-$Na_2HPO_4 + 0{\cdot}002$ M-NaH_2PO_4; $I = 1{\cdot}0$ NaCl; pH 7·40).

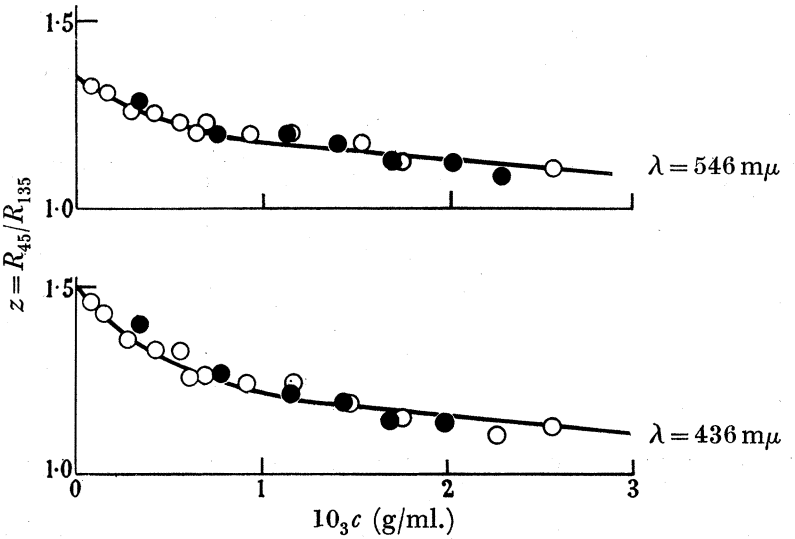

FIGURE 2. Plot of z against c for sodium polyglutamate at ionic strength 1·1. ○, original sodium salt; ●, regenerated salt.

Refractive increment

Values of the refractive increment of *PGA* 1 were determined on three separately prepared stock solutions over the concentration range 0·5 to 2·0 g/100 ml. No concentration dependence was observed, and mean values of 0·1764, 0·1719 and 0·1703 were calculated at wavelengths 436, 546 and 589 mμ. At each wavelength the standard deviation of the observations from the mean was 0·0004 and the standard error of the mean was 0·0001. Values of the refractive increment of *PGA* 2 are shown in table 3, where it is seen that these values significantly exceed those for *PGA* 1, possibly due to the variation of the sodium contents of the two samples.

Dissymmetry measurements

The relation between z and c is shown in figure 2. Plots of Kc/R_{90} against c are, within experimental error, linear below a concentration of 0·1 g/100 ml., so justifying a linear extrapolation of $(z-1)^{-1}$ against c to zero concentration to obtain the value of the intrinsic dissymmetry, $[z]$, at each wavelength. Plots of $(z-1)^{-1}$ against c for *PGA* 1 are satisfactorily linear at both wavelengths, and extrapolation of the lines calculated by the

TABLE 3. LIGHT-SCATTERING DATA

ionic strength	...	1·1		1·1		0·2			
sample	...	*PGA* 1		*PGA* 2		*PGA* 2			
wavelength (mμ)	...	436	546	436	546	436	436	546	546
polarization	...	*U*	*U*	*V*	*V*	*U*	*V*	*U*	*V*
dn/dc	...	0·1764	0·1719	0·1817	0·1770	0·1919	0·1919	0·1872	0·1872
$10^7 K$	...	5·097	1·968	10·656	4·114	5·951	11·902	2·302	4·604
$10^5\left(\frac{Kc}{R_{90,u/v}}\right)_{c=0}$		0·632	0·601	0·643	0·606	0·870	0·886	0·750	0·755
$[z]$		1·50	1·34	1·43	1·26	1·77	1·83	1·61	1·81
$P^{-1}(90)$ (polydisperse coil)		1·38	1·26	1·33	1·19	1·62	1·67	1·48	1·66
$10^5\left(\frac{Kc}{R_{90,u/v}}\right)_{c=0} P(90)$		0·458	0·477	0·483	0·509	0·537	0·531	0·507	0·454
$10^{-3}\left(\frac{R_{90,u/v}}{Kc}\right)_{c=0} P^{-1}(90)$		218	210	207	196	186	188	197	220
$10^5\left(\frac{Kc}{R_{0,u/v}}\right)_{c=0}$		—	—	0·43	0·45	0·41	0·40	0·41	0·34
$10^{-3} M_w$		—	—	233	222	244	250	244	294
$10^5 \times$ intercept asymptote, $c=0$, $\theta=0$		—	—	0·50	0·54	0·63	0·62	0·58	0·55
$10^{-3} M_n$		—	—	100	93	80	81	86	92
$\sqrt{(\overline{r_z^2})}$ (Å)		—	—	1590	1450	1890	1980	2200	2420
$\sqrt{(r_n^2)}$ (Å)		—	—	490	400	560	540	620	700
$\sqrt{(r^2)}$ (Å) (polydisperse coil)		800	820	700	740	1020	1070	1120	1320
$10^3 A_2$ ($\theta=0$)		—	—	0·90	0·86	1·37	1·25	1·10	1·50
$10^3 A_2$ ($\theta=90$)		1·36	1·38	1·40	1·24	—	—	—	—
A_3		—	—	0·112	0·085	—	—	—	—

method of least squares gave values of $[z] = 1{\cdot}50$ and $1{\cdot}34$ at wavelengths 436 and 546 mμ respectively.

Values of $P^{-1}(90)$ corresponding to these dissymmetries and a polydisperse coil as model are $1{\cdot}38$ (436 mμ) and $1{\cdot}26$ (546 mμ). Comparison of these values of $[z]$ and $P^{-1}(90)$ for samples *PGA* 1 and *PGA* 2 may be made from table 3. In making this comparison it should be remembered that the data were gathered at only 45, 90 and 135° but at several concentrations below 0·1 g/100 ml. for *PGA* 1, whereas for *PGA* 2 the values of $[z]$ reported were derived from only a small number of concentrations and values of $(R_{45}/R_{135})_{c=0}$ taken from smoothed lines drawn to form a family with other lines through many different angles. Hence it may be concluded that the true values of the intrinsic dissymmetries of the two samples are probably very close.

Depolarization ratios

Depolarization ratios $\rho_u = H_u/V_u$ were determined only for sample *PGA* 1 and showed some concentration dependence. Linear extrapolation to zero concentration gave values of $(\rho_u)_{c=0}$ of 0·0195 and 0·0126 at $\lambda = 436$ and 546 mμ respectively. Depolarization correction factors corresponding to these values were obtained from insertion of $(\rho_u)_{c=0}$ in place of ρ in the expression $f_\rho = (6-7\rho)/(6+6\rho)$, giving 0·959 and 0·973 at 436 and 546 mμ respectively. Values of molecular weights have not been corrected by these factors in tables 3 or 4, but in view of the close similarities of the two samples it may be assumed that the values given above would apply to both.

Scattering data on PGA 1

Scattering data on sample *PGA* 1 were obtained at 90° only over the concentration range 0·008 to 0·3 g/100 ml. The experimental points in figure 3 show the relation between Kc/R'_{90} and c and are those from two completely independent experiments. Comparison of the 90° scattering from samples *PGA* 1 and *PGA* 2 may be made in figure 4, where the

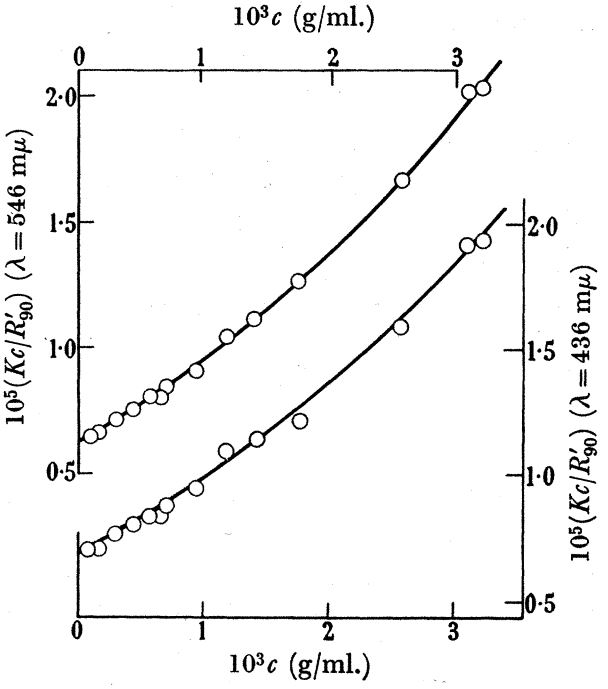

FIGURE 3. Plots of Kc/R'_{90} against c for sodium polyglutamate at ionic strength 1·1.

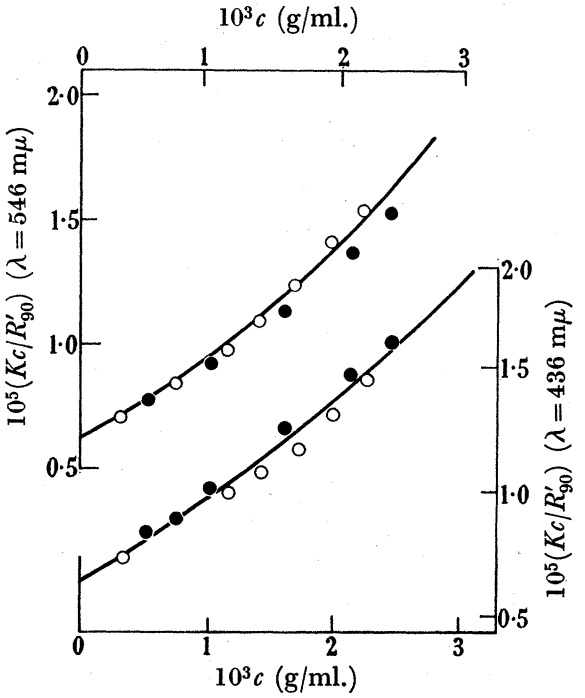

FIGURE 4. Plots of Kc/R'_{90} against c for original sodium polyglutamate (——), regenerated sodium polyglutamate (○) and *PGA* 2 (●) at ionic strength 1·1.

full lines, represent values from *PGA* 1 and the filled circles are experimental points taken from the work on *PGA* 2. At 436 mμ, where the accuracy is highest, the two samples could not be differentiated from a knowledge of their 90° scattering.

At concentrations below 0·1 g/100 ml. the relation between Kc/R_{90} and c is, within experimental error, linear and extrapolation to zero concentration yields a value $(Kc/R_{90})_{c=0}$, which is at both wavelengths in excellent agreement with the corresponding value from 90° measurements on *PGA* 2 (table 3).

Regenerated sodium polyglutamate

Scattering measurements were made on a sample of sodium polyglutamate which had been formed by the neutralization of a sample of polyglutamic acid which had itself been prepared from *PGA* 1. It was hoped in this way to demonstrate any possible degradation occurring in the preparation of the free acid from the sodium salt.

Experimental conditions were exactly as described above for *PGA* 1, and values of the refractive increments and depolarization factors were assumed to be identical for both the regenerated and the original sodium polyglutamate.

Plots of z against c (figure 2) are in excellent agreement at both wavelengths with corresponding data for *PGA* 1.

Values of Kc/R'_{90} for the regenerated salt are plotted against concentration in figure 4, where the full lines represent the data for *PGA* 1. It appears reasonable to deduce from the close coincidence of these two sets of experimental data that little degradation had occurred in the preparation of the regenerated salt or in the preparation of the acid from *PGA* 1.

Scattering data on PGA 2

Intensities of light scattered were examined over the angular range 30–130° from solutions of *PGA* 2 over the concentration range 0·05 to 0·25 g/100 ml. at wavelengths 436 and 546 mμ, using vertically polarized light. The experimental results are illustrated in figure 5, where the data are shown as a double plot according to Zimm's (1948*b*) method, in which $Kc/R_{\theta,v}$ is plotted against $\sin^2\frac{1}{2}\theta+100c$. The curvature of the line $(Kc/R_{\theta,v})_{c=0}$ against $\sin^2\frac{1}{2}\theta$ is clearly seen, and the ease of location of the asymptote to the curve at high values of $\sin^2\frac{1}{2}\theta$ apparent.

Provided (as stated by Schneider & Doty 1954) that

(1) the chain is Gaussian in the sense that the mean segment density about the centre of gravity is not more concentrated 'near the centre than in the usual case',

(2) that stiffness of the links of the chain is not contributing to the curvature of the plot of $P^{-1}(\theta)$ against $\sin^2\frac{1}{2}\theta$,

(3) the molecule is non-branched,

(4) the distribution of segments in the chain is Gaussian in the sense that the ratio of the contour length to the root-mean-square end-to-end length exceeds a value of 3 to 4, and

(5) the dimension $\sqrt{(\overline{r_z^2})}$ is between 800 and 3000 Å,

then the curvature in the plot of $(Kc/R_{\theta,v})_{c=0}$ against $\sin^2\frac{1}{2}\theta$ may be interpreted as being due to the form of distribution of molecular species forming the sample, as shown by Benoit *et al.* (1954). Conditions (4) and (5) may be acknowledged as valid from an examination of scattering data whilst condition (3) is justified from end-group analysis (see below).

Geometrically, polyglutamate will differ from a hindered polymethylene chain only as a result of the partial double-bond character of the amide link, provided that no large additional restriction is introduced by the effects of electrostatic repulsion due to the high charge density in this polymer. Schneider & Doty (1954) have shown that conditions (1) and (2) hold for a sodium carboxymethylcellulose of weight-average degree of polymerization double the value which will be deduced for *PGA* 2, but a value for the length of the statistical chain element which is also twice that for *PGA* 2. Furthermore, the overall dimensions and the value of the second virial coefficient which we shall deduce for *PGA* 2 suggest that the degree of local shielding in the solvents we have used is as good as in the studies on sodium carboxymethylcellulose. It seems reasonable, therefore, to assume that conditions (1) and (2) also hold for polyglutamate.

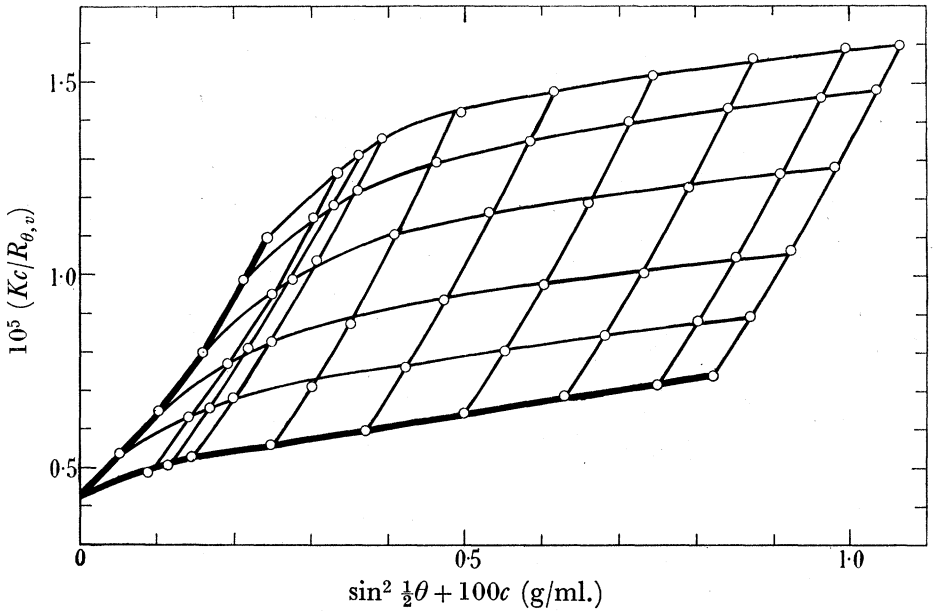

FIGURE 5. Light scattering of *PGA* 2 using vertically polarized light of wavelength 436 mμ. Ionic strength 1·1.

Table 3 accordingly shows values of M_n, M_w, $\sqrt{(\overline{r_n^2})}$ and $\sqrt{(\overline{r_z^2})}$ calculated from the relations

$$M_w = \frac{1}{(Kc/R_0)_{c=0}}, \quad \overline{r_z^2} = \frac{9\lambda'^2\,(\text{initial slope})}{8\pi^2 (Kc/R_0)_{c=0}},$$

and

$$M_n = \frac{1}{2\,(\text{intercept of asymptote})},$$

$$\overline{r_n^2} = \frac{3\lambda'^2\,(\text{slope of asymptote})}{8\pi^2\,(\text{intercept of asymptote})},$$

and it may be seen that the value deduced for $\sqrt{(\overline{r_z^2})}$ does in fact fall in the region 800 to 3000 Å.

Table 3 also gives values of $(Kc/R_{90,v})_{c=0} P(90)$ for *PGA* 2 and shows that at ionic strength 1·1 these values are close to the value of the intercept of the asymptote to the line $(Kc/R_{\theta,v})_{c=0}$ against $\sin^2 \frac{1}{2}\theta$. Accordingly, the value $(Kc/R_{90})_{c=0} P(90)$ for *PGA* 1 is most correctly interpreted as being approximately equal to the reciprocal of twice the number-

average molecular weight, but having regard to the close similarities of the two samples with respect to light scattering, viscosity and diffusion behaviour it is reasonable to assign to *PGA* 1 the properties deduced for *PGA* 2.

Scattering data at ionic strength 0·2

Intensities of light scattered from *PGA* 2 dissolved in phosphate buffer

($I = 0{\cdot}1$ phosphate, $= 0{\cdot}0326$ M-$Na_2HPO_4 + 0{\cdot}002$ M-NaH_2PO_4; $I = 0{\cdot}1$ NaCl)

were examined over the concentration range 0·02 to 0·14 g/100 ml. and over the angular range 30 to 130° using unpolarized and vertically polarized light at wavelengths 436 and 546 mμ. Results are given in table 3 and illustrated in figure 6. The curvature of the $(Kc/R_\theta)_{c=0}$ against $\sin^2 \frac{1}{2}\theta$ is again evident and again the asymptote is easily located.

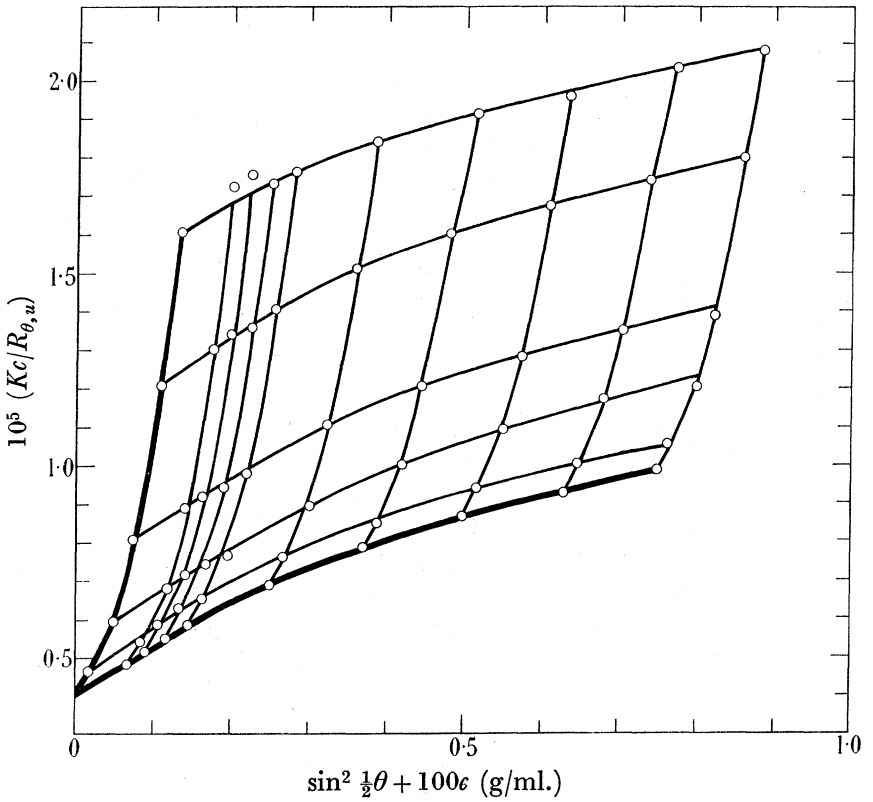

FIGURE 6. Light scattering of *PGA* 2 using unpolarized light of wavelength 436 mμ. Ionic strength 0·2.

(*d*) *Discussion*

It is implicit in the theory of the analysis which we have made (Benoit 1953) that b, the length of the statistical chain element defined by the equation

$$b = \sqrt{(\overline{r^2}/N)},$$

where $\overline{r^2}$ is the mean-square end-to-end distance of the coil and N the degree of polymerization, is for a given polymer under given experimental conditions, independent of the size of the molecule. It is also helpful to emphasize the definition of $\overline{r^2}$ given by Benoit

and others by the example: $\sqrt{(\overline{r_z^2})}$ is the root-mean-square end-to-end distance of a molecule possessing the z-average molecular weight of the distribution. If b is not independent of size then the theory is invalid, but if b is independent of size then the relations

$$b = \sqrt{(\overline{r_n^2}/N_n)} = \sqrt{(\overline{r_z^2}/N_z)} = \sqrt{(\overline{r_w^2}/N_w)}$$

are also true.

Hence, having justified the analysis of the data of *PGA* 2, a value of b may be calculated from $\overline{r_n^2}$ and M_n at each ionic strength and values of M_z and $\overline{r_w^2}$ calculated from the equalities above and experimental values of $\overline{r_z^2}$ and M_w.

Molecular weights

The values of the weight- and number-average molecular weights are shown in table 3 and are in good agreement at both ionic strengths. Table 4 gives values of M_n and M_w which are the means of all experiments on *PGA* 2 except that in which there is least confidence, 546 V ($I = 0{\cdot}2$) and which have not been corrected for depolarization effects. These mean values have been used to calculate values for M_z and N_z (using dimensional data at $I = 1{\cdot}1$) and $\sqrt{(\overline{r_w^2})}$ (using dimensional data at the appropriate ionic strength). The values of the ratio $\sqrt{(\overline{r_z^2})}/\sqrt{(\overline{r_n^2})}$ are in reasonable agreement (3·54 and 3·42) at ionic strengths 0·2 and 1·1 respectively.

TABLE 4. LIGHT-SCATTERING RESULTS (MEAN VALUES) FOR SODIUM POLYGLUTAMATE, *PGA* 2

	number-average	weight-average	z-average
$10^{-3}M$	88	238	1030
N	583	1576	6800
contour length (Å)	4960	13400	57800
b (Å), $I=1{\cdot}1$	18·43	—	—
$\sqrt{(\overline{r^2})}$ (Å), $I=1{\cdot}1$	445	732	1520
b (Å), $I=0{\cdot}2$	23·73	—	—
$\sqrt{(\overline{r^2})}$ (Å), $I=0{\cdot}2$	573	942	2030

The distribution assigned to both samples *PGA* 1 and *PGA* 2 is therefore of the form

$$M_n : M_w : M_z :: 1 : 2{\cdot}7 : 11{\cdot}7.$$

This result shows a wider distribution than that recorded for sodium carboxymethylcellulose, for which the distribution was described as

$$M_n : M_w : M_z :: 1 : 2{\cdot}65 : 5{\cdot}15$$

and was regarded as larger than the most probable distribution of sizes. The samples of polyglutamate examined are possibly breakdown products of a much larger native molecule, and no detailed descriptions of such degradation products are available for comparison.

It is customarily assumed (see, for example, Doty & Edsall 1950) that the effective scattering unit in charged macromolecules in solutions of moderate ionic strengths is the charged macro-ion plus its 'gegen-ion' atmosphere, and that it is the molecular weight of this neutral entity which is deduced from light-scattering data extrapolated to zero concentration. In the experiments described above therefore it may be assumed that the molecular weights quoted refer to the sodium salt of the polyglutamic acid.

If the scattering results were those from experiments involving solution of the macro-ion in water, the molecular weight deduced could be reasonably interpreted as an anhydrous one. In the case of selective solvation from a multi-component solvent the precise interpretation of results is rendered somewhat uncertain. In the experiments described above, however, the molecular weights deduced from solvents of widely different ionic strength were in good agreement and such errors are likely to be small.

Molecular dimensions

The root-mean-square end-to-end dimensions of molecules possessing the variously averaged molecular weights of the distribution are given in table 4 and are the means of the values given in table 3 at each ionic strength (experiment 546 V ($I = 0{\cdot}2$) has been omitted in arriving at these means). The expansion of the coil with decreasing ionic strength is illustrated by the increase of b from 18·43 to 23·73 as the ionic strength decreases from 1·1 to 0·2. Extrapolation of these figures to infinite ionic strength yields the value $b = 13{\cdot}1$ Å.

It is interesting to note that the values of $\overline{r_w^2}$ obtained are, at both ionic strengths, in reasonable agreement with the value of $\overline{r^2}$ deduced from dissymmetry data using a polydisperse coil as model (cf. Doty & Steiner 1950), in spite of the fact that this model assumes a distribution of characteristics different from those we have deduced for *PGA* 2.

With the variously averaged degrees of polymerization and assuming the length of the repeating unit to be 8·5 Å, the contour lengths shown in table 4 may be calculated. The ratios of these dimensions to the appropriately averaged values of $\sqrt{(\overline{r^2})}$ are, at both ionic strengths, greatly in excess of the minimum value (3 to 4) which is usually held as a criterion of a Gaussian distribution of segments within the coil.

Over a limited molecular weight range (see below) the intrinsic viscosity is related to the molecular weight of polyglutamate by the relation

$$[\eta] = KM^{1{\cdot}06}$$

when values of $[\eta]$ are measured at ionic strength 1·1. The proximity of the value of the power 1·06 to unity is indicative of a Gaussian coil of a free-draining character.

The data presented here are insufficient to support a theoretical investigation into the dependence of molecular dimensions upon ionic strength, and in particular it may be felt that reliable information concerning the net charge residing on the molecule is a necessity if more is to be accomplished than is given by Schneider & Doty (1954).

Second virial coefficient

Fleming, Peacocke & Wallis (1956) derived a value for A_2 from 90° scattering from solutions of *PGA* 1 which depended upon application of the equation originally due to Zimm (1948*a*)

$$\frac{1}{c}\left[\frac{Kc}{R_{90}} - \left(\frac{Kc}{R_{90}}\right)_{c=0}\right] = 2A_2 + Yc, \qquad (2)$$

where $Y = 3A_3 Q(\theta) - 4A_2^2 P(\theta)\{1 - P(\theta)\}$. The values of $10^3 A_2$ deduced from this application were 1·36 and 1·38 at $\lambda = 436$ and 546 mμ, whilst a reciprocal form of equation (2) gave values of 1·38 and 1·42 at $\lambda = 436$ and 546 mμ. From these a mean value of 1·38 was taken.

Values of A_2 deduced from 90° scattering by application of equation (2) are given in table 3 for *PGA* 2 at ionic strengths 1·1 and 0·2. It appears that the agreement of values of A_2 demonstrated for *PGA* 1 may have been fortuitous, since a similar close correspondence

is not found for *PGA* 2. It should, however, be noted that in the case of *PGA* 2 the weight of experimental data is considerably less and may account for the discrepancy. The mean value of A_2 deduced for *PGA* 2 from 90° scattering ($1{\cdot}32 \times 10^{-3}$) is in fair agreement with that for *PGA* 1 of $1{\cdot}37 \times 10^{-3}$.

Determination of A_2 from 0° data by application of equation (2) leads to values which are considerably lower than those obtained from 90° data, and are in excellent agreement at the two wavelengths studied. Such a variation of A_2 may not be unexpected for a distribution of the characteristics described here. The variation of second virial coefficient with ionic strength is of the sign and magnitude to be expected. Schneider & Doty (1954) have made theoretical estimates of the variation of the second virial coefficient with ionic strength which depend principally upon the assumption that the dominant contribution to A_2 is from steric interactions, and that the shielding of the macromolecule by a sheath of counter-ions held close to the macromolecule is complete so that the value of A_2 is not affected by charge effects. Experimental observations on carboxymethylcellulose agreed well with theory, but the data for *PGA* 2 are, unfortunately, in too narrow an ionic strength range (where the change in A_2 is small and not greatly in excess of experimental error) to offer any further information in this respect.

Third virial coefficient

The application of equation (2) to 0° data enables a value of A_3 to be deduced, since at zero angle the function $Q(\theta)$ has the value unity. The values so derived at ionic strength 1·1 are given in table 3. It is difficult with these limited data to decide whether the discrepancies in A_3 are due to experimental error or to a wavelength dependence. The values of A_3 deduced lead to values of 'g' in Flory's equation (1953, p. 299)

$$H(c/\tau) = H(c/\tau)_0 \,(1 + 2\Gamma_2 c + 3g\Gamma_2^2 c^2)$$

of 0·59 and 0·52 at wavelengths 436 and 546 mμ, but these values cannot be considered to be sufficiently reliable to displace the currently accepted value of $g = 0{\cdot}25$ for a 'soft' sphere.

At ionic strength 0·2 a plot of the left-hand side of equation (2) against c is no longer linear, and values of A_3 deduced from the limiting slope of such a plot as $c \to 0$ show wide fluctuations.

5. Diffusion

(*a*) *Experimental details*

Preliminary studies in the Hilger model of the Tiselius apparatus (using the inclined-edge schlieren optical system) of the diffusion of sodium polyglutamate at 0·75, 0·5 and 0·25 % concentration in phosphate buffer $I = 0{\cdot}2$, pH 8, showed skew distributions due to the variation of diffusion coefficient with concentration. These observations were later verified using the more sensitive optical system of the Spinco electrophoresis apparatus and an inclined slit schlieren system. The need for measuring diffusion coefficients at the lowest possible concentrations has been indicated by Mandelkern & Flory (1951), and further diffusion studies were carried out in a Gouy interference diffusiometer.

The Gouy apparatus used was closely similar to that described by Gosting, Hanson, Kegeles & Morris (1949) and the cell boundary-forming device was modelled on lines similar to those described by Coulson, Cox, Ogston & Philpot (1948), Ogston (1949*a*) and Creeth

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(1952). The cell and boundary-forming device were almost completely immersed in a very well stirred thermostat which was maintained at either 20 or 25° C ± 0·01° C. Solutions were dialyzed at 0° C for at least 3 days, the dialysis sac being continuously rotated during this period. In the case of polyglutamic acid, the solutions of which are at a low pH value, solutions were made up immediately before use so that any degradation of the macromolecule might be minimized. Precautions were taken at all stages to prevent concentration of the solutions by evaporation.

Diffusion coefficients were calculated from measurements of the Gouy interference patterns by the formulae of Kegeles & Gosting (1947)

$$D = \frac{(j_m \lambda b)^2}{4\pi C_t^2 t}, \tag{3}$$

and

$$D = \frac{(j_m \lambda b)^2 (e^{-z_j^2})^2}{4\pi Y_{j_0}^2 t}. \tag{4}$$

In these equations j_m is given by $j_m = a(n_s - n_0)/\lambda$, in which n_s and n_0 are the refractive indices of the solution and solvent, a is the width of the diffusing column in the direction of the light beam and λ is the wavelength (*in vacuo*) of the light used for the observations. j_m can be determined experimentally from an observation of the sum of the integral number of fringes and the fractional part of a fringe which together determine the interference pattern (see, for example, Gosting *et al.* 1949; Coulson *et al.* 1948). b is the optical distance from the centre of the diffusion cell to the photographic plate and t is the time elapsed between the formation of the infinitely sharp boundary and the time of observation. Y_{j_0} is the distance of the outermost minimum from the normal slit image position and the quantity C_t defined by $C_t = Y_j/e^{-z_j^2}$ represents the maximum downwards deviation of light if the light followed geometrical optics; for an ideal diffusion C_t should be constant when calculated from each fringe of a Gouy pattern at a given time. The quantity $e^{-z_j^2}$ was obtained from tables relating $e^{-z_j^2}$ to a function $f(z_j)$, where

$$f(z_j) = \frac{2}{\sqrt{\pi}} \int_0^{z_j} e^{-\beta^2} d\beta - \frac{2}{\sqrt{\pi}} z_j e^{-z_j^2},$$

and where $f(z_j)$ was derived from calculaionts of the interference conditions by use of the Airy integral (see Gosting & Morris 1949).

Diffusion coefficients were usually calculated by equation 4 and additionally, in some experiments, by equation (3). In the latter case, values of C_t were derived from experimental data by the method of Akely & Gosting (1953). Arithmetic mean diffusion coefficients were also calculated according to the method described by Ogston (1949*b*) for some experiments at the higher concentrations. Values of diffusion coefficients were all finally corrected to standard conditions, water at 20° C.

In addition to the evaluation of j_m whilst the boundary was sharpening as described above, less precise values of j_m (j'_m) were derived from the Gouy patterns at different stages of each diffusion experiment. It was found that in diffusion experiments at the higher ionic strength ($I = 1{\cdot}1$) a decrease in j'_m occurred, though in comparable experiments with bovine plasma albumin j'_m was constant and in good agreement with j_m. In these experiments (sodium polyglutamate and polyglutamic acid at $I = 1{\cdot}1$) the rate of loss of j'_m was greatest

at the beginning of the experiment, and for the calculation of diffusion coefficients a single value of j'_m was used for each experiment which corresponded with a time (late in the diffusion experiment) when the rate of change of j'_m was zero or at a minimum. In solutions of low ionic strength ($I = 0{\cdot}2$) the loss in j'_m was observed but to a much smaller extent; derivation of the value of j_m to be used for a given experiment was as described for high ionic strength solutions.

(*b*) *Experimental results and discussion*

Diffusion coefficients in standard phosphate buffer + sodium chloride ($I = 1{\cdot}0$) solutions were markedly concentration-dependent. Figure 7 (constructed from a diagonal-slit schlieren photograph) illustrates the diffusion of 0·4 % solution 179 600 s after the formation of the boundary at this ionic strength; the distribution is shown in normal co-ordinates and

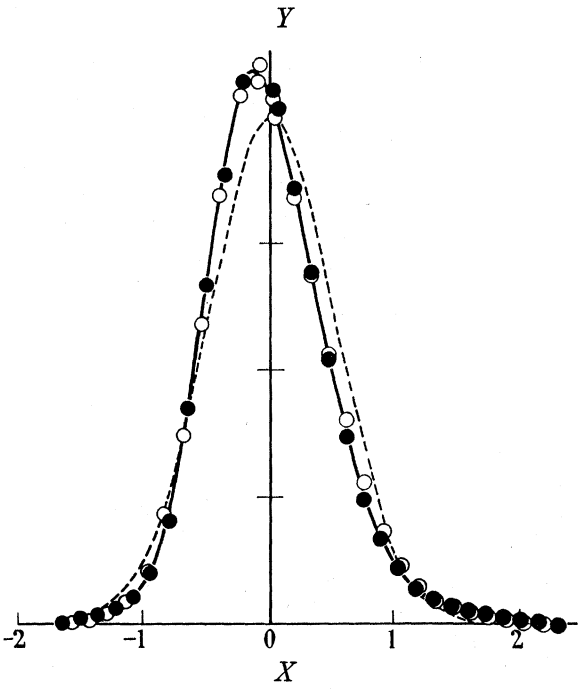

FIGURE 7. The diffusion of sodium polyglutamate in normal co-ordinates from a diagonal-slit schlieren photograph. Measurements from upper (●) and lower (○) edges of the trace.

it may be compared with an ideal normal curve. The diffusion curve is skewed into the solvent region and demonstrates an increase in diffusion coefficient with increase in concentration. Further illustration of this concentration-dependence is found in figure 8*b*, where diffusion coefficients in the concentration range 0 to 0·65 g/100 ml. are calculated from Gouy interference patterns by the aid of equation (4) (Y_{j_0} method) and plotted against j'_m (which is proportional to the solute concentration), and in figure 9*b*, where diffusion coefficients are calculated by equation (3) (C_t method). Extrapolation of the lines calculated by the method of least squares lead to the values $D_{20,w} = 1{\cdot}33 \times 10^{-7}(Y_{j_0})$ and $1{\cdot}25 \times 10^{-7}(C_t)$. Both methods, Y_{j_0} or C_t, correspond to height-area methods, and the C_t method utilizing more experimental measurements from a given exposure may be considered rather more precise.

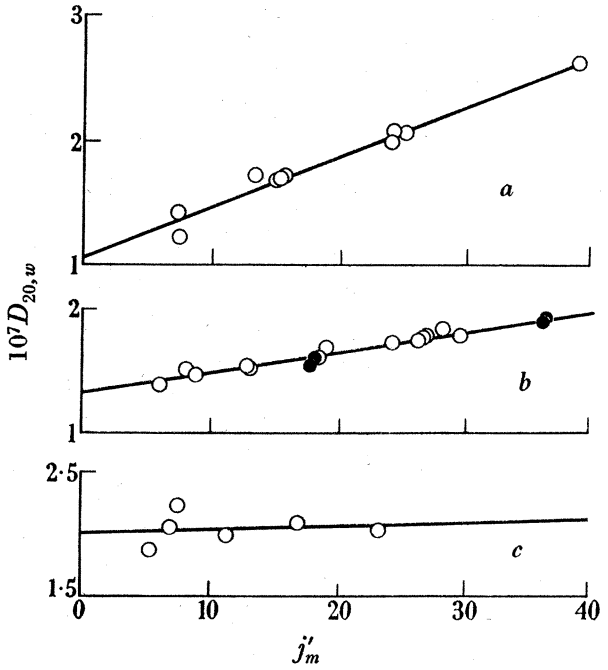

FIGURE 8. Diffusion coefficients calculated by equation (4) (see text). (*a*) Sodium polyglutamate, $I = 0{\cdot}2$. (*b*) Sodium polyglutamate, $I = 1{\cdot}1$. ○, with sodium chloride; ●, with potassium chloride. (*c*) Polyglutamic acid in sodium chloride solution, $I = 1{\cdot}0$.

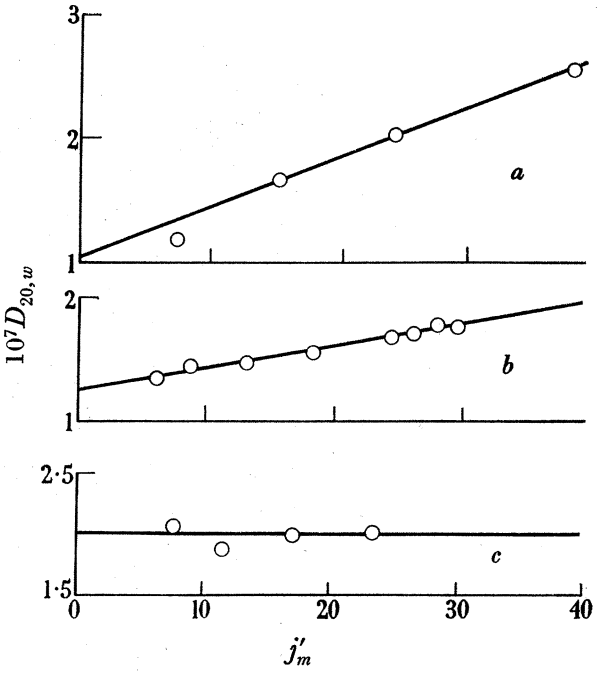

FIGURE 9. Diffusion coefficients calculated by equation (3) (see text). (*a*) Sodium polyglutamate, $I = 0{\cdot}2$. (*b*) Sodium polyglutamate, $I = 1{\cdot}1$. (*c*) Polyglutamic acid in sodium chloride solution, $I = 1{\cdot}0$.

Figure 8*b* also shows diffusion coefficients of sodium polyglutamate dissolved in a phosphate+potassium chloride buffer ($I = 0{\cdot}1$ phosphate buffer already described, $I = 1{\cdot}0$ potassium chloride; pH 7·52). It is not possible to differentiate these diffusion rates from those in sodium chloride solutions of equal ionic strength.

Mandelkern & Flory (1951) have pointed out that the linear extrapolation of diffusion coefficient against concentration to infinite dilution is theoretically unsound even if a linear relation of these quantities is observed at higher concentrations. Provided that the frictional coefficient varies linearly with concentration ($f = f_{c=0}(1+k_1c)$) it is deduced that the variation of D with c is given by

$$\frac{(1+2\Gamma_2 c+3g\Gamma_2^2c^2)}{D} = \frac{(1+k_1c)}{D_0} \tag{5}$$

at concentrations sufficiently low to satisfy the inequality $\Gamma_2 c<1$. For sodium polyglutamate in phosphate buffer ($I = 0{\cdot}1$ phosphate, $I = 1{\cdot}0$ sodium chloride), sedimentation experiments show a linear variation of $1/s$ with c and appear to justify the assumption of

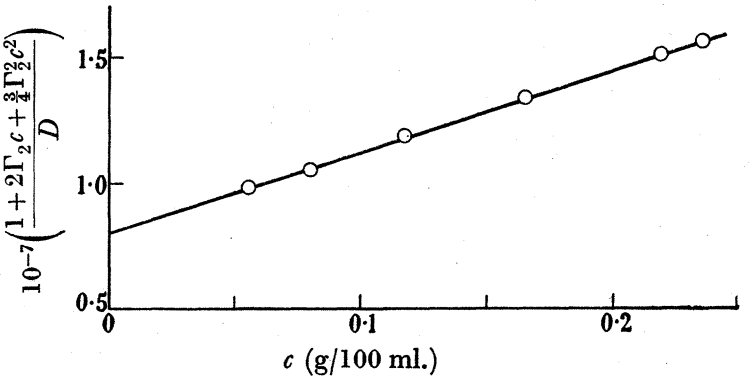

FIGURE 10. Relation of $(1+2\Gamma_2 c+3g\Gamma_2^2c^2)/D$ to concentration for sodium polyglutamate at ionic strength 1·1.

a linear dependence of f upon c, light-scattering studies offer a value for Γ_2 ($= MA_2/100$) of 2·83 and, following Flory, a value for g of 0·25 has been assumed for this, as for other flexible chain molecules. A plot of the left-hand side of equation (5) against c, for values of c such that $\Gamma_2 c<1$, is shown in figure 10.* Linear extrapolation of the line in figure 10 to infinite dilution leads to a value of $1{\cdot}25\times10^{-7}$ which is identical with that from the ordinary linear extrapolation through the experimental points of figure 9*b*.†

Values of the arithmetic mean diffusion coefficient have been calculated from the Gouy records of the diffusion at the two highest concentrations (figure 9*b*) and indicate that the ratios of the arithmetic mean to the C_i value of D are 1·048 at j'_m 24·81 and 1·055 at j'_m 36·60. Taking the mean of these observations as 1·05 and assuming that the ratio is independent of concentration, a value of $D_{20,w} = 1{\cdot}31\times10^{-7}$ is obtained as the arithmetic mean diffusion

* In the calculations leading to figure 10 values of D (calculated by equation (3)) corresponding to mean concentrations of polyglutamate in each diffusion experiment have been used. On the other hand, in sedimentation and, of course, in light scattering, concentrations refer to total polyglutamate concentration.

† The use of the value of A_2 derived from zero-angle scattering measurements on *PGA*2 ($0{\cdot}88\times10^{-3}$ ml. mole/g) in place of 1·38 would lead to a value of $D_0 = 1{\cdot}24\times10^{-7}$; since no zero-angle data were obtained with this sample of polyglutamate the value of 1·38 has been retained.

coefficient at zero concentration for use in the calculation of molecular weight with sedimentation data.

Diffusion coefficients of sodium polyglutamate in the concentration range 0 to 0·7 g/100 ml. were also estimated by the Gouy method in phosphate buffer ($I = 0{\cdot}1$ phosphate as above, $I = 0{\cdot}1$ sodium chloride) solutions pH = 7·88. Concentration dependence was greater at the lower ionic strength and is illustrated in figure 8*a* (Y_j method) and in figure 9*a* (C_t method). The straight lines drawn in both figures 8*a* and 9*a* are the same and represent the line (calculated by the method of least squares) through the experimental points of figure 9*a*. In this case the values calculated by the C_t method are very close to those predicted by the Y_{j_0} method but are rather few in number. The value of $D_{20,w}$ at zero concentration has therefore been taken as the same by either method of calculation and amounts to $1{\cdot}05 \times 10^{-7}$.

No values are available for the second virial coefficient of *PGA* 1 at ionic strength 0·2 and the validity of the linear extrapolation of D has not been tested. Values of the ratio of arithmetic mean to the C_t diffusion coefficient were 1·32 at j'_m 38·84 and 1·09 at j'_m 24·55.

Measurements of the diffusion coefficients of polyglutamic acid (prepared from the stock polyglutamate) at different concentrations in the range 0 to 0·4 g/100 ml. were made in sodium chloride solutions, ionic strength 1·0, without added buffering ions. Figures 8*c* and 9*c* show that in contrast to the sodium salt, diffusion coefficients of polyglutamic acid were almost independent of concentration. Extrapolation of the lines in figures 8*c* and 9*c* (calculated by the method of least squares) leads to the values of $D_{20,w}$ $2{\cdot}01 \times 10^{-7}$ (Y_{j_0} method) and $2{\cdot}00 \times 10^{-7}$ (C_t method) at zero concentration. The ratio of the arithmetic mean to the C_t diffusion coefficient was 1·14 at j'_m 23·32, and again assuming this ratio to be concentration-independent, a value of $2{\cdot}28 \times 10^{-7}$ is obtained for the arithmetic mean diffusion coefficient at zero concentration.

Measurements of the diffusion coefficients of *PGA* 2 were made in phosphate buffer ($I = 0{\cdot}1$ phosphate, $I = 0{\cdot}1$ sodium chloride) at two concentrations only. Comparison of these coefficients with those of *PGA* 1 at the same concentration (at $j'_m = 44{\cdot}67$, $D_{20,w}$ *PGA* 2 $= 2{\cdot}75 \times 10^{-7}$, *PGA* 1 $= 2{\cdot}80 \times 10^{-7}$; at $j'_m = 19{\cdot}76$, $D_{20,w}$ *PGA* 2 $= 1{\cdot}83 \times 10^{-7}$, *PGA* 1 $= 1{\cdot}83 \times 10^{-7}$) shows that the two samples have very similar diffusion characteristics.

6. Sedimentation

(*a*) *Experimental*

Measurements were made with a Spinco electrically driven ultracentrifuge using the diagonal bar schlieren optical system. Much of the work, notably the measurements in solvents of high density, could hardly have been carried out without the aid of the synthetic boundary cell designed by Pickels, Harrington & Schachman (1952). Except for a few runs at 42 000 rev/min in high molarity potassium iodide and rubidium iodide, a rotor speed of 59 780 was used throughout. No correction has been applied for the effect of stress on change of rotor temperature. Solutions were made up directly by weight from the dried sample (stored at 0·01 mm Hg over phosphorus pentoxide), taking precautions against absorption of atmospheric moisture. No precautions have been taken against the possibility of small errors arising from electrostatic effects when weighing milligram quantities of the material. Where possible a stock solution was prepared and dilutions made by weight, and

this procedure generally gave more concordant results. Except in measurements on the free acid, the standard pH 8 phosphate buffer was incorporated in all solutions examined.

Partial specific volume measurements were made in a pyknometer of 12·5 ml. capacity at 20° C. The results are given in table 5. Calculations of the partial specific volume of the polyglutamate ion are given in appendix B.

TABLE 5. PARTIAL SPECIFIC VOLUMES OF POLYGLUTAMIC ACID AND ITS SODIUM AND POTASSIUM SALTS

solvent	solvent density	solute	solute concentration	partial specific volume	
0·1 M-NaCl, standard phosphate buffer $I=0·1$	1·007	sodium polyglutamate	1·7 % w/v	0·484	0·486
			0·86 % w/v	0·489	
1·0 M-NaCl, standard phosphate buffer $I=0·1$	1·045		1·56 % w/v	0·510	0·506
			0·78 % w/v	0·503	
2·2 M-KI, standard phosphate buffer $I=0·1$	1·26		0·63 % w/v	0·525	—
2·2 M-KI, standard phosphate buffer $I=0·1$	1·26	potassium polyglutamate	0·70 % w/v	0·539	—
4·3 M-KI, standard phosphate buffer $I=0·1$	1·51		0·32 % w/v	0·561	
1·0 M-NaCl	1·042	polyglutamic acid	1·24 % w/v	0·666	0·663
			0·62 % w/v	0·659	

(*b*) *Theoretical considerations*

Polyglutamic acid is a typical polyelectrolyte and, as various recent investigations have emphasized (see, for example, Howard & Jordan 1954, sedimentation studies on polymethacrylic acid), this class of substance presents special difficulties in physico-chemical investigations. The high charge carried by such substances raises special problems in sedimentation studies. Being macromolecular, they may exhibit in addition all the anomalies associated with particles having a large effective volume, e.g. pronounced decrease in sedimentation rate with rising concentration and, as a result, enhanced artificial sharpening of the sedimenting boundary.

(i) *Charge effects*

Svedberg & Pedersen (1940) have shown how electric charge effects influencing the rate of sedimentation of an ionized macromolecule can arise from two distinct causes. First, the tendency of the slowly sedimenting 'gegen-ions' to retard the sedimentation of the poly-ion, which effect they designate the primary charge effect. Secondly, the effect of the electric field set up by the differing sedimentation rates of the oppositely charged ions comprising the buffer or salt solution. This so-called secondary charge effect may either retard or speed up the sedimentation of the poly-ion depending on the relative sedimentation rates of the buffer ions.

(ii) *The primary charge effect*

This decreases with increasing ionic strength of the medium and the usual procedure for reducing it to negligible proportions is to observe the sedimentation rate in a medium of increasing ionic strength until a constant rate is attained. Under these conditions the observed rate of sedimentation of the poly-ion is presumed to be that which it would have

if sedimenting as an uncharged particle. The method tacitly ignores, however, the possibility of any change in the effective size of the particle with changing ionic strength of the medium. In the case of polyglutamic acid viscosity measurements show that a marked decrease in hydrodynamic volume occurs with increasing ionic strength so that the frictional force opposing sedimentation will not become constant except possibly at very high ionic strength. Furthermore, the density of the sedimenting particle in solution is not known in this case, so that sedimentation rates in media of different density cannot be reduced to standard conditions for comparison.

The magnitude of the primary charge effect may, however, be calculated from equation (43) of Svedberg & Pedersen (1940) if the electrophoretic mobilities of the various charged species comprising the system are known. Using the simplified expression due to Kraut (1954),

$$\frac{1}{s_1'} = \frac{1}{s_1}\left\{1 - \frac{m_1 c_1 u_1}{m_1 c_1 u_2 + m_3 c_3 (u_2 - u_3)}\right\}, \tag{6}$$

where s_1' is the observed rate of sedimentation of the polyanion, s_1 the rate it would have in the absence of a primary charge effect, and m, c and u are the valence, concentration and electrophoretic mobility of the ions respectively, the subscript 1 referring to the polyglutamate ion, 2 the cation and 3 the anion of the solvent electrolyte. A value of about $1{\cdot}3 \times 10^{-4}\,\mathrm{cm^2 s^{-1} V^{-1}}$ was found for the mobility of the present sample of sodium polyglutamate in 0·1 M-sodium chloride, pH 8 phosphate, $I = 0{\cdot}1$ buffer, and this value did not greatly alter on raising the sodium chloride concentration to 1·0 M. Substitution in equation (6) shows that the primary charge effect for a 0·3 % solution of sodium polyglutamate in the 0·1 M-sodium chloride buffer does not reduce the sedimentation rate by more than 3 % and that it is negligible in M-sodium chloride. In the event of ion-pair formation the primary charge effect will be even further reduced. In the present studies, however, we are concerned for the most part only with sedimentation rates at infinite dilution where the primary charge effect vanishes even in dilute salt.

(iii) *The secondary charge effect*

This does not vanish at zero concentration of polyelectrolyte but only when the rate of sedimentation of the anion and cation comprising the solvent electrolyte are equal. In a unit centrifugal field, the rate of sedimentation of a particle is

$$s = \frac{M(1 - \rho_s/\rho_p)}{f}, \tag{7}$$

where M is the anhydrous molecular weight of the particles, ρ_p the density of the sedimenting unit in a solvent of density ρ_s, and f is the frictional coefficient per mole opposing sedimentation.

Estimates of the magnitude of the secondary charge effects can be made for sodium and potassium chlorides and potassium iodide by combining the results of Des Coudres (1896) and those of Tolman (1911).* Tolman's figures predict an electrophoretic mobility in potassium iodide under the conditions of our experiments equivalent to $\frac{1}{10}$S (S = Svedberg unit), and the figures of Des Coudres that the potential gradient in a given centrifugal field

* The results of Des Coudres are incorrectly quoted by Tolman (by a factor of 10).

would be for sodium chloride 7·3 % and for potassium chloride 13·7 % of that found for potassium iodide. Evidence is presented in this paper that the secondary charge effect in potassium iodide is considerably greater than the predicted figure and of the order of 0·5 to 1·0 S. Even so, the secondary charge effect in sodium chloride and potassium chloride should still be negligible.

(iv) *The density of the sedimenting particle*

Whatever the true state of the molecule in solution (whether, for example, it is or is not a free-draining molecule) any selective solvation can be regarded as made up of a volume of unchanged solvent together with an excess (positive or negative) of water. If a large volume of solvate consisting mainly of solvent accompanies the molecule in sedimentation then the density of the moving unit will approximate to that of the solvent. Despite this statement, the driving force in sedimentation is determined by the mean density of the anhydrous skeleton together with its excess water, and it is this selectively solvated skeleton which we define as the sedimenting particle and with which we are primarily concerned in these studies (cf. Ogston 1953).

The usual Svedberg equation for the molecular weight

$$M = \frac{RTs}{D(1-V\rho_s)} \tag{8}$$

(where s and D are the measured values ($c \to 0$) at $T°$ K in a medium of density ρ_s and V is the measured pyknometric (anhydrous) partial specific volume) gives the true anhydrous molecular weight only in the special case where the particle is unsolvated, or, if solvated, only when the solvate has the same composition as the medium.

In the general case it may be shown (see appendix A) that

$$M = \frac{RTs}{D\{1-V\rho_s+a(\rho_a-\rho_s)\}}, \tag{9}$$

where a is the volume in millilitres (in free solution) of solvate per gram anhydrous solute and ρ_a is the density of the solvate in free solution (that is, the density the solvate would possess if it were removed from the influence of the particle). With a slight difference in the definition of a and ρ_a, equation (9) is identical in form with that given by Schachman & Lauffer (1950) who did not specifically consider electrostriction. The question of electrostriction effects which are present in the case of salts of polyglutamic acid as can be seen from the variation in V with ionic strength (table 5) is included in the treatment given in appendix A, where it is shown that any such influences on the particle density are corrected by the use of the measured partial specific volume in the V term if it is assumed that the entire effect is limited to the solvate.

Schachman & Lauffer point out that the error in using equation (8) is usually of a small order when s and D are measured in dilute buffers of density 1·01 g/ml. and that when such dilute buffers cannot be used, an attempt should be made to determine the particle density. To minimize errors in extrapolation to zero concentration we have preferred to use s and D measurements carried out in 1·0 M-salt for assessing the molecular weight. The errors likely to be caused by solvation effects can then become serious, and we have therefore attempted

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to obtain information on the actual density of the particle in M-salt from its sedimentation behaviour in solutions covering a range of density. Unfortunately, the procedure of extrapolation or interpolation to $s\eta_0 = 0$ to get ρ_p, the density of the particle (η_0 = viscosity of the medium), only provides with certainty the density of the particle in that particular medium at that density, even when, as Schachman & Lauffer have shown, there is a linear relation between $s\eta_0$ and ρ_s. Such a linear relation can be expected not only when m, v, a, ρ_a and f are all constant in media of different densities but also when ρ_a changes with ρ_s, provided it does so in a linear fashion. Measurements of intrinsic viscosity at different ionic strengths (table 1) show that for salts of polyglutamic acid, f is by no means constant as the salt strength varies, a change which could result in the case of a polyelectrolyte from change in the effective (hydrodynamic) volume of the particle as distinct from any change in v or a. It is also evident from table 5 that electrostriction effects diminish with rising salt concentration, so diminishing the apparent (pyknometric) density. While it is true that these effects tend to neutralize each other as far as their effect on sedimentation rate is concerned, it is perhaps remarkable that the experimental plots of $s\eta_0$ against ρ_s in, for example, rubidium bromide (see figure 13) are linear within the accuracy of measurement over the wide range of density explored. In spite of these difficulties, it is, nevertheless, possible to estimate the degree of hydration of the particle in 1·0 M-salt from data in different media at different densities and hence to estimate the anhydrous molecular weight.

In order to avoid the additional complications which may be caused by the changing ionic strength of the medium, various workers, in attempts to determine the density of virus particles, have used such substances as serum albumin and sucrose to raise the density of the medium. These substances do not provide the wide range of density required in the present investigation and have the disadvantage of raising considerably the viscosity of the medium. Chloral hydrate has been chosen as a suitable non-electrolyte for the present studies; it provides large density increments with only a relatively small rise in viscosity.

(*c*) *Results and discussion*

The sedimentation data obtained with sodium polyglutamate in a number of electrolytes at different ionic strengths are collected in figure 11, which presents the reciprocals of the sedimentation coefficient, corrected only for the viscosity of the medium, against concentration. In every case, and especially in the more dilute salt solutions, the change in s with c is considerable, and in consequence pronounced artificial boundary sharpening occurs, thus facilitating measurements at concentrations of 0·05 % or lower. Another consequence of the rapid fall in s with c is a tendency for s to increase during the course of an experiment as a result of progressive dilution of the cell contents with shift of the boundary. The effect becomes pronounced only over large boundary movements when a suitable correction may be applied (see, for example, Record & Stacey 1948). Within the limits of accuracy of measurement, the relation $1/s$ against c appears to be linear in every case over the range examined, although the possibility of curvature at very low concentrations, such as has been observed, for example, in the case of deoxypentose nucleic acid (Peacocke & Schachman 1954), cannot be entirely ruled out. In the present work the synthetic boundary cell (Pickels *et al.* 1952) has permitted measurements at lower concentrations than would have been possible in the usual cell. Even so, no real evidence has been obtained to cast

any doubt on the validity of the procedure of extrapolating linearly the plots of $1/s$ against c to obtain the sedimentation coefficient at zero concentration; and this method has been adopted throughout the work. Table 6 gives the extrapolated ($c \to 0$) values of s corrected for the viscosity of the medium. Buoyancy corrections have not been applied owing to doubt about the density of the sedimenting particle in each case.

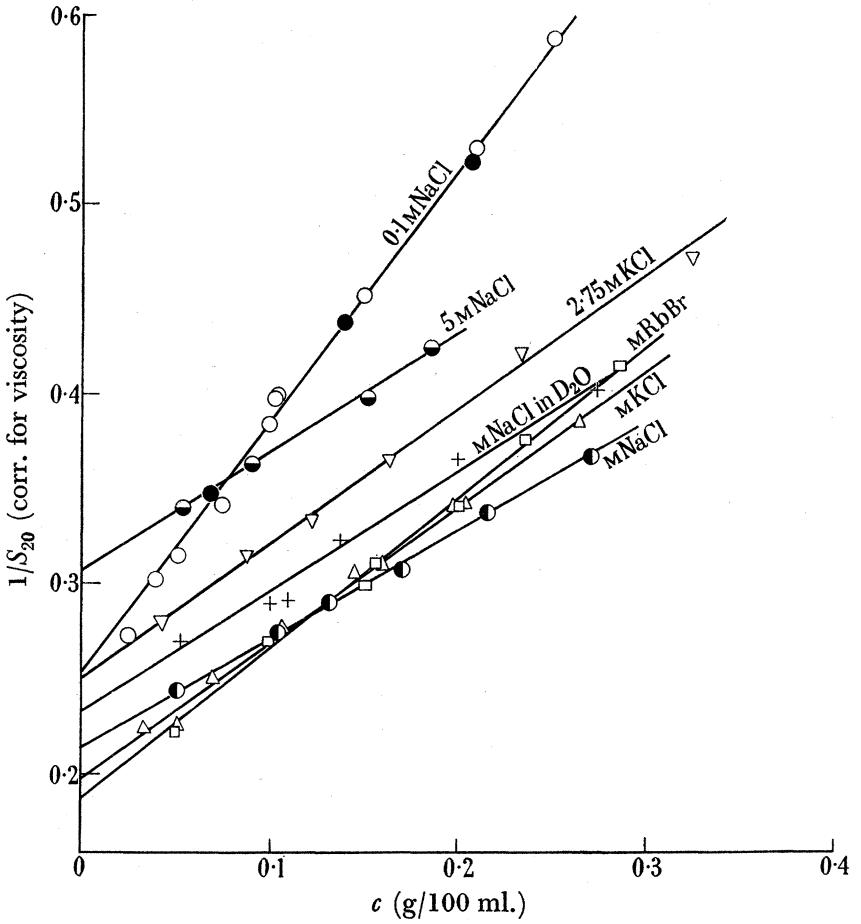

FIGURE 11. Plot of $1/s$ against c for sodium polyglutamate in different solvents: ○, 0·1 M-NaCl; ●, 0·1 M-NaCl (regenerated Na salt); ◐, M-NaCl; ◒, 5 M-NaCl; +, M-NaCl in D_2O; △, M-KCl; ▽, 2·75 M-KCl; □, M-RbBr.

(i) *Sedimentation in different salt solutions at the same ionic strength*

The question of what, in the case of a polyelectrolyte, is actually producing the observed sedimenting boundary, whether the colloid ion or the ion pair, appears to be somewhat controversial at present. If we look at the sedimentation values (table 6) of sodium polyglutamate in the three salts, sodium chloride, potassium chloride and rubidium bromide, at molar strength, where both primary and secondary charge effects are negligible, it is difficult to correlate them with the conception that the sedimenting species is the same, e.g. the polyglutamate ion, in each case. The measured intrinsic viscosity is practically identical in the three solvents, and since they are all of the same ionic strength one would expect such an ion to be but little affected by the change in environment from one solvent to another. It is thus a matter of difficulty to explain the difference in sedimentation

rate on going from sodium chloride to potassium chloride, since these two solvents have practically the same density. The marked increase in sedimentation rate on going over to rubidium bromide is even more difficult to explain, since the medium has a markedly higher density which should reduce the observed sedimentation rate. These considerations present strong evidence therefore that ion-pair formation between the polyglutamate ion and the more abundant cation present occurs in large degree.

TABLE 6. SEDIMENTATION COEFFICIENTS OF POLYGLUTAMATE ($c \rightarrow 0$) IN VARIOUS SOLVENTS

solvent	specific gravity (20° C) ρ_s/ρ_{H_2O}	relative viscosity (20° C) η_s/η_{H_2O}	$10^{-13}/s$	$10^{13} s\eta_{rel.}$
0·1 M-NaCl	1·0074	1·0316	0·254	3·94
1·0 M-NaCl	1·043	1·1116	0·213	4·70
5·0 M-NaCl	1·193	1·897	0·308	3·25
1·0 M-KCl	1·048	1·009	0·198	5·05
2·75 M-KCl	1·125	1·029	0·250	4·00
1·0 M-RbBr	1·127	0·968	0·188	5·33
1·0 M-NaCl in D_2O	1·148	1·371	0·235	4·25

Precise knowledge of the extent to which ion-pair formation occurs can hardly be obtained from these data, but recent work on other polyelectrolytes suggests that it may be considerable. For example, Kraut (1954) has shown by analysis of the results of Howard & Jordan (1954) on sodium polymethacrylate that an effective degree of ionization of 0·37 must represent a maximum value, and that in the presence of moderate salt concentrations it is probably much less than this. Electrolytic transference experiments on sodium polyacrylate (Huizenga, Greiger & Wall 1950) point to a similar state of affairs in this case also. It seems possible therefore, by analogy, that polyglutamic acid may be expected to behave in a similar fashion and the sedimentation results fall into line with this point of view.

(ii) *Sedimentation in sodium chloride at different salt concentrations*

The sedimentation coefficients ($c \rightarrow 0$) at different ionic strengths appear at first sight anomalous. This is due to the fact that changing ionic strength results in changes in two factors which affect the rate of sedimentation in opposite senses. Increasing ionic strength results in increasing density of solvent which retards sedimentation but also in contraction of the coil with accompanying decrease in the frictional factor and hence acceleration of sedimentation. There is good reason, both from the practical and the theoretical standpoint, for regarding the frictional force opposing sedimentation as being proportional to the linear dimensions of the particle (see, for example, Flory 1953). Thus the sedimentation coefficients may be corrected to rates in 0·1 M-sodium chloride as far as frictional effects are concerned by multiplying by the one-third power of the ratio of the intrinsic viscosities (table 7). Assuming a linear dependence of $s\eta_0$ (where s has been so corrected) on the density of the medium, extrapolation of data at 1 M- and 5 M-sodium chloride gives a value of 1·44 as the density at which zero sedimentation may be expected to occur.

Applying a buoyancy correction to these figures on the assumption that the sedimenting particle has a constant density of 1·44 over the range 0·1 to 5 M (see below), the s values corrected to sedimentation in 0·1 M-NaCl are obtained (see last column of table 7). Fairly

good agreement thus results, and this supports the belief that the density of the sedimenting particle of sodium polyglutamate is of the order of 1·5 (the small effects due to electrostriction (table 5) have been ignored in this treatment).

TABLE 7. SEDIMENTATION COEFFICIENTS OF SODIUM POLYGLUTAMATE IN SODIUM CHLORIDE

solvent	intrinsic viscosity	$\frac{[\eta_0]}{[\eta_0']}$	$\left(\frac{[\eta_0]}{[\eta_0']}\right)^{\frac{1}{3}}$	$10^{13}s\eta_0$	$10^{13}s\eta_0\left(\frac{[\eta_0]}{[\eta_0']}\right)^{\frac{1}{3}}$	buoyancy correction factor	$10^{13}s$ (corr.)
0·1 M-NaCl	4·00	1·00	1·00	3·94	3·94	1·00	3·94
1·0 M-NaCl	2·20	1·82	1·22	4·70	3·85	1·08	4·17
5·0 M-NaCl	1·55	2·58	1·37	3·25	2·37	1·60	3·96

(iii) *Sedimentation in heavy water*

From equation (9) it is evident that the numerical value for the correction factor for solvation ($k = a(\rho_a - \rho_s)$) will be sensitively dependent on the values of s, D and V. Nevertheless, provided that D_2O quantitatively replaces H_2O, an estimate of the order of magnitude of this correction should be possible by comparing the rates of sedimentation of polyglutamate at zero concentration in H_2O and D_2O under otherwise identical conditions. Assuming that a is unchanged in going from H_2O to D_2O,

$$\frac{s_{(H_2O)}\eta_{0(H_2O)}}{s_{(D_2O)}\eta_{0(D_2O)}} = \frac{M_{(H_2O)}\{1-V_{(H_2O)}\rho_{s(H_2O)}+a(\rho_{a(H_2O)}-\rho_{s(H_2O)})\}}{M_{(D_2O)}\{1-V_{(D_2O)}\rho_{s(D_2O)}+a(\rho_{a(D_2O)}-\rho_{s(D_2O)})\}}$$
$$= \frac{M_{(H_2O)}(1-V_{(H_2O)}\rho_{s(H_2O)}+k)}{M_{(D_2O)}(1-V_{(D_2O)}\rho_{s(D_2O)}+k)},$$

if D_2O quantitatively replaces H_2O in the solvate. Morowitz & Chapman (1955) have shown that the hydrogen atom of the amide link in peptides is freely exchangeable with deuterium. At infinite dilution of the solute this exchange must be complete, and hence there will be one deuterium atom per repeating unit and $M_{(D_2O)}/M_{(H_2O)}$ will have the value 1·006. If, as seems probable, the molar volume is unaltered by this exchange, the apparent partial specific volume will be reduced by the same factor. $V_{(H_2O)}$ is 0·506 and hence $V_{(D_2O)}$ will be 0·503. Substituting these values together with figures from table 6 gives $k = +0{\cdot}01$, a value which, in view of the experimental errors involved, is probably not distinguishable from zero. It appears from further investigation that k probably should have a small negative value ($-0{\cdot}027$). An error of 1 % in the ratio of the sedimentation constants would be sufficient to account for this discrepancy. Despite the uncertainties of the treatment, it is evident that the correction factor for selective solvation at an ionic strength of 1·1 is not large.

(iv) *Sedimentation in electrolyte solutions at higher densities*

Experiments have been made in potassium iodide, rubidium iodide and rubidium bromide over a wide range of density. In addition to their high solubility, these alkali halide solutions have the virtue in the present studies of small viscosity decrements even at high molarities. Pronounced concentration gradients were set up in the medium itself in these experiments and the synthetic boundary cell was essential in obtaining a clear picture of the sedimenting polyglutamate boundary. The sedimentation coefficients, corrected in each case for the viscosity of the medium, are plotted against the density of

the medium in figure 12 for potassium iodide and rubidium iodide. It is seen that the relationship is linear in each case but that the two lines are displaced from each other showing zero sedimentation at a density of 1·32 in potassium iodide and 1·47 in rubidium iodide. This discrepancy may be due in part to the greater secondary charge effect in the case of potassium iodide. With potassium iodide of density 1·40 negative sedimentation of polyglutamate was evident from its accumulation at the meniscus, although the calculation of the sedimentation coefficient was hardly possible on account of the extremely rapid spread of the boundary.* Evidence of a large secondary charge effect has been obtained in studies on a different sample of sodium polyglutamate (*PGA* 2). With this sample, sedimentation measurements in 0·1 M-sodium chloride and 0·1 M-sodium iodide (each containing $I = 0{\cdot}01$, pH 8 phosphate buffer), in which solvents buoyancy corrections are negligible,

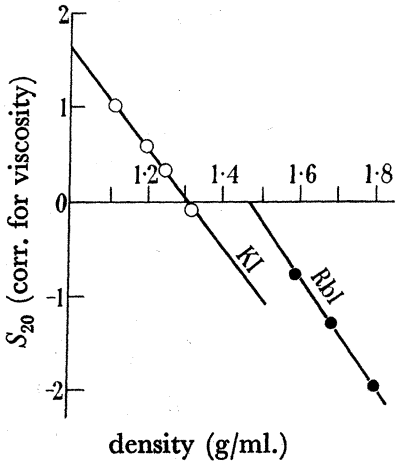

FIGURE 12. Plot of $s\eta_0$ against ρ_s (the density of the medium) for sodium polyglutamate (0·5 g/100 ml.) in solutions of potassium and rubidium iodides.

lead to values of $(s_{20})_{c\to 0}$ of 3·85 and 2·905 S, differing by 0·85 S. This difference is to be attributed to secondary charge effects, and a secondary charge effect of this magnitude is to be expected in a 0·5 % solution of polyglutamate in the high concentration of potassium iodide at density 1·4 (4·4 M).

All attempts to form a boundary in potassium iodide of density 1·5 failed, whether the polyglutamate solution occupied the upper or lower section of the cell. The experiments at densities in excess of 1·5 were carried out in rubidium iodide. Sedimentation took place towards the meniscus in each case, and the schlieren pictures showed the sedimenting boundary as a peak below the base-line instead of in the normal position above owing to the refractive index of the solvent exceeding that of the sedimenting material.

As already pointed out, the sedimenting substance probably consists largely of the ion pair rather than the polyglutamate ion, and the discrepancy in the apparent particle density in potassium iodide and rubidium iodide may be partly the result of the differing densities of the potassium and rubidium salts of polyglutamic acid.

* In this case the rapidly spreading boundary observed was probably due largely to an inequality in the potassium iodide concentrations between solution and solvent as a result of the hydration of the initially anhydrous sodium polyglutamate added, together with the very low refractive increment of polyglutamate in potassium iodide of this concentration.

The complications due to secondary charge effects in the two solvents potassium iodide and rubidium iodide are practically absent in rubidium bromide. The variation in the sedimentation coefficient of a 0·2 % solution of sodium polyglutamate (corrected only for the viscosity of the medium) in rubidium bromide over a wide range of density is plotted in figure 13. Here again the relationship over the density range examined (1·125 to 1·62) is linear within the limits of error of measurement. The point showing negative sedimentation at density 1·625 also shows an inverted peak, and it is evident that in the neighbourhood of the particle density the particle and the solvent have the same refractive index. At such a point even if the difference in density between particle and solvent were enough to permit the formation of a stable boundary it would not in fact be observed.

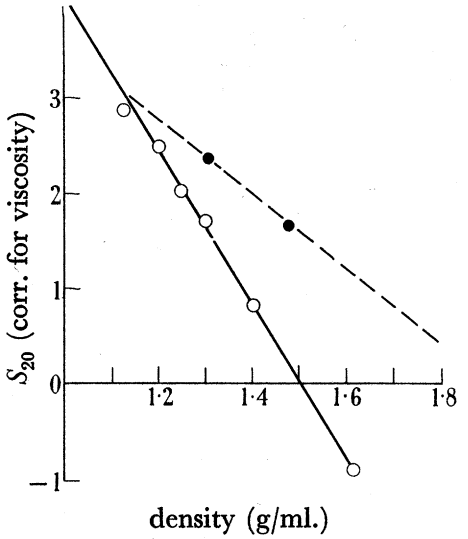

FIGURE 13. Plot of $s\eta_0$ against ρ_s (the density of the medium) for sodium polyglutamate (0·2 g/100 ml.) in solutions of rubidium bromide (○); ●, M-RbBr + chloral hydrate.

The straight line through the experimental points indicates zero sedimentation at a density of 1·505, and it may at least be said with certainty that in rubidium bromide solution of this composition the particle has a density of 1·505. If, as we believe is the case, the particle consists very largely of the ion pair, rubidium polyglutamate, its anhydrous density may be calculated from the measured value of the potassium salt in 4·3 M-potassium iodide, i.e. 0·561 (table 5), and the known difference for the potassium and rubidium ions (Harned & Owen 1950) as 2·12. It is evident therefore that the particle in 4 M-rubidium bromide must carry with it as solvate a surplus of about 58 % of its own weight of water attached to it, in addition to an unknown volume of the solvent which may be entrained, enmeshed or attached. As Schachman & Lauffer (1950) have stressed, one cannot conclude from this result that the sedimenting particle has the same surplus amount of water associated with it in say 1·0 M-rubidium bromide, and they point out that a linear relationship between $s\eta_0$ and ρ_s can still exist even when the density of the solvate changes with ρ_s provided it does so in a linear manner.

If such a change is taking place in this case, it may reasonably be expected that the amount of surplus water in the solvate would increase with falling concentrations of salt in the medium. However, a consideration of the experimental results leads one to conclude

that the amount is unlikely to vary over very wide limits. For example, the fact that the particle is sedimenting rapidly inwards in rubidium iodide at density 1·8 shows that it still has a substantial surplus of water attached to it, since the anhydrous density of the particle is 2·12. A linear change in the amount of this surplus water with ρ_s, the density of the medium, could not therefore result in more than about 100 % surplus water of solvation in molar salt, and the actual figure is almost certainly less than this.

It seems reasonable to argue from these results therefore that the surplus amount of water attached to the particle in molar salt is unlikely to exceed the weight of the particle and is probably nearer the minimum figure of 60 %.

(v) *Sedimentation in non-electrolyte solutions at higher densities*

The effect of change in the density of the medium on the sedimentation rate in the presence of molar potassium chloride and sodium chloride without appreciable change in ionic strength has been studied with the aid of chloral hydrate. The results are shown in figure 14 for sodium polyglutamate at a concentration of 0·15 %. As already seen from figure 11, there is very little difference in the sedimentation rate in 1·0 M-potassium chloride and 1·0 M-sodium chloride over a range of concentration and particularly at 0·15 %. In the presence of substantial concentrations of chloral hydrate, however, a very pronounced difference is observed. A linear relation between $s\eta_0$ and the density of the medium is again observed in both cases, but while with sodium chloride zero sedimentation occurs at a density of 1·46, in good agreement with the value already deduced from measurements in sodium chloride at different molarities, the particle in potassium chloride is still sedimenting rapidly (1·8 S) at this density, and zero sedimentation, as seen by extrapolation, does not occur until the high density of 1·94 is reached. The only significant difference in the two experiments at density 1·4 (figure 14) lies in the different cation, sodium and potassium, present, and it would appear that in the presence of high concentrations of chloral hydrate of high osmotic activity water still remains associated with the sodium polyglutamate complex, while in the case of potassium polyglutamate it does not. It must follow in the latter case that the potassium polyglutamate particle is losing water with increasing concentration of chloral hydrate at a rate proportional to the chloral hydrate concentration. Rubidium polyglutamate behaves in a similar fashion to the potassium salt as seen from the line included in figure 13, extrapolation giving a value of 1·9 for the density at zero sedimentation.

The density of anhydrous sodium polyglutamate in 1·0 M-sodium chloride was measured as 1/0·506 (table 5), from which the value for potassium polyglutamate in 1·0 M-salt can be calculated (Harned & Owen 1950) as 1·94, in excellent agreement with the value extrapolated from the chloral hydrate/potassium chloride line (figure 14).

(vi) *The slope, K, of* $1/s$ *against* c

As already pointed out, the change in $1/s$ with c appears to be linear over the concentration range and in the various solvents examined, i.e.

$$\frac{1}{s} = \frac{1}{s_0}(1+Kc),$$

where K is the specific slope, i.e. $(1/s - 1/s_0)/(1/s_0)$ in each case.

Newman & Eirich (1950) have shown that in the case of polystyrene fractions in various solvents a strong parallelism exists between K and the intrinsic viscosity, $[\eta]$ (see also Bisschops 1955; Wales & van Holde 1954).

It is of interest to note that a similar parallelism exists in the case of sodium polyglutamate in different concentrations of sodium chloride, e.g. 0·1, 1·0 and 5·0 M (see table 1). The ratio $K/[\eta]$ is unchanged when D_2O is substituted for H_2O in 1·0 M-sodium chloride.

A similar parallelism holds also in the case of potassium chloride (1·0 and 2·75 M), although the ratio $K/[\eta]$ is different from that in sodium chloride.

It appears therefore that whatever governs $[\eta]$ in any given salt is also responsible for the proportional change in $1/s$ with c.

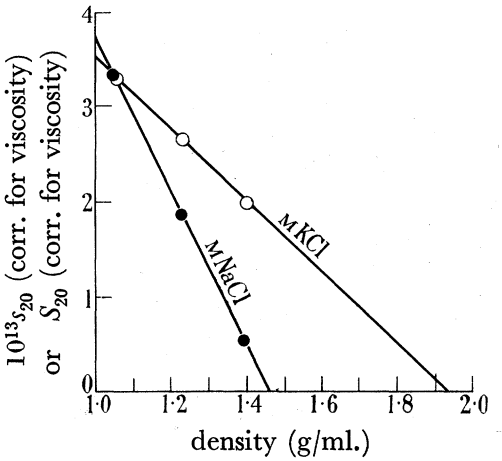

FIGURE 14. Plots of $s\eta_0$ against ρ_s (the density of the medium) for sodium polyglutamate (0·15 g/100 ml.) in solutions of chloral hydrate (of varying concentration) which were also molar with respect to sodium chloride or potassium chloride.

(vii) *The molecular weight*

The identity of the values for the particle density of the sodium salt of polyglutamic acid as deduced both from the sedimentation behaviour in sodium chloride of different density and in 1·0 M-sodium chloride plus chloral hydrate supports the view that the particle has a constant density of 1·45. On the assumption that it has in fact this density in 1·0 M-sodium chloride, and taking $4{\cdot}70 \times 10^{-13}$ as the value of s_{20} at infinite dilution and $D_0 = 1{\cdot}31 \times 10^{-7}$ in 1·0 M-sodium chloride, both being corrected for the viscosity of the medium to water, we obtain for the anhydrous molecular weight (equation (9)) the figure 197 000, where $a = 0{\cdot}59$, the net surplus volume of water, over and above any solvation by the medium, attached to each gram of the anhydrous particle to reduce its density to 1·45.

As already pointed out, the linearity of $s\eta_0$ against ρ_s up to densities closely approaching $1/V$ (1·975) (see figure 12) shows that the particle still retains appreciable water of hydration even at these high densities. Taking the extreme case in which hydration approaches zero at density $1/V$, the linear change in a with ρ_s required by the linear $s\eta_0$ against ρ_s plots found by experiment leads to $a = 1{\cdot}0$ in 1·0 M-sodium chloride corresponding to a particle density of 1·33 and $M = 205\,000$. Thus, assuming precise values for s, D and V it can be stated with some certainty that the anhydrous molecular weight lies between 197 000 and 205 000 and is very probably near the lower figure corresponding to a particle which has firmly attached

to it a net surplus of water, over and above any solvation by the medium, equal to about 60 % of the dry weight of the particle.

From what has already been said, we believe these molecular weights approximate to those of the sodium salt rather than the polyglutamate ion owing to extensive ion-pair formation. On this basis the molecular weight of this sample of polyglutamic acid lies between 170 000 and 177 000, with the emphasis on the lower figure.

(viii) *Sedimentation measurements on the free acid*

The free polyglutamic acid was prepared from the sodium salt (same sample as used throughout this work) by an ion-exchange method in order to avoid any degradation resulting from low pH. Reconversion of the acid back to the sodium salt showed the absence of any degradation by its identity in respect of viscosity, sedimentation (see figure 11) and light scattering with the sodium salt from which it was originally prepared.

TABLE 8. SEDIMENTATION COEFFICIENTS OF POLYGLUTAMIC ACID IN 1·0 M-SODIUM CHLORIDE

concentration (g/100 ml.)	$10^{13}s_{20}$ (corr. for viscosity only)
0·254	5·00
0·342	4·73
0·540	4·84
0·735	4·77
0·958	4·71

$$(s_{20})_{c\to 0} = 5{\cdot}00.$$

The sedimentation coefficients at various concentrations in 1·0 M-sodium chloride are given in table 8. In sharp contrast to the sodium salt, only a small variation of s with c is found. A consequence of this very low concentration effect is the absence of any appreciable boundary sharpening, and the rapid spread of the sedimenting boundary compared with the low natural diffusion constant (2×10^{-7}) is strong evidence of the polydisperse nature of the acid and therefore presumably of its sodium salt. The value of s_{20} ($c\to 0$) extrapolated from the results of table 8 is $5{\cdot}00\times 10^{-13}$ (corrected only for the viscosity of the medium). A mean value of 0·663 (table 5) was obtained for the partial specific volume in 1·0 M-sodium chloride, which in the absence of solvation corresponds to a particle density of 1·51. On the assumption that no selective solvation occurs in the case of the free acid, these data, together with a value $D_0 = 2{\cdot}28\times 10^{-7}$ substituted in the usual Svedberg formula, give a value of 172 000 for the molecular weight. The question of hydration and its effect on this estimate of the molecular weight of the free acid has not been further explored. The value given is a minimum and agrees well with the estimate derived from the value for the sodium salt.

(ix) *The frictional ratio and the particle volume*

Taking 197 000 as the molecular weight of the anhydrous sodium salt, the frictional ratio f/f_0 may be calculated as 5·0. This high value is in accord with the high intrinsic viscosity of the sodium salt and points to a particle occupying a volume many times greater than the actual volume of sodium polyglutamate comprising it. In view of the absence of any marked dependence of viscosity on shear, the degree of asymmetry of the particle is evidently not large.

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Direct evidence of the large volume occupied by the particle is seen in the large 'pellet' volume, i.e. the volume of the sediment at the bottom of the cell in the sedimentation studies on salts of polyglutamic acid, e.g. at $c = 0{\cdot}105$ g/100 ml. in 0·1 M-sodium chloride the pellet deposit has a volume of 76 ml./g of the dry sodium polyglutamate. This large particle volume is not, as we have already shown, the result of hydration, since this amounts to only about 60 % of the dry weight of the particle. It must therefore be made up by the solvent, either attached to the particle or merely occupying the free space within its boundaries.

The free polyglutamic acid has a frictional ratio of 2·6 suggesting, in accord with viscosity measurements, that the particle in this case, although occupying a volume which is large in relation to that of the polyglutamic acid skeleton, is considerably smaller than that of the ionized form. The variation in s and D with concentration is also correspondingly less.

(x) *Sedimentation: résumé*

Sedimentation studies on the sodium, potassium and rubidium salts of polyglutamic acid show in every case a large increase in s with falling concentration. Plotted as $1/s$ against c a good linear relation is observed in various salt solutions of various molarities over the range of concentration examined. Perhaps the most striking feature of the sedimentation results is the role played by the cation even when primary and secondary charge effects are negligible. Thus, in salt solutions at molar strength we find the s_{20} $(c \to 0)$ value (corrected only for the viscosity of the medium) increases on going from sodium chloride to potassium chloride to rubidium bromide in spite of the increasing density of the medium. The effect of the cation is even more pronounced in the measurements in molar sodium chloride and potassium chloride in the presence of chloral hydrate; where sodium is the cation sedimentation ceases at a density of 1·46, while in the presence of potassium the particle is still sedimenting at a rapid rate. A substantial degree of ion-pair formation between the polyglutamate ion and the cation present in large excess appears to be the most likely explanation of these results. The chloral hydrate results are explicable on this basis if it is supposed that the sodium ions retain the solvated water in the complex against the osmotic effects of chloral hydrate while potassium ions fail to do so. Further evidence of the effect of the cation is seen in the ratio of the slope K of the $1/s$-c lines (table 1) to the intrinsic viscosity. This quotient, which is substantially constant in either sodium chloride or potassium chloride, is different in the two solvents, even though at molar strength the intrinsic viscosity, the ionic strength and the solvent density are practically the same in both cases. These changes are difficult to account for if the same species is sedimenting in every case.

Sedimentation studies over a range of density show that, in the case of sodium polyglutamate, the particle is solvated with a surplus of between 60 and 100 % of its dry weight of water over and above any of the medium so attached. This conclusion allows the particle weight of the sodium salt of this particular specimen of polyglutamic acid to be calculated as within the range of 197 000 to 205 000, and this estimate agrees well with the value of 172 000 obtained for the free acid if extensive ion-pair formation in the case of its salts is assumed to occur. The error in M resulting from uncertainty in the degree of hydration is, in this case, comparable in magnitude with the cumulative errors of s, D and V.

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The large frictional ratio, as also the large intrinsic viscosity of sodium polyglutamate, point to a particle possessing a very large effective volume since, in view of the absence of any marked dependence of viscosity on shear rate, there is no pronounced asymmetry of shape. Indications of polydispersity are deduced from sedimentation and diffusion studies on the free acid.

7. General discussion

The chemical evidence on which the structure of bacterial poly-D-glutamic acid is based has been recently reviewed (Waley 1955). There seems little doubt that the glutamic acid units are γ linked and that no detectable amount of α linkage occurs. Sachs & Brand (1953, 1954) have shown that the γ peptide bond in small glutamyl residues is not remarkably more susceptible to hydrolysis than a normal amide linkage, and this conclusion is supported by partial acid hydrolysis studies on polyglutamic acid by Zwartouw (1955). On the other hand, the results of Hanby & Rydon (1946) and some viscosity results reported in this paper indicate the existence of bonds relatively susceptible to acid hydrolysis. Whilst it is possible to conceive a mechanism whereby certain γ peptide linkages in a long polypeptide could become more susceptible to hydrolysis than the same linkage in smaller polypeptides, it remains possible that there are a few acid-sensitive links of a non-amide type in the polypeptide isolated from natural sources.

The absence of detectable amounts of α linkage precludes significant amounts of chain-branching by condensation between the α carboxyl groups of one chain with the free amino group of another. Waley (1955) has reported a number-average molecular weight for the sample of polyglutamic acid *PGA* 1 of 100 000 by end-group assay of free amino groups. This value is consistent with the sedimentation-diffusion average molecular weight of 172 000 reported in this paper and agrees well with a number-average molecular weight derived from light-scattering data. The agreement of the light-scattering number average, the evaluation of which rests on the assumption of non-branched chains, with the end-group-assay number average, offers additional confirmation of the statement that there is no substantial amount of chain branching due to the condensation of the carboxyl groups of one chain with any other grouping of another chain, since there would then be more than one NH_2 group per molecule. It seems reasonable, therefore, to conclude that natural polyglutamic acid as isolated is essentially a linear polymer.

It is of some interest to calculate Flory's constant (1953, p. 627), $P^{-1}\Phi^{\frac{1}{3}}$, from the equation

$$P^{-1}\Phi^{\frac{1}{3}} = \frac{\eta_0 (M[\eta])^{\frac{1}{3}}}{f'_{c=0}}.$$

In 1·0 M-salt, $f'_{c=0}$ cannot be evaluated by means of the Svedberg equation, since this has been shown not to apply on account of selective solvation, but it can be replaced by kT/D_0, giving

$$P^{-1}\Phi^{\frac{1}{3}} = \frac{D_0 \eta_0 (M[\eta])^{\frac{1}{3}}}{kT}.$$

At ionic strength 1·1, $D_0\eta_0 = 1{\cdot}31 \times 10^{-9}$, $M = 2{\cdot}05 \times 10^5$, $[\eta] = 2{\cdot}2$ leads to a value of $P^{-1}\Phi^{\frac{1}{3}} = 2{\cdot}5 \times 10^6$, in good agreement with other values of this constant for random coil molecules.

Some information concerning the properties of the polyglutamate coil at ionic strength 1·1 can be obtained by comparing the intrinsic viscosity of the present sample in 1·0 M-potassium chloride (2·25) with the values for two specimens of bacterial polyglutamic acid already examined by Record & Wallis (1956). These samples, of molecular weights 96 000 and 278 000, gave intrinsic viscosities of 0·97 and 3·00 respectively. A plot of log $[\eta]$ against log M is linear for these figures, and from the slope a value of 1·06 is obtained for the value of the exponent a in the empirical relation

$$[\eta] = KM^a.$$

This value is higher than the average for uncharged random coils and approximates to the free-draining model.

We have not been able to make a precise estimate of the degree of polydispersity of this sample. The spread of the peak in the ultracentrifuge in the case of the free acid suggests a considerable degree of polydispersity, but, obviously, information derived from specimens of the alkali salts would be of greater value. Unfortunately, however, the artificial sharpening that takes place with such salts in the ultracentrifuge prevents a similar analysis. The curvature of the plots of Kc/R_{90} against c in the light-scattering studies suggests that plots of Π/c against c would also exhibit considerable curvature, and thus preclude an accurate estimate of the number-average molecular weight by osmotic pressure measurements. A reasonable estimate of the polydispersity is, however, possible if the interpretation of the light-scattering data is correct, and this suggests a distribution approximating to $M_z : M_w : M_n :: 11{\cdot}7 : 2{\cdot}7 : 1$.

Two points of considerable practical importance emerge from this work. The charge density in this polymer is as high as appears probable for any naturally occurring material. Despite this, the degree of selective solvation in solution is not large. The use of the Svedberg formula, using values of the sedimentation and diffusion coefficients suitably extrapolated to infinite dilution and neglecting the effect of selective solvation leads to values for the anhydrous molecular weight of the alkali salts which are not grossly in error. For example, at ionic strength 1·1 they are about 10 % low and at lower ionic strengths the error will be less. While it would be dangerous to generalize from this one case, it seems likely that similar results would be obtained in comparable cases.

The second point concerns the secondary charge effect, which is shown in § 6 to be of considerable magnitude, even at relatively low ionic strengths (0·11). At this ionic strength, sodium phosphate itself produces a secondary charge effect of about the same magnitude and sign as sodium iodide. Evidently in sedimentation studies, buffers must either be chosen with great care to minimize this effect or used in the presence of a substantial excess of electrolytes such as sodium or potassium chlorides or rubidium bromide.

It is not established that polyglutamic acid is the only constituent of the capsule of *B. anthracis*, and, in the absence of a detailed knowledge of the structure of the capsule, any discussion of its function therein must be speculative.

Provided that the molecular weight of the native material in the capsule is not many times greater than that of the isolated material it may appear surprising that a substance as water-soluble as polyglutamic acid should be present in massive amounts in a bacterial capsule. It is, of course, possible that every molecule is attached, directly or indirectly, to

the cell wall by covalent links, but the physical characteristics of the material appear to make such a universal assumption unnecessary. Strong electrostatic forces will exist between polyglutamic acid and the polar regions of the cell surface and/or some protein framework in the capsule and hydrogen bonding may play a part. Unless this (conjectured) protein framework were very extensive, we are of the opinion that ordinary diffusive forces would preclude the retention in the capsule of massive amounts of a molecule of the size of the isolated material. It seems likely, therefore, that either the molecules of polyglutamic acid in the capsule are much larger or that they are constrained. It is probable that the native material has a more rigidly defined configuration than that of the random coil structure that is present in solutions of the isolated material, since the latter is non-antigenic whereas injection of whole capsulated organisms of *B. anthracis* will give rise to antibodies which precipitate with polyglutamic acid. It appears likely that *some* covalent links are involved in the retention of the native configurations.

It seems pertinent to consider briefly if the physical or chemical properties of polyglutamic acid are consistent with the known biological properties of the bacterial capsule. Despite the high macroscopic viscosity of solutions of polyglutamate, the 'open-work' structure shown by this work suggests that small ions and molecules may be able to move freely through the macromolecular domain, a possibility that is in accord with the fact that the organisms continue to metabolize freely despite heavy capsulation. Possible interactions between polyglutamic acid and naturally occurring basic polypeptides have been discussed by Katchalski (1950). We have observed that polyglutamic acid precipitates with basic polypeptides (e.g. clupeine, licheniformin and lysozyme), and it seems likely that the presence of this material in the capsule will prevent or impede the attack of basic lytic agents. However, it has not, as yet, been established that lysis plays any major role in the removal of invading organisms *in vivo*. On the other hand, Zwartouw & Smith (1956) have shown that polyglutamic acid has an antiphagocytic action. It is, however, certain that polyglutamic acid is not solely responsible for virulence in *B. anthracis* infections. Capsulated strains have been reported which, though resistant to phagocytosis, are either non-virulent or of low virulence (Sterne 1937; Bruce White 1946) and Smith, Keppie, Ross & Stanley (1954) have shown that a specific toxin plays a major part.

To sum up, polyglutamic acid seems to play a contributory role in virulence, and its physical and chemical characteristics do not appear to be at variance with the known properties of the bacterial capsule of *B. anthracis*.

We wish to thank Dr A. G. Ogston, F.R.S., and Dr A. R. Peacocke for helpful discussions and express our indebtedness to Messrs P. Fleming, K. H. Grinstead and J. A. Stirrup for much expert technical assistance.

Appendix A. The effect of electrostriction in solvation

Schachman & Lauffer (1950) have considered the effect of solvation on sedimentation without explicitly examining the effect of electrostriction. A result of the same general form as that of these authors can be obtained even if electrostriction occurs. Consider a particle of anhydrous mass, m, which would occupy a volume in solution, v, in the absence of solvation. Let it be placed in a solvent of two components (water and salt) of density ρ_s and let

it become solvated with a ml./g of a mixture of components, the density of which in free solution would be ρ_a. Let this solvate become electrostricted so that it has a mean density ρ_e, and let the mean density of the resulting solvated particle be ρ_p. The volume of the solvated particle is $v+(am\rho_a/\rho_e)$ and the driving force in sedimentation is proportional to

$$\left(v+am\frac{\rho_a}{\rho_e}\right)(\rho_p-\rho_s).$$

If s is the sedimentation coefficient and f' the frictional coefficient per molecule

$$s=\left(v+am\frac{\rho_a}{\rho_e}\right)(\rho_p-\rho_s)/f'.$$

Multiplying top and bottom of the right-hand side by Avogadro's number and denoting the anhydrous molecular weight by M and the molar frictional coefficient by f,

$$s=M\left(\frac{v}{m}+a\frac{\rho_a}{\rho_e}\right)(\rho_p-\rho_s)/f.$$

The apparent partial specific volume V of the solute is given by

$$V=\frac{v}{m}-a+a\frac{\rho_a}{\rho_e},$$

assuming that the effects of electrostriction in such cases are limited to the solvate and do not affect the free solvent.

Hence

$$\frac{v}{m}+a\frac{\rho_a}{\rho_e}=V+a.$$

Substituting for f, RT/D, where D is the diffusion coefficient and converting to zero concentration of solute, leads to

$$s_0=\frac{MD_0}{RT}(V+a)(\rho_p-\rho_s).$$

ρ_p is given by

$$\rho_p=(1+a\rho_a)\Big/\left(\frac{v}{m}+a\frac{\rho_a}{\rho_e}\right). \tag{10}$$

Hence

$$s_0=\frac{MD_0}{RT}(1+a\rho_a-V\rho_s-a\rho_s)$$

$$=\frac{MD_0}{RT}\{1-V\rho_s+a(\rho_a-\rho_s)\}, \tag{11}$$

which, with a slight difference in the definition of a and ρ_a is identical with the equation of Schachman & Lauffer. This result is in accord with the conclusions of Baldwin & Ogston (1954) who by thermodynamic reasoning showed that electrostriction effects do not alter the sedimentation equations in the case of a two-component system.

It should be noted that if highly accurate values of s_0, D_0 and M can be obtained a minimum value for a can be deduced, since ρ_a cannot be less than 0·998, the density of pure water at 20° C. If in addition a reliable value of ρ_p can be deduced at any point other than the point of zero sedimentation, equations (10) and (11) are soluble for both ρ_a and a.

Appendix B. The partial specific volume of the polyglutamate ion

The fact that charged organic molecules have smaller partial specific volumes than their uncharged isomers containing the same or very similar groupings has long been known and gives rise to the phenomenon known as 'electrostriction'. However, no values for ions as such have been calculated. Provided the value for one ion were determinable, others should be deducible in view of the well established fact that molecular volumes are in the main additive. There is some qualitative evidence that the size of the potassium ion and of the chloride ion in solution is similar and since both are of similar weight and lightly, and probably about equally, hydrated (Glueckauf 1955), their densities are probably nearly equal. Quantitative support for this conclusion is given by the application of Gorin's (1939) equation to the data of Shedlovsky (1932) and Longsworth (1932). This leads to values of the ionic radii in solution of 1·978 Å for K^+ ion and 1·933 Å for Cl^- ion. The ratio of the density of the K^+ ion, ρ_{K^+} to that of the chloride ion ρ_{Cl^-} is then given by

$$\frac{\rho_{K^+}}{\rho_{Cl^-}} = \frac{39}{1{\cdot}978^3} \Big/ \frac{35{\cdot}5}{1{\cdot}933^3} = 1{\cdot}02.$$

To a first approximation it seems permissible to regard the two ions as of equal density. From the known differences in molecular volumes of the series of alkali halides it can also be shown that if the densities of the K^+ ion and Cl^- ion are approximately equal so are the densities of the Rb^+ ion and of the Br^- ion, a conclusion which is used in considering the secondary charge effect in sedimentation.

The use of Masson's (1929) equation

$$\phi_v = \phi_v^0 + S_v\sqrt{c},$$

with values of ϕ_v^0 and S_v for potassium chloride (see, for example, Harned & Owen 1950) leads to a molal volume of potassium chloride at 1·0 M strength of 28·85. The equivalent volumes of K^+ ion and Cl^- ion, assuming equal densities, would therefore be 15·12 and 13·73 respectively at this concentration. The molal volume of sodium chloride can be similarly calculated and is 18·55, leading to a partial specific volume of the Na^+ ion of 0·209. Using this value, a sodium content of 13·6 % and the above-quoted value for the partial specific volume for sodium polyglutamate in 1 M-sodium chloride (0·506) leads to a value of 0·55(4) for the partial specific volume of the polyglutamate ion.

Weber's statement (1930) that ionization of an organic acid is accompanied by a contraction of 10·3 ml. per mole offers an alternative derivation of a value for the partial specific volume of the polyglutamate ion from that of the free acid, namely, 0·663. According to published values, the molal volume of hydrochloric acid at 0·1 M concentration is 18·45. This figure, combined with the equivalent volume of the chloride ion at 0·1 M concentration, gives a value of 5·5 for the equivalent volume of the H^+ ion at this concentration. Since the equivalent of polyglutamic acid is 141 and its partial specific volume is 0·663, its molal volume is 93·4, and the equivalent volume of the polyglutamate ion is

$$93{\cdot}4 - 10{\cdot}3 - 5{\cdot}5 = 77{\cdot}6.$$

This, divided by its equivalent, 140, gives 0·55(4) as the partial specific volume of the ion.

In order to avoid the errors involved in the use of derived values for the partial specific volumes of the Na^+ and H^+ ions, an attempt has been made to relate the volume of the polyglutamate ion directly to that of the K^+ ion. The volume increase attendant on the dissolution of free polyglutamic acid in a $\frac{1}{3}$ M solution of dipotassium hydrogen phosphate has been measured. It is 0·758 ml./g determined by the usual pyknometric method. The change involved can be represented by the equation

$$HPO_4^{2-} + H\bar{A} = H_2PO_4^- + \bar{A}^-$$

($\bar{A}$ = polyglutamate radical), and the volume increase represents the sum of the volumes occupied by the polyglutamate ion and of the change HPO_4^{2-} to $H_2PO_4^-$. The volume accompanying the latter change can be calculated from a knowledge of the molecular volumes of dipotassium hydrogen phosphate and potassium dihydrogen phosphate and the calculated value of the K^+ ion which corrected to M/5 is 14·4. The measured values at M/5 (the mean in the final solution) of the partial specific volume of dipotassium hydrogen phosphate and potassium dihydrogen phosphate were respectively 0·306 and 0·157, giving molecular volumes of 27·3 and 41·6 respectively, thus

$$2\phi_v(K^+) + \phi_v(HPO_4^{2-}) = 27{\cdot}3,$$
$$\phi_v(K^+) + \phi_v(H_2PO_4^-) = 41{\cdot}6.$$

The volume change accompanying the conversion of 1 equivalent of HPO_4^{2-} to $H_2PO_4^-$ is thus an increase of $41{\cdot}6 - 27{\cdot}3 + \phi_v(K^+) = 14{\cdot}3 + 14{\cdot}4$, i.e. 28·7 ml. Since the equivalent of polyglutamic acid is 141, the volume increase due to the conversion of HPO_4^{2-} to $H_2PO_4^-$ resulting from the dissolution of 1 g polyglutamic acid will be $28{\cdot}7/141 = 0{\cdot}204$ ml. Hence the partial specific volume of the polyglutamate ion is $0{\cdot}758 - 0{\cdot}204 = 0{\cdot}55(4)$. The coincidence to the third significant figure of the values by the three methods is certainly fortuitous but it seems that this figure represents a reasonable approximation.

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