POLYELECTROLYTES AND THEIR BIOLOGICAL INTERACTIONS

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ABSTRACT Polyelectrolytes are water-soluble electrically charged polymers. Their properties are determined by the interplay of the electrical forces, the Brownian motion of the macromolecular chain, and intermolecular Van der Waals forces. Charged polyacids or polybases are stretched by the electrostatic forces, as evidenced by increase in solution viscosity, or by the stretching of polyelectrolyte gels. The electrical field of the polyions is neutralized by a dense atmosphere of counter-ions. The counter-ion attraction to the polyions is expressed by a reduction of the osmotic activity of the polyion—the osmotic pressure being only 15 to 20 per cent of the ideal in highly charged polyelectrolytes neutralized by monovalent counter-ions, and as low as 1 to 3 per cent of the ideal for polyvalent counter-ions. Since the ionic atmosphere is only slightly dependent on added low molecular salt, the osmotic pressure of polyelectrolyte salt mixtures is approximately equal to the sum of the osmotic pressure of polyelectrolyte and salt alone. Acidic and basic polyelectrolytes interact electrostatically with precipitation at the point of polymeric electroneutrality. At higher salt concentrations the interaction is inhibited by the screening of polymeric fixed charges. The importance of these interactions in enzymatic processes is discussed. The electrical double layer is polarizable as may be deduced from dielectric and conductometric studies. The polarizability leads to strong dipole formation in an electrical field. These macromolecular dipoles may play a role in the adsorption of polyelectrolytes on charged surfaces. The final part of the paper is devoted to interactions of polyelectrolytes with cell membranes and the gluing of cells to higher aggregates by charged biocolloids.

1. INTRODUCTION

1.1 Classical colloid chemistry profoundly influenced biological thought in the early twentieth century and then seemed to undergo an irreversible decline during the nineteen thirties. Both biochemists and biophysicists lost interest in the "World of Neglected Dimensions" (1) and turned their attention to more rewarding fields of research. It was felt that a direct study of cell components and their metabolic transformations could provide deeper insights into living processes than the physicochemical characterization of ill-defined particles with colloidal dimensions. The situation changed drastically, however, in the early thirties when polymer chemists

and physicists provided a conceptual framework for the understanding of the behavior of biological macromolecules. The realization that biocolloids are chain molecules, the structure of which can be analyzed in terms of well defined patterns of chemical bonds, has bridged the gap betweeen molecular physics and chemistry and cellular biology. It has paved the way for a new colloid chemistry—the colloid chemistry of macromolecules.

In 1938 Kern (2) published a couple of papers devoted to the study of electrically charged synthetic polymers. The polyacids studied by him exhibited the normal properties of polymers in solution, such as high specific viscosity, but were in addition typical electrolytes capable of conducting electrical current and giving electrode reactions. It was soon realized that the polymeric electrolytes are suitable models for the understanding of the electrochemical behavior of biopolymers; in 1947 several laboratories launched a long range investigation of the solutions and gels of these new colloids (3–7). Fuoss proposed the suitably fitting name of "polyelectrolytes" for the group of substances which, from the molecular point of view, are polymers while their electrochemical properties bring them into the realm of electrolytes. The subject of this paper is to review simply the results of the study of polyelectrolytes based primarily on the work done in the author's laboratory, although the writer recognizes clearly the conscious and subconscious impact of the ideas and results of other workers to whom only inadequate tribute can be paid in this short review.

1.2 From the electrochemical standpoint polyelectrolytes can be classified as polyacids, polybases, or polyampholytes. Their ionizable groups may be acidic-carboxylic, sulfonic, or phosphoric groups, or they may be basic amino groups, free or substituted. The ionizable groups may be ionized to various degrees and thus behave as partial or full salts. Around the fixed ionized polymeric groups there is generally to be found an atmosphere of small ions composed primarily of *counterions* and to a smaller extent of *coions* due to low molecular weight salts which may be added to the polyelectrolyte solution.

On the other hand, for the polymer chemist, polyelectrolyte molecules may be highly polymerized, or less so, and their backbone may be linear or branched. The monomeric units composing the macromolecular chain may be arranged in random sequence or they may be built in a stereospecific array. Some of the polyelectrolytes, and notably numerous biocolloids, have a crystalline, helical, pattern and may melt to form amorphous, more or less randomly kinked macromolecules.

The notion of a polyelectrolyte has a classificatory advantage and enables us to study diverse substances within a common methodological framework.

Thus, many of the gums and resins carried by the caravans of the Far East since early antiquity owe their usefulness to their polyelectrolyte components. Many important plant products such as the pectic acids of fruit jellies and alginic acid of seaweed are typical polyelectrolytes. The important polyuronic acids from animal

organisms such as chondroitin sulfuric acid, mucoitin sulfuric acid, hyaluronic acid, and heparin can be regarded as polyelectrolytes. The polyelectrolyte nature of some polyacid and polybase polypeptides endows them with inhibitory activity in enzymatic reactions; and the electrochemistry of nucleic acids is determined to a large extent by the interplay of the numerous charged groups situated on their polyelectrolytic macromolecular helix.

The interactions of natural polyelectrolytes play an important role in the inner organization of the cell and the binding of cells in the organism. It is not only the chromosomes, which can be regarded as a reaction product of a polybase with a polyacid, but the structure of many cell membranes and the intracellular reticulum can be attributed to forces operating between oppositely charged polyions. In addition, as polyelectrolytes and their interactions are responsible for many of the properties of connective tissue, it is appropriate for the present symposium to get a more general view of the physical chemistry of charged macromolecules and of their interactions.

2. SHAPE DYNAMICS OF POLYELECTROLYTE MOLECULES

2.1 As a first demonstration of the joint operation of polymeric and electrical properties within polyelectrolyte molecules, we shall consider the shape dynamics of charged polymolecules. A "geometrical" macromolecule—one in which intramolecular forces play no role, performs a very complicated set of movements around its carbon-to-carbon bonds. Thermal collisions with the medium cause the molecular chains to perform rotary movements which cause constant alterations in the shape of the macromolecule; this dynamic shape resembles the random walk of the statisticians, or the Brownian movement of a single colloid particle. In the polymeric coil the steps of the random walk are the directions of the units or segments of the polymeric chains, while the statistical average of the macromolecular dimension is proportional to the square root of the number of units.

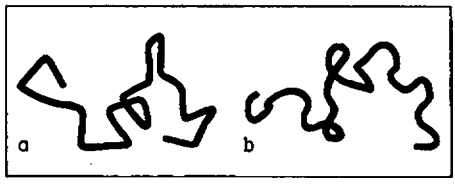

FIGURE 1 Model representation of randomly kinked macromolecules.

In a "physical" macromolecule the monomeric units of the polymer interact with each other and it is to be expected that in a polyacid of the type of polyacrylic acid

$$-(CH_2-\underset{\displaystyle COOH}{\underset{|}{CH}}-)_n$$

there will exist numerous hydrogen bonds between the carboxylic groups, which will tend to contract the molecular chain and diminish its dimensions below the statistical average. On the other hand, if some of the acidic groups be ionized, by the addition of a base such as sodium hydroxide to the solution, a strong repulsion develops between the negative carboxylate ions—COO^- which tends to stretch the macromolecule and open up the random coil. Further ionization of the acidic groups stretches the molecules to a large extent and endows parts of the chain with a cylindrical symmetry.

A suitable indicator for the changes in shape occurring upon ionization is the specific viscosity η_{sp} of polyelectrolyte solutions.[1]

Solutions of spherical macromolecules present a lower resistance to flow so that the η_{sp} of their solutions is small; on the other hand, if the molecules stretch and approach a rod-like shape the frictional dissipation of energy accompanying their flow increases and correspondingly η_{sp} reaches high values. This behavior is shown in Fig. 2 based on the measurements of Eisenberg where the specific viscosity of polymethacrylic acid is plotted against the degree of ionization.[2]

It will be observed that at all polymer concentrations, the specific viscosity changes slowly up to $\alpha = 0.15$. This slow change indicates that in this range of ionization the electrostatic repulsion is insufficient to overcome the hydrogen bonding of the carboxylic groups keeping the molecules coiled. From $\alpha = 0.15$ to $\alpha = 0.8$ there is a rapid and marked increase in η_{sp} which indicates that molecules have opened up and stretched; finally at still higher degrees of ionization there is a slight decrease in η_{sp} due to the increase in the concentration of counter-ions. Indeed, the effect of the electrostatic stretch is highest at lowest polymer and counter-ion concentration. High concentrations of counter-ions mask the repulsive effect of the counter-ions so that lower stretching effects are to be expected.

Even if the polymeric molecules have an organized helical structure, such as is found in many polypeptides, the electrostatic repulsion of charged groups may suffice to overcome the attractive forces and bring the helix to melting with subsequent stretching. This is shown in the recent measurements of Goldstein (8) on polyglutamic acid. Fig. 3 shows that up to a degree of ionization of 50 per cent, the helical structure can withstand the strong repulsion of the fixed charges. At higher α's the helix breaks down and the molecules stretch to an appreciable extent. As expected, the addition of neutral salt screens off the polymeric charges and diminishes the repulsion so that the stretching effect is lower at higher salt concentrations.[3]

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[1] Specific viscosity η_{sp} is defined as the ratio of viscosity of the solution η minus the viscosity of the solvent to that of the solvent η_o; $\eta_{sp} = \eta - \eta_o/\eta_o$. The ratio of specific viscosity and polymer concentration, at very low concentration, is known as "intrinsic viscosity" $\mathrm{Lim}_{c \to o} \eta_{sp}/c = [\eta]$ and it is this quantity which is directly related to polymer volume (*cf.* Flory).

[2] $\alpha = \dfrac{\text{No. of ionized carboxylic groups}}{\text{Total No. of carboxylic groups}} = \dfrac{\nu}{P}$

[3] A word of caution should be interjected on the viscometric measurements: the data underly-

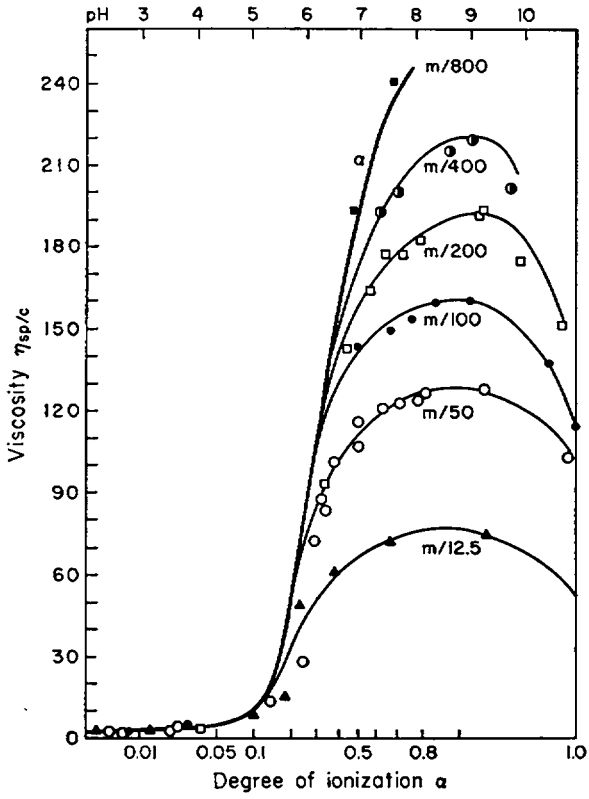


FIGURE 2 The specific viscosity of polymethacrylic acid solutions (η_{sp}/c) as functions of the logarithm of the degree of ionization. Each curve represents a different concentration of the poly acid (16).

2.2 The interplay of electrostatic repulsion and thermal coiling or intramolecular attraction makes it possible to convert chemical into mechanical energy by changing the shape of the macromolecules. This conversion can be explained by means of imaginary operation of a single macromolecule. Let the macromolecule be hooked up by one end and let the other end carry a commensurately small weight. Let the polymolecule be a negatively charged polyacid in a stretched state and have a length 1. Now let us add to the molecule a mineral acid to provide hydrogen ions to combine with the ionized carboxylate groups and transform them into undis-

ing Figs. 2 and 3 were obtained with the ordinary Ostwald viscometer in which rather large stresses develop in the solution. Several authors and notably Eisenberg (9) and Hermans and Hermans (10) have shown that the viscosity of polyelectrolytes is non-Newtonian, the results being strongly dependent on the rate of shear. Valid results can be obtained by measuring the viscosity at low velocity gradients and extrapolating the results to gradient zero. Although the absolute values of the extrapolated values differ markedly from those given in Figs. 2 and 3 the dependence on α remains essentially of the same type.

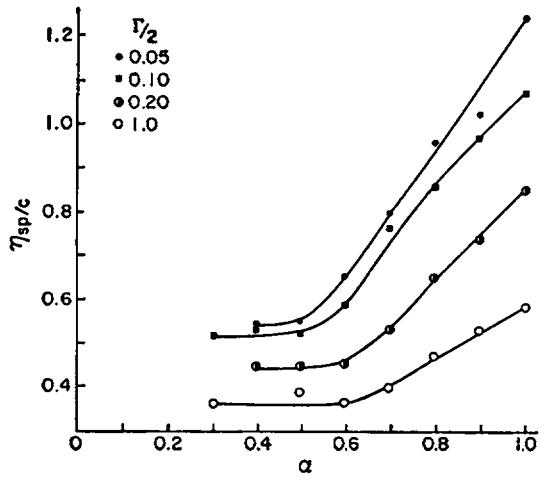


FIGURE 3 The specific viscosity of linear polyglutamic acid in aqueous solutions of sodium chloride. ($\Gamma/2$ = 0.05; 0.10; 0.20 and 1.0 molar) (8).

sociated carboxylic groups according to the reaction $RCOO^- + H^+ \rightleftharpoons RCOOH$. By means of this reaction, the electrostatic repulsion which kept the macromolecule in a highly stretched state vanishes and instead the Brownian motion and intramolecular attraction cause a coiling up of the polymeric chains. Upon coiling, the polymolecule contracts and lifts the attached weight through a distance Δl. On lifting the weight, mechanical work $f \cdot \Delta l$ was performed, the origin of the work being the chemical interaction of, say, Δn moles of hydrogen ions, with the fixed charges of the polyion.

This mental experiment demonstrates the possibility of transforming chemical energy into mechanical work in an isothermal process. Such mechanochemical transformations, which are the basis of all mechanical performances of the living body, were first carried out on synthetic polyelectrolyte systems (11–13). There is a profounder reason as to why chemical energy can be converted directly and readily into mechanical energy by means of reactive polymers and cannot be observed in every chemical reaction, despite the fact that there is no thermodynamical restriction on the transformation of any chemical free energy into useful work. The possibility of mechanochemical conversion is determined by the existence of long range interactions within the molecules of the system. These long range interactions must be able to change the dimensions of the system upon changing the extent of reaction and, *vice versa,* a change in dimensions must cause a corresponding variation in the degree of reaction. Such behavior is expected from cooperative systems, made of an ensemble of numerous interacting units, in which interactions depend not only on local events but also on the system as a whole. From a cybernetic point of view, the molecular conversion of chemical into mechanical energy requires a feed-back mechanism which can provoke an over-all response of the system to events taking place at any point within the molecules. Polyelectrolytes are admirably suited to ful-

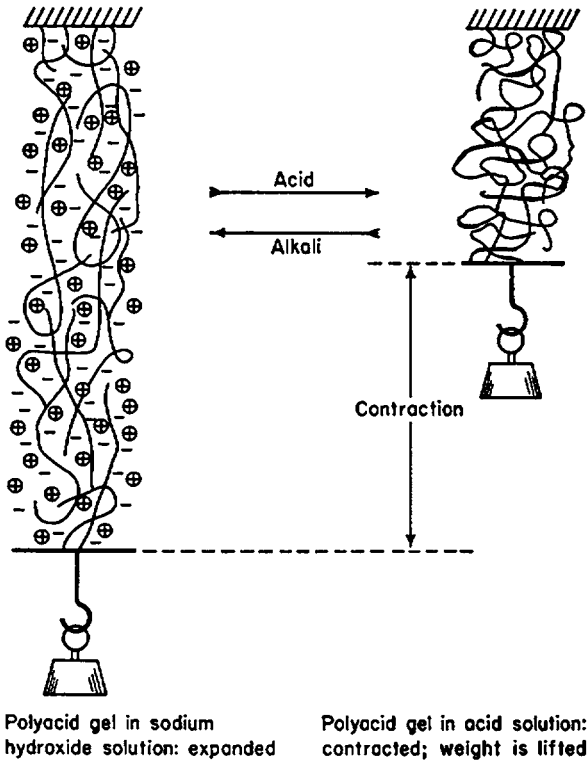


FIGURE 4

fil these requirements, for electrostatic forces are long range forces and any change in the local degree of ionization causes a corresponding change in the over-all electrostatic field. This change in field intensity causes in its turn either a change in dimensions or the development of a contractile force. It is, however, not the performance of a single molecule which interests the biophysicist and the polymer chemist. To make the phenomena observable on a macroscopic scale, one has to treat a larger assembly of macromolecules, cross-linked with suitable bonds. Fig. 5 shows that analogous to the operation of single macromolecules gels expand upon ionization, and contract upon diminishing the number of charged groups (14). In the presence of neutral salts, which screen the fixed charges and reduce the repulsive forces, the degree of expansion decreases so that maximum swelling and contraction are obtained in salt-free systems. A further analysis of the equilibrium and kinetic behavior of mechanochemical systems (15) is beyond the scope of this paper.

Having thus become acquainted with the regulation of shape of polyelectrolyte molecules by electrostatic fields, we may turn our attention to more conventional aspects of the physical chemistry of polyelectrolyte solutions.

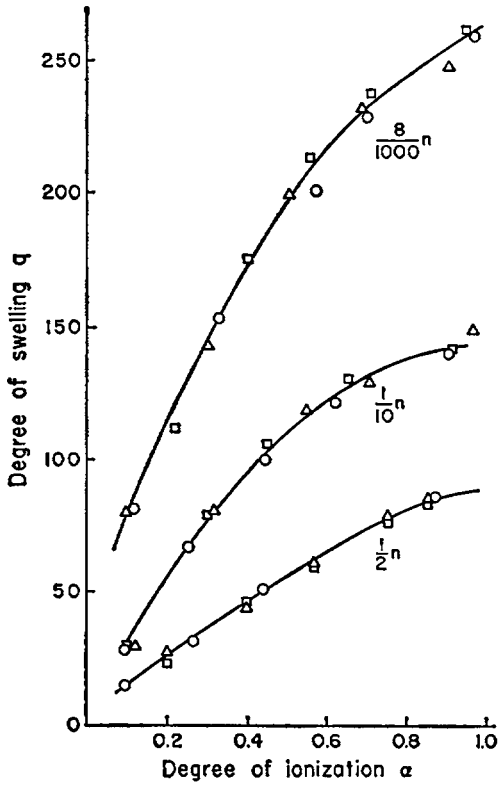


FIGURE 5 Degree of swelling (q) of a cross-linked polymethacrylic acid gel *versus* degree of ionization (α). Each curve was obtained at different ionic strengths of a monomonovalent salt with which the gel maintained equilibrium. □, LiCl; △, NaCl; ○, KCl (14).

3. OSMOTIC PROPERTIES OF SALT-FREE POLYELECTROLYTE SOLUTIONS

3.1 The theoretical analysis of an electrochemical system such as a polyelectrolyte solution is generally based on the evaluation of the electrostatic potential created by the charged molecules. If the potential is known it is possible to evaluate the distribution of the counter-ions in solution, the density of the double layer surrounding the polymeric ion, and the osmotic pressure of the solution at any concentration and degree of ionization. There exists, however, no general method for calculating the potential of macromolecules with a variable random shape. For lack of any better alternative, we attribute to the molecule some symmetry properties which allow the solution of the equations of electrostatics and the evaluation of the electrostatic potential. Earlier studies of colloid particles often used the assumption that the surface of the particle is endowed with planar symmetry (*cf.* Verwey and Overbeek (16) in their fundamental treatment of stability of colloidal solutions). Polyelectrolyte molecules were often treated as permeable spheres (3, 17, 18). More recently however, there has been a growing recognition that the symmetry closest to reality is the cylindrical. There is now little doubt that at higher degrees

of ionization, sections of the polymeric chains can be treated as little rods surrounded by a cylindrical atmosphere even if the macromolecule as a whole has a more or less random shape. A rigorous solution for the potential ψ is available (19) for charged cylindrical polyions in salt-free solutions. It is depicted in Fig. 6. In the figure we have plotted the potential ψ *versus* the distance r from the center of the cylinder.[4]

It will be observed that the potential drops rapidly with distance from the polyion

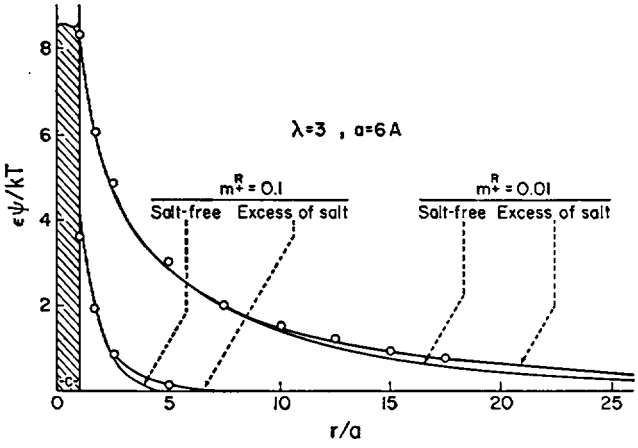


FIGURE 6 The dependence of the electrostatic potential ψ on the distance r from the center of the macroion. Relative units were used for both the potential ψ ($e\psi/kT$) and for the distance r (r/a) where a is the radius of the polyelectrolyte cylinder ($a =$ 6 A). The curves were calculated for both salt-free solutions and in excess of salt when the counter-ions were maintained at 0.01 molar or 0.1 molar concentration (25).

and approaches constant (zero) values at a distance of 15 to 50 A from the center of the polyelectrolyte depending on concentration. An illuminating aspect of this picture is the electrostatic field in the neighborhood of the polyion. The field intensity is the gradient of the electrostatic potential and is given in our case by $\partial\psi/\partial r$. For highly charged polyelectrolytes the electrical forces reach the tremendous values of 300 million volts per cm at the surface of the macromolecules! There is no wonder that these powerful electrostatic forces attract an appreciable fraction of the counter-ions and create a rather dense atmosphere of lower mobility.

3.2 A direct expression of counter-ion immobilization, or "ion fixation," by the polyion is given by the osmotic pressure of a polyelectrolyte solution. An ideal solution containing n_p moles of polymeric ions per unit volume, with each polymer

[4] For the sake of convenience we use the ratio of electrical and thermal energy $e\psi/kT$ instead of the potential where e— is the unit electrical charge, and the distance (r) is given in units of the radius of the cylinder a; *i.e.* r/a.

ion carrying ν charges and neutralized by ν counter-ions should have an osmotic pressure given by van't Hoff's law as

$$\pi_{\text{ideal}} = (n_p + n_p\nu)RT \tag{1}$$

In equation (1) we assume that the polymeric ions and the counter-ions contribute fully and independently to the osmotic pressure, and since generally a polyelectrolyte molecule carries many hundreds of fixed charges $n_p\nu \gg n_p$ or

$$\pi_{\text{ideal}} = n_p\nu RT \tag{2}$$

It will be noted that equation (2) indicates that practically all the osmotic pressure of a salt-free polyelectrolyte solution may be attributed to the small counter-ions—the contribution of the large polyions being a negligible fraction. Moreover, since the number of counter-ions per macromolecule is large, we would expect neutral polyelectrolytes such as nucleic acids or polyuronic acids to have osmotic pressures comparable to those of low molecular weight neutral salts. Experimentally it is found that equations (1) and (2) do not fit the measured osmotic pressures and hence, in order to "save the phenomena" we have to introduce a coefficient, the osmotic coefficient φ_p, which, when multiplied by the ideal value, should give the observed osmotic pressure:

$$\pi = n_p\nu\varphi_p RT \tag{3}$$

As expected, the values of φ_p are appreciably lower than unity, which means that a large fraction of the counter-ions is immobilized and does not contribute to the osmotic pressure. Theory predicts that for highly charged polymers φ_p should approach values of 0.1 to 0.2, so that only 10 to 20 per cent of the counter-ions are osmotically active and up to 90 per cent are fixed in the neighborhood of the polyion. My colleague Alexandrowicz has carried out a large series of measurements on ionized polyacids to determine the values of φ_p as a function of concentration and degree of ionization (20). He found that φ_p is only slightly dependent on concentration while it decreases markedly with ionization. Indeed in a wide range φ_p is almost inversely proportional to the number of charged groups per polymer (ν) so that the product $\nu\varphi_p$ is approximately constant. From the point of view of osmotic measurements $\nu\varphi_p$ is the effective charge density of the polyelectrolyte and equals the number of counter-ions (per polymolecule) effective osmotically. It is thus found that polyelectrolytes and charged biocolloids have, in a wide range of ionization, a more or less constant osmotic contribution per macromolecule: you may add a neutralizing reagent and change the actual degree of ionization but the polyelectrolyte acts as an osmotic buffer and opposes any change in osmotic pressure.

Several values of φ_p for different polyelectrolytes are assembled in Fig. 7. It will be observed that all the values fall on two curves which can be interpreted theoretically. The upper curve fits the more rigid macromolecules whose average end-to-end distance is about three-fourth of the fully stretched length, while the lower curve is

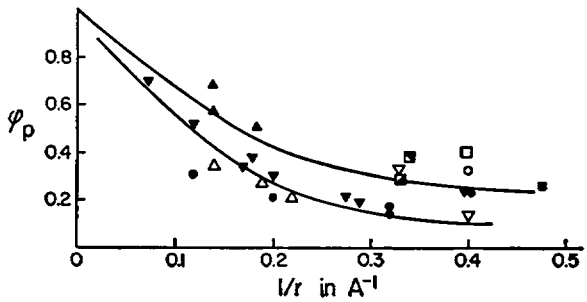


FIGURE 7 The osmotic coefficient φ of various polyelectrolytes as a function of the degree of ionization. Upper curve for stiffer polymers, lower curve for flexible polymers.

▲ NaCMC, sodium carboxymethyl cellulose
△ AgCMC, silver salt of carboxymethyl cellulose
▽ NaCS, sodium salt of cellulose sulfate
○ Na(MA-VA), sodium salt of copolymers of maleic anhydride and vinyl alcohol
□ Cl-PVA, polyvinyl amine chlorohydrate
◪ NaPSS, sodium polystyrene sulfonate
◨ H PSS, hydrogen polystyrene sulfonate
▼ NaPVS, sodium salt of polyvinyl sulfonic acid
● NaPMA, NaPAA, sodium salts of polymethacrylic and polyacrylic acids
■ NaPP, sodium polyphosphate

The points are from various sources, the curves are calculated (57).

that of flexible molecules whose length even at high degrees of ionization is only half that of the fully stretched one. The remarkable fact about all the points assembled in the figure is the slight dependence of the osmotic behavior on the specific properties of the polyions. Polyacids of different acidic groups and various polymer backbones, as well as polyamines of comparable degrees of ionization, exhibit very similar osmotic behavior. It is thus clear that φ_p is controlled primarily by electrostatic interactions rather than by specific bonding or association. Another noteworthy conclusion is that φ_p is independent of molecular weight; it is the degree of ionization and extent of molecular stretching which control the osmotic activity but not the absolute length of the polyions or the total number of the ionized groups on the macromolecule.

3.3 The osmotic results are corroborated by measurements of light-scattering by polyelectrolyte solutions. According to a well known theory of Einstein (21) adapted by Debye to polymer solutions the fraction of the light scattered at 90° — R^{90}—is given by the following equation

$$1 + \nu\varphi_p = \frac{K \cdot c \cdot M_p}{R^{90}} \tag{4}$$

where K is a constant dependent on temperature, refractive index, and wavelength, c is the polyelectrolyte concentration in grams per milliliter, and M_p is the molecular weight of the polymer. It is evident that R^{90} is maximal when $\nu = 0$. In this case the

polyelectrolyte solutions show the high values of light-scattering found in ordinary polymer solutions. With increasing ν, R^{90} goes down and at high degrees of ionization, the light-scattering drops to the low values observed in simple salt solutions. This is the reason why slight contaminations such as those caused by traces of dust or bacterial growth distort the experimental results in highly ionized solutions. If, however, due precautions are taken, equation (4) becomes a useful tool for the determination of the osmotic coefficient for polyelectrolytes of known M_p and ν. The results obtained by Alexandrowicz (20) correspond closely to those of direct osmometry and have been put on the same curves in Fig. 7.

4. COLLIGATIVE PROPERTIES OF POLYELECTROLYTE SOLUTIONS CONTAINING LOW MOLECULAR WEIGHT SALTS

4.1 The interest of the physiologist and biochemist in salt-free solutions of polyelectrolytes is rather limited. Native polyelectrolyte biocolloids are generally surrounded by a complicated mixture of ionic components so that pure polyion solutions are regarded primarily as a theoretical reference state. To approach more closely systems of biological importance, we shall consider here the osmotic behavior of mixtures of highly charged macromolecules with low molecular weight salt. This paragraph is devoted to the influence of monomonovalent salt on the osmotic pressure of the mixture.

A beginner in physical chemistry would expect the osmotic behavior of a polyelectrolyte salt mixture to be simply additive. Indeed, the very name "colligative properties" indicates the collaborative additivity of the partial osmotic pressures contributed by the different solutes in solution. The more sophisticated electrochemist is more cautious: he might suspect screening effects based on the electrostatic interaction of polyelectrolyte and low molecular weight salt and would expect these interactions to obviate simple additivity. Experiments and subsequent theory have proven, however, the beginner to be correct. From the work of Mock and Marshal (21), the measurements of Nagasawa *et al.* (22), and the extensive study of Alexandrowicz (23), it can be stated that the osmotic pressure of a mixture of polyelectrolyte and monovalent salt is almost exactly the sum of the partial osmotic pressures of the components. If the osmotic pressure of the salt-free polyelectrolyte solution be denoted by π_p, and that of the corresponding salt solution, free of polyelectrolytes, be denoted by π_s then the osmotic pressure of the mixture is

$$\pi = \pi_p + \pi_s \tag{5}$$

Introducing for π_p its value from equation (2) and for π_s

$$\pi_s = 2n_s\varphi_s RT \tag{6}$$

we get for the osmotic pressure of the mixture

$$\pi = RT(n_p + n_p\nu\varphi_p + 2n_s\varphi_s) \tag{7}$$

For practical use n_p may be neglected and φ_s may be set as approximately equal to unity. Then.

$$\pi = RT(n_p \nu \varphi_p + 2n_s) \tag{8}$$

The striking fact about equation (7) is that the osmotic coefficient φ_p is the same for salt-free and salt-containing polyelectrolyte solutions. Thus a knowledge of the φ_p's given in Fig. 8 permits the evaluation of the colligative behavior of polyelectrolytes in a wide range of salt concentrations, comprising *inter alia* the physiological isotonic case. The extent of agreement of measured with calculated values can be seen in Fig. 8 where the full curves are calculated from equation (7) and

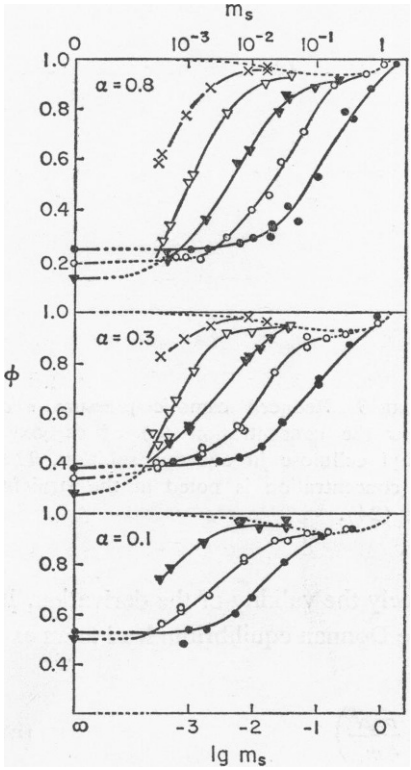


FIGURE 8 The osmotic coefficient φ of solution mixtures of polymethacrylic acid and sodium bromide. The x axis is the logarithm of the salt concentration log m_s and the y axis gives the values of φ. Three degrees of ionization $\alpha = 0.1$, 0.3, and 0.8 of the polyacid are represented at different monomolar concentrations, ●, 0.45; ○, 0.09; ▼, 0.018; ∇, 0.0036; and ×, 0.0007. The points are experimental while solid lines are calculated from osmotic additivity (23).

the points are experimentally determined. The fit is very satisfactory in the range of salt concentrations from 1 molar to 10^{-4} molar, polymer concentrations from 0.45 to 7×10^{-4} molar, and α's 0.1, 0.3, and 0.8.

4.2 An important quantity which can be readily derived from equation (7) is the Donnan osmotic or "oncotic" pressure. This is the difference in osmotic pressure between a polyelectrolyte-salt solution and an external salt solution with which it

maintains equilibrium across a semipermeable membrane. The calculated value of the osmotic pressure π_{on} is

$$\pi_{on} = \frac{RTm_u}{1000}\left(\frac{1}{P} + \frac{m_u(\alpha\varphi_p)^2}{4m_s}\right) \tag{9}$$

where m_u is the monomolar concentration[5] of the polyelectrolyte, P its degree of polymerization, and m_s the molality of the salt in the external solution. Upon plotting (π_{on}/m_u) $(1000/RT)$ *versus* m_u for a series of polyion solutions which maintain equilibrium with the same salt solution, there result straight lines whose intercept gives the molecular weight of the polymer $(1/P)$ and whose slope $((\alpha\varphi_p)^2/4m_s)$ provides further means of determining the factor φ_p. Fig. 9 reproduces the results of

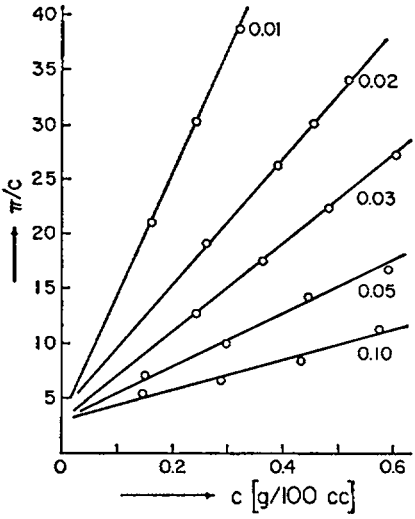


FIGURE 9 Reduced osmotic pressure π/c *versus* the concentration (c) of carboxymethyl cellulose in aqueous solution. The salt concentration is noted at the straight lines (24).

Inagaki and Hirami (24) which demonstrate nicely the validity of the derivation. It is worth noting that the classical equations for the Donnan equilibrium lead to an expression similar to equation (9)

$$\pi_{on} = \frac{RTm_u}{1000}\left(\frac{1}{P} + \frac{m_u\alpha^2}{4m_s}\right) \tag{10}$$

The factor missing in equation (10) is φ_p^2 so that the ideal straight lines obtained with equation (10) should have a slope $1/\varphi_p^2$ times larger than those represented in Fig. 9. Since, for highly charged polyelectrolytes $1/\varphi_p^2 = 25\text{-}100$, the discrepancy between the real and ideal cases is rather large.

4.3 A stringent test for the validity of equation (7) is the Donnan distribu-

[5] Monomolar means molar concentration in monomer units.

tion of salt across a semipermeable membrane. The distribution is generally characterized by the coefficient

$$\Gamma = \frac{m_s' - m_s}{m_p} \tag{11}$$

where m_s' is the concentration of salt in the polyelectrolyte compartment and m_p the molar concentration of the polymer. The classical colloid chemical expression is $\Gamma = \frac{1}{2}$; however, equation (7) leads to the much lower value of $\Gamma = \varphi_p/2$. Many experimental studies have shown that although Γ is definitely lower than $\frac{1}{2}$, it is neither equal to $\varphi_p/2$ nor constant, but increases with increasing salt concentration. Although a fuller analysis of the Donnan distribution coefficient would take us too far afield, it is important to learn that equation (7) does not withstand the "Γ-test" and cannot be regarded as precise enough for all purposes. To cover the whole field of the colligative properties of polyelectrolyte solutions more comprehensive, though less convenient, expressions are required (25).

5. INTERACTION OF POLYELECTROLYTES WITH DIVALENT COUNTER-IONS

5.1 Throughout the discussion of the previous paragraphs we assume the counter-ions to be non-specific electrical point charges which create an atmosphere around the central polyion. For inorganic monovalent ions this assumption is a fair approximation to reality and any differences which were observed could be attributed to the different radii of the hydrated counter-ions (26).[6] On turning our attention however, to divalent ions, specific factors become very prominent. As an indicator of specificity we may consider the osmotic coefficient φ_p for various ions. Table I gives the values of φ_p for the interaction of several monovalent and divalent ions

TABLE I

Monovalent ions	φ_p	Divalent ions	φ_p
Na^+	0.40 ± 0.05	Mg^{++}	0.15 ± 0.2
K^+	0.35 ± 0.05	Ca^{++}	0.01 ± 0.05
NH_4^+	0.27 ± 0.05	Cu^{++}	0.03

with alginic acid (27). It is readily seen that while the φ_p's for the monovalent ions do not differ appreciably and indicate that in all cases about 30 per cent of the counter-

[6] With polarizable ions and organic counter-ions there is specificity of binding even with monovalent counter-ions. Van der Waals interactions of these ions with biocolloids may give rise to large changes in free energy ΔG, enthalpy (ΔH), and entropy (ΔS). Thus Klotz (28) found that the reaction of sulfonated azo dyes with proteins is accompanied by $\Delta G = -6$ -7 kcal/mol dye, ΔH — 2kcal/mole, and ΔS — 14-17 e.u./mole. These interesting and important effects will not be followed up in this paper

ions are "free," in the case of divalent ions there are about 15 per cent free Mg^{++} in solution while for Ca^{++} only 1 per cent is osmotically active and 99 per cent are bound to the alginate ion.

For such strong degrees of ion binding it is appropriate to assume an association presumably localized at definite sites along the polymeric chain. This general idea of ion association with the formation of neutral ion pairs was advanced several years ago by Harris and Rice (29). Although association in the strict sense does not seem to hold for most monovalent counter-ions, there is no doubt of its validity and usefulness for divalent counter-ions. Morawetz (30), Gregor (31), Wall (32), and their coworkers studied complexes of polyions with strongly complexing ions and derived the equations for the determination of the equilibrium constants characterizing the interaction. In some cases, there is a superposition of a well defined complex formation with a more diffuse attraction of the divalent ions to non-specific groups. Thus Carr (33) proved rather conclusively that out of the 100 carboxylic groups of bovine serum albumin only 8 participate in true complex formation with Ca^{++} ions and the other 92 groups form loose combinations of the ordinary electrostatic type. It may be assumed that the 8 complexing groups are situated so as to favor stereochemical fitting of the Ca^{++} into the reaction sites. It seems that the strong and specific combination of Ca^{++} with polyions may be responsible for some of the biological properties of this ion. Since the work of Roux (34) and Herbst (35), it has been known that Ca^{++} is an essential constituent for the adhesion of cells and its removal by oxalic acid or by versene (36) causes disaggregation of tissue into single cells. On noticing that the intracellular cement is rich in mucopolysaccharides of polyelectrolyte nature, we may speculate that the role of Ca^{++} in this case is the formation of intermolecular bridges gluing the cells together. In order to get a better grasp of the ionic cross-linking of polyelectrolyte ions, we have to discuss the precipitating and bonding action of the divalent ions.

Precipitation of polycarboxylic acids by alkaline earth cations has been known for many years. Thus alginic and pectic acids precipitate with Ca^{++}, presumably by the formation of fixed cross-links (37). Wall and Drenan (38) studied the precipitation of polyacrylic acid with Ca^{++}, Sr^{++}, and Ba^{++} ions. This latter study was followed further by the work of Michaeli (39) who determined the critical concentration of the divalent ions required to cause the precipitation of a polyelectrolyte in the presence of a given concentration of mono-monovalent salt. It was found that in all cases the concentration of divalent ions required to produce an incipient precipitate increases linearly with the concentration of the polyion. At higher sodium chloride concentrations, larger amounts of calcium ion were required to cause precipitation. At zero monovalent salt concentration and for higher degrees of ionization, the critical concentration of Ca^{++} bears a strikingly simple relation to the concentration of the polymer.

$$n_{Ca^{++}}^{crit.} \simeq 0.8 \cdot n_p \cdot \nu$$

Since $n_p\nu$ is the total number of fixed polymeric charges, the last equation means that when the Ca++ has neutralized over 80 per cent of the charged polymeric groups, precipitation sets in. This remarkable observation is corroborated by independent measurements on swelling and contraction of polyelectrolyte gels in mixtures of mono- and divalent counter-ions. Zwick (40) found that a highly swollen gel of polymethacrylic acid neutralized by sodium hydroxide undergoes gradual shrinkage when part of the sodium counter-ions are exchanged by Ca++ or Ba++. When the divalent ions have exchanged with 80 to 85 per cent of the monovalent counter-ions, there is a dramatic reduction of the degree of swelling from 90 times to 4 times the dry volume. This contraction by Ba++ ions is shown in Fig. 10. In

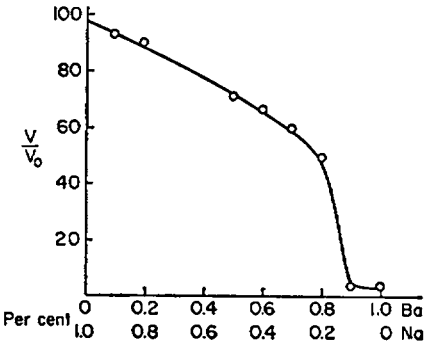
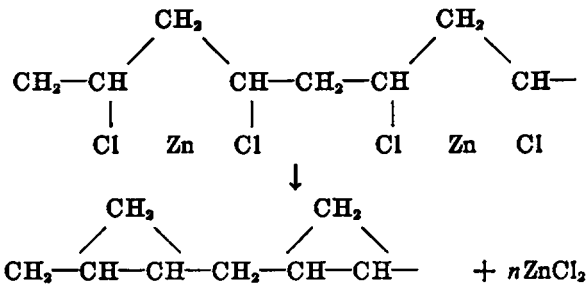


FIGURE 10 The swelling of a fully ionized polymethacrylic acid gel as functions of ratio of barium to sodium counter-ions in the gel (40).

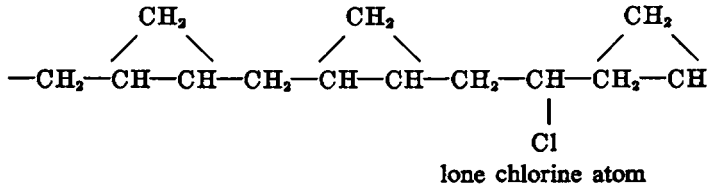
the case of the gels, too, the shrinkage, which is equivalent to precipitation in solution, takes place when over 80 to 85 per cent of the fixed charges are neutralized by divalent counter-ions.

The surprising constancy of the fraction of the charged polymeric groups which have to be bound before precipitation or gel shrinkage sets in is still open to fuller theoretical analysis. As pointed out by Michaeli, Overbeek, and Voorn (41), phase separation is expected in the case of polyelectrolytes partially neutralized by counter-ions of valencies higher than one. It is difficult, however, to see from their theory why precipitation and gel contraction should take place when 80 to 85 per cent of the fixed charges are neutralized by divalent ions. A very tentative explanation may be advanced by assuming that the alkaline earth cations are so strongly bound to sites on the polyion that statistical methods developed for local covalent bonds could apply in this case too. Although the explanation proposed may not withstand more careful theoretical analysis, it is worth stating for use in more appropriate cases.

The chemical example which will serve as a model for our purpose is the removal of chlorine atoms from polyvinylchloride. Marvel, Sample, and Roy (42) treated dioxane solutions of PVC with excess of zinc in order to obtain polymeric cyclopropane rings according to the scheme



No matter what amount of Zn was added to the reaction mixture, only 84 to 86 per cent of the chlorine atoms could be removed from the polymer molecules. Flory (43) showed statistically that there is always a probability for some chlorine atoms, left on the polymer chain and lacking a Cl partner, with which additional ring formation could take place.



lone chlorine atom

Calculations lead to the conclusion that $1/e^2$ or 13.5 per cent of the halogens remain alone while 86.5 per cent may undergo cyclization.

Let us assume that this primary act of the combination of Ca++ with a polyelectrolyte molecule leads to the formation of rather stable bonds with two neighboring fixed charges. Such combinations will consume over 80 per cent of all the groups amenable to statistical pairing and will leave less than 20 per cent of lone charges. In order to utilize further the interaction with the lone charges, any additional Ca++ ions have to join two groups situated on different polymolecules—or intermolecular cross-links have to be formed. The formation of cross-links would cause both precipitation in polyelectrolyte solutions and shrinkage in polyelectrolyte gels, and, what is more interesting for the subject of this colloquium, would provide the bonds for the intercellular cement.

6. MUTUAL INTERACTION OF BASIC AND ACIDIC POLYELECTROLYTES

6.1 Phase separation in polyelectrolyte systems is more easily brought about by interacting polyelectrolytes of opposite charge than by interaction with small ions. Already some seventy years ago Kossel (44) observed that proteins interact with nucleic acid and form heavy precipitates when mixed even at very high dilutions. Since then, a very large amount of data have accumulated on the coprecipitation, or coacervation, of oppositely charged biocolloids. Bungenberg de Jong was especially

active in studying the effect of various factors such as pH, salt concentration, and hydration on phase separation and constitution of the precipitated phase of the reaction product (45). He pointed out features of morphogenetic interest in coacervation and showed that there is a far reaching parallelism between the complexes and the behavior of biological membranes. Thus, there exists an antagonism between Ca++ and Na+, the divalent ion producing shrinkage and the monovalent ion causing swelling of both precipitate and membrane. More recently, Troschin and his coworkers have attempted to relate the behavior of cell coacervates with active transport and supported their views with numerous additional studies on model systems (46).

In 1949 Fuoss and Sadek (47) showed that similar behavior is exhibited by synthetic polyelectrolytes and since these systems have the advantage of simpler chemical structure, they make a better starting point for a physicochemical investigation.

The simplest indication of the interaction is the coprecipitation which is readily observed turbidimetrically (Fig. 11). Upon the addition of polyacid to a

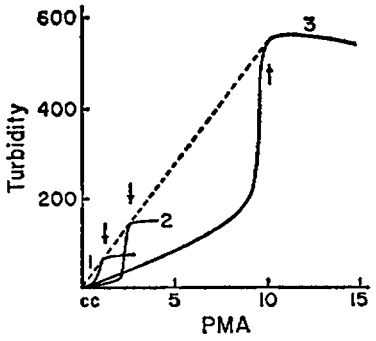


FIGURE 11 Turbidimetric titration of 20 ml of three polylysine solutions (1) 1 μg/ml; (2) 2 μg/ml, and (3) 8 μg/ml by a 10_m^{-3} solution of polymethacrylic acid. The initial pH in all three cases was 6.8 (51).

polybase solution, there is first a slow increase in the turbidity until the point of electrochemical equivalence is attained. At this point, when the number of negative charges on the polyacid equals the number of positive polybase groups, a sharp increase in turbidity is observed and precipitation takes place. The precipitation point is so sharp that it can be used with advantage for the titration of minute quantities of biocolloids (48). It is noteworthy that the precipitation is an "all or none" reaction; *i.e.*, before the precipitation all of the polymer is in solution and after precipitation practically none of it remains in the supernatant. It is rather suggestive that polymeric systems exhibit this "razor edge" behavior at the phase separation. Hill pointed out (49) that at the point of phase separation there should be a drastic increase in the combining capacity of the coacervate, a point which might be of profound interest for the absorption of ions by natural coacervates.

6.2 The theoretical treatment of these complex and interesting phenomena is still lagging behind the extensive experimental data found in the literature. An im-

portant step in the attempt to describe polyelectrolyte phase separation in more fundamental terms is due to Overbeek and Voorn (50). Their treatment considers the competition between electrical attraction which tends to aggregate the particles and the entropy which tends to disperse them. Among the predictions of this theory we shall consider the influence of the ionic strength on the extent of complex formation. The addition of salt of low molecular weight screens the polyelectrolyte field and decreases the probability of coacervation and at sufficiently high ionic strength no precipitation takes place. This can be shown experimentally by a potentiometric method developed by Spitnik *et al.* (51). If a neutral polybase is added to a partially neutralized, salt-free, polyacid solution, there is a gradual decrease in pH until the equivalence point is attained. At this point, there is a strong drop in pH of over 1.5 pH units, indicating enhanced intramolecular combination as predicted by Hill, and precipitation takes place. If the same reaction is carried out in a molar sodium chloride solution, only a slight change in pH is observed and only traces of precipitation are visible.

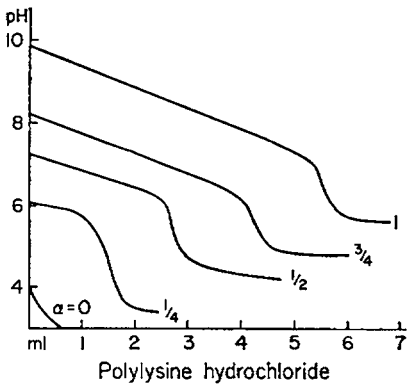


FIGURE 12 Potentiometric titration of 10 ml polymethacrylic acid (10^{-3} molar) of different degrees of ionization ($\alpha = 0$, 0.25, 0.5, 0.75, and 1.0) by 2.10^{-3} molar solutions of salt-free polylysine hydrochloride (51).

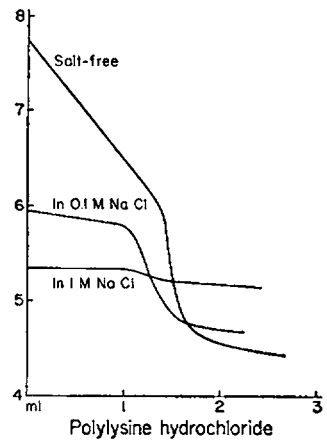


FIGURE 12A Potentiometric titration of 10 ml half-neutralized ($\alpha = \frac{1}{2}$) polymethacrylic acid 10^{-3} molar, by polylysine hydrochloride, 5.10^{-3} molar in solutions of different salt content (51).

6.3 It is often of great advantage to use, as one of the components in the interaction, a charged enzyme, the activity of which can be masked by combination with an oppositely charged polyelectrolyte. It is possible to measure the extent of interaction by the residual enzymatic activity. Thus, the activity of the acidic pepsin can be inhibited by the interaction with basic polymers and the activity restored by competitive treatment with heparin; muscle phosphorylase behaves similarly; on the other hand, ribonuclease and hyaluronidase are inhibited by interaction with acid polyelectrolytes and their activity regenerated by polybases. Figs. 13 and 14 give an example of an enzyme-polyelectrolyte interaction from the recent work of Regelson (54).

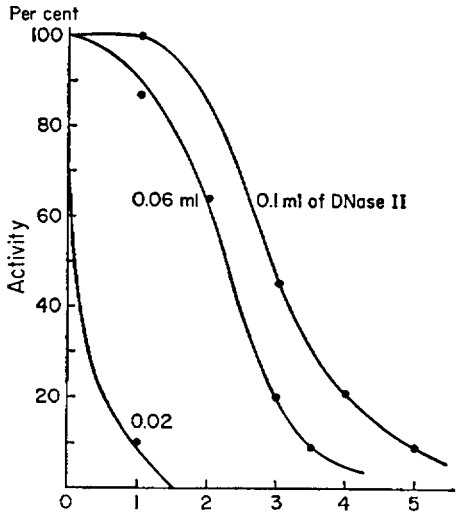


FIGURE 13 Activity of DNase II *versus* concentration of hydrolyzed ethylene maleic-anhydride.

Fig. 13 is a "titration" of DNase II with an acidic polyelectrolyte–hydrolyzed maleic anhydride. It is a typical curve of polybase-polyacid interaction now conveniently determined by the diminution of enzyme activity. Fig. 14 shows, as expected, that with increasing ionic strength the complex is loosened so that enzymatic activity increases with increasing ionic strength.

6.4 The interactions discussed hitherto are essentially due to a random combination of polyelectrolyte chains forming a mixed coacervate phase resembling, from some points of view, a concentrated solution of ordinary salt (50). It has been known, however, to biochemists for many years that the natural complexes of, say, nucleic acid with basic proteins, such as the nucleoproteins, are not randomly cross-linked polyelectrolyte gels. The nucleoproteins are well organized systems, fairly soluble in aqueous solution, while man-made mixtures of the isolated components

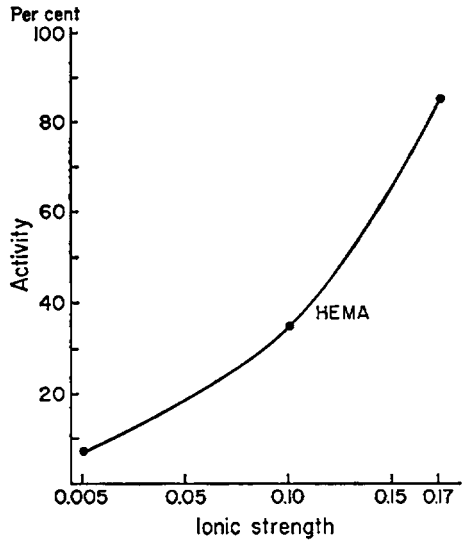


FIGURE 14 Effect of ionic strength on the combination of DNase II and amidated ethylene-maleic anhydride.

give insoluble and amorphous precipitates. With the advent of modern recognition of the importance of crystallographic structure of the biocolloids, there is also a growing interest in the structure of the natural polybase-polyacid interacts.

It will take us too far to discuss recent work on nucleoproteins; we shall mention, however, one interesting observation made by Felsenfeld (52) on the formation of organized complexes by the interaction of synthetic polynucleotides with polybases. Felsenfeld started with solutions of the double helical complex of polyadenylic and polyuridilic acid, the phosphate groups of which were neutralized by divalent manganese ions. The manganese ions were so strongly bound that free manganese ions could be detected in solution. When, however, the polybase protamine was added, it combined avidly with the polynucleotide complex and replaced the manganese ions. Thus, the liberation of Mn^{++}, which could be easily followed, indicated extent of interaction. Since a soluble complex forms, it is believed that there is no random combination but the polybase "creeps" into the open groove of the double helix of the polynucleotide and forms a triple helix. The movement of the polybase along the helix and its adaptation to the preformed structure of the nucleotide are similar to the adaptation of the nucleic acid to the preformed protein pattern of the viruses.

6.5 In concluding this chapter, it is important to stress that the interactions of oppositely charged biocolloids have additional functions in the living organism and their role is not exhausted by forming structures and regulating enzymatic activity. Polyelectrolyte interactions are often mobilized for *ad hoc* operations in cases when the

defense mechanism of the organism requires the participation of highly charged particles. Thus Bloom, Cromartie *et al.* (53) observed several years ago that when *Bacillus anthracis* penetrates mammalian tissues, it produces large amounts of polyglutamic acid, an acidic polyelectrolyte, which acts as a spreading factor, opening up channels between the cells and facilitating the bacterial penetration. It was found that the action of the polyglutamic acid is counteracted by a polybasic tissue factor which combines with the polyacid and neutralizes its action. This is still an open field for investigation and one can expect to find other interesting polyelectrolytes which play a role in biological defense mechanisms. The uninitiated should, however, not be misled into thinking that the simple injection of a basic polyelectrolyte is going to cure infectious diseases in a straightforward manner. Since the majority of the natural colloids are acidic, the injection of a polybase will generally result in strong combination with tissue components and will kill the animal before the bacteria. Even negative polyelectrolytes, which are safer to use, exhibit numerous toxic effects. Thus Regelson *et al.* (54), in their study on possible effects of anionic polyelectrolytes on tumour growth, found that polyethylene sulfonate, which bears some similarity to heparin, has both acute and chronic toxic effects. The electrostatic interaction discussed in this paper is only one component of the highly selective and specific interactions encountered in the organism; wider research will be required before any application of coacervation in the organism could be contemplated.

7. ON THE POLARIZABILITY OF THE POLYELECTROLYTE DOUBLE LAYER

7.1 It might be natural to proceed from the mutual interaction of polyelectrolytes to the interaction of polyelectrolytes with membranes and cell surfaces. In the interaction of charged macromolecules with layer structures, we encounter, however, additional physicochemical factors which should be considered before continuing with the consideration of biological interactions.

In the first part of this paper we paid special attention to the atmosphere of small ions surrounding the central polyion. It was found that an adequate means for counter-ion fixation in the atmosphere is the osmotic coefficient φ_p which might be roughly interpreted as that fraction of the counter-ions left to roam freely in solution and to exert an osmotic pressure. The convenient features of φ_p are its independence of polymer molecular weight and relative independence of polymer and salt concentration. Our confidence in the significance of the osmotic coefficient was based on results from a limited number of experimental methods depending on equilibrium properties only. There is, however, at the disposal of the experimentalist another group of non-equilibrium methods which also allow the evaluation of the degree of ion fixation. These are the measurements of transport phenomena such as conductance, electrophoresis, and diffusion, and more indirectly, the studies of the dielectric constant and its frequency dependence.

Wall and his coworkers (55) and Eisenberg (56) have carried out extensive conductance measurements of the polyelectrolyte solutions and determined the fraction of the free counter-ions in solution. It was found that, like φ_p, the fraction f of the free counter-ions in solution is independent of molecular weight and polymer concentration, but its value is entirely different from the equilibrium φ_p.

TABLE II

α	0.3	0.4	0.6	0.8
φ_p	0.30	0.27	0.21	0.15
f	0.70	0.58	0.45	0.38

In all cases f is larger than φ_p by a factor of 2 which lies much beyond any possible experimental error. From the table, it appears as if the transport measurements "liberate" part of the fixed counter-ions and let the atmosphere contribute to conductance and electrophoresis. In order to remove the discrepancy between these two groups of results we have to clarify more precisely the nature of the counter-ion fixation in the atmosphere. An analysis by the methods of irreversible thermodynamics (Katchalsky, Alexandrowicz, and Kedem (57)) leads to the conclusion that the fixation consists of a strong immobilization of the counter-ion movement in a direction perpendicular to the axis of the polyion. There remains, however, a relatively free mobility parallel to the axis. To put it in a more precise form—the charged macromolecule is surrounded by cylindrical equipotential surfaces; any counter-ion in the atmosphere is free to move along the equipotentials but is restricted in its movements to a direction perpendicular to equipotential surfaces. Now in the measurement of colligative properties, both the central ion and its atmosphere move as a single particle and act osmotically as a unit—in other words, the atmospheric ions do not contribute to the osmotic pressure. On the other hand, in an electrical field the atmospheres and polyelectrolyte molecules are attracted in opposite directions and the fixed ions are made to move relative to the central polyion. Had the "fixed ions" been associated rigidly, their contribution would have been zero in conductivity measurements too and f would equal φ_p. Due to their relative mobility, the counter-ions in the atmosphere take part, however, in the transport of electricity and behave as partially free particles. This is the reason why f is always larger than φ_p although smaller than unity.

7.2 The displacement of the atmosphere in an electrical field may be regarded as a surface conductance of the type known for many years in colloid chemistry (58). This mobility of the atmosphere allows the polyions to become polarized in an electrical field. The conventional polarizability used in the characterization of organic molecules is based on the mobility of electrons relative to the atomic skeleton of the molecules. Here we encounter a new polarizability based on the mobility of counter-ions relative to the macromolecular chain. In spite of the slowness of

ionic mobility, as compared to electronic motion, the electric dipoles induced even by small electric fields may reach very large values. This is indeed observed in the study of the dielectric constant of polyelectrolyte solutions (59).

Particularly illuminating in this respect is the demonstration of an anisotropic mobility of counter-ions in their atmospheres by means of conductometric measurement of oriented polyphosphates and nucleic acids (60). Schindewolf brought about the orientation by making the polyelectrolyte solution flow in a capillary under relatively high gradients of velocity. The capillary was fitted with two pairs of electrodes parallel and perpendicular to the direction of flow. As expected, with increasing velocity of flow, the conductance increased parallel to the axis of the capillary and dropped perpendicular to it. In sufficiently dilute solutions (10^{-4} — 10^{-5} molar) the relative increase in conductance due to orientation reached a value of 15 per cent.

Eigen and Schwartz (61) studied the induced polarizability of polyions in an electrical field and found that the factor of polarizability, α, of polyphosphates reaches the tremendous value of 10^{-12} cm^3 which is about 10^{10} times larger than that observed in ordinary organic molecules. To appreciate the significance of the findings of Eigen and Schwartz, let us consider a polyelectrolyte molecule in a field of 1000 volt cm. The magnitude of the dipole $\vec{p}$ induced by the field ($\vec{E}$) is $\bar{p} = \alpha\bar{E}$, or expressing the field intensity in electrostatic units

$$\bar{p} = 10^{-12} \cdot \frac{1000}{300} = 3.3 \times 10^{-12} \text{ e.s.u.} \times \text{cm}.$$

Now a Debye unit is 10^{-18} e.s.u. $\times$ cm so that our polyion assumes a dipole of 3.3×10^6 Debye units. It will be noted that the largest permanent dipoles measured in low molecular molecules are of the order of magnitude of a few Debyes, while the induced dipoles in polyelectrolyte solutions approach several million.

7.3 It is known from the laws of electrostatics that inhomogeneous electrical fields produce not only an orientation of dipoles, but develop also ponderomotive forces which cause an oriented movement of the dipoles towards the center of inhomogeneity. Such oriented movements have indeed been demonstrated by Kruyt and others (62) on colloidal suspensions in organic solvents. The living cell abounds in powerful and inhomogeneous electrostatic fields. Even if we consider the low electrophoretic potentials of cell membranes, the field intensity accompanying them is very high. It is estimated that the drop of the cell surface potential to zero takes place along a distance of about 200 A—hence, the electrostatic field of a cell having a potential of, say, 10 mv is $10 \cdot 10^{-3}/200 \cdot 10^{-8} = 5000$ volts/cm. Such strong and inhomogeneous fields should produce appreciable induced dipoles in the neighboring macromolecules and should cause a directed movement perpendicular to the biological membrane. Cell membranes and the intracellular reticulum may therefore act as an organizing element which would bring the biocolloids together in an orderly

array. Moreover, they would exert attractive polarization forces which will contribute to the interaction of natural polyelectrolytes with membranes and other preformed elements of the cell.[7]

8. THE INTERACTION OF POLYELECTROLYTES WITH CELL SURFACES

8.1 Minute quantities of basic polyelectrolytes at concentrations of a few micrograms per milliliter cause strong agglutination of bacterial, plant, and animal cells (62). The agglutination is preceded by an adsorption of the polyions to the cell surface. This leads to numerous biological changes, such as inhibition of growth, lowering of toxicity, and hemolysis of erythrocytes (63).

On *a priori* grounds this adsorption of polyelectrolytes is another case of the adsorption of a polymer from solution to a solid surface. These adsorption phenomena have been studied rather extensively during the last ten years, both experimentally and theoretically. Adsorption isotherms were worked out by Mackor and van der Waals (64) for the adsorption of rod-like macromolecules and by Sarolea (65) for the adsorption of flexible macromolecules lying entirely in the adsorbing surface. Frisch, Simha, and Eirich (66) have investigated the adsorption of macromolecules in which part of the chain lies in the surface and part is floating in solution.

Silberberg (67) summarized the major results obtained by various workers and found that a careful statistical analysis of the macromolecular behavior in the surface accounts satisfactorily for the experimental observations. We would like to mention a few of his conclusions:

(*a*) The amount of polymer adsorbed per unit area increases rapidly with polymer concentration in the equilibrium solution. Then it reaches a plateau value which does not change appreciably with further increase in bulk concentration;
(*b*) The polymer molecule is adsorbed with a large number of its segments in direct contact with surface;
(*c*) The adsorbed polymer layer is often impossible to remove by washing with pure solvent;
(*d*) In general, the adsorption data can be fitted to a Langmuir isotherm.

The behavior of uncharged polymers is only partially similar to that of polyelectrolytes adsorbing to cell surfaces. In a study on the interaction of polylysine with the surface of red blood cells, it was found that the adsorption is not so strong and does not reach limiting saturation values at the low concentrations predicted by theory for ordinary polymers (68). On the other hand, the adsorption isotherm fits a Langmuir isotherm well, as can be seen by inspecting Fig. 15. The adsorbed

[7] It is worth noting that the orientational attraction due to induced polarization is a dynamic phenomenon and may disappear with the change in the membrane potential.

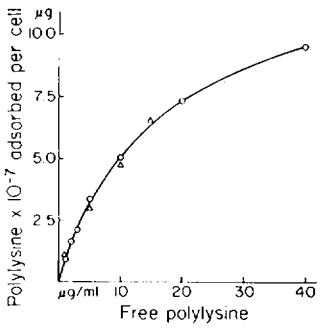


FIGURE 15 An adsorption isotherm for polylysine bromhydrate on red blood cells and erythrocyte ghosts. *x* axis, concentration of free polylysine in solution in μg/ml; *y* axis, amounts adsorbed per cell in $\mu g\ 10^{-7}$ ○, experiments with red blood cells. △, experiments with ghosts (68).

polybase profoundly influences the electrophoretic potential of the cells, as may be seen in Fig. 16.

As expected, the electrophoretic cell potential becomes more positive as the polybase is adsorbed. It is remarkable that the adsorption does not stop when the potential becomes zero, but proceeds smoothly, in spite of the electrostatic repulsion, to high positive values of the surface potential. This fact, as well as the finding

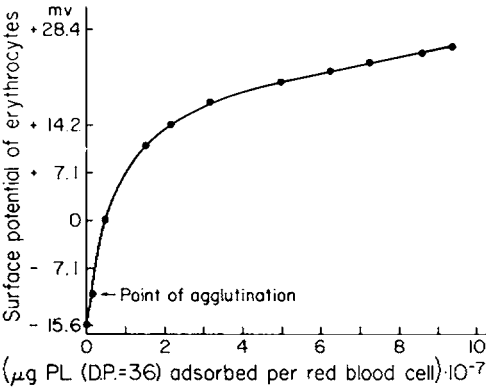


FIGURE 16 Electrophoretic potential of erythrocytes (in millivolts) *versus* amount of adsorbed polylysine per cell (69).

that the amounts adsorbed are several times larger than those required to cover the cell surface with a flat monolayer, indicates that the polyions are at least partially perpendicular to the cell surface. Presumably this orientation is due to the polarization effects discussed in the previous paragraph. The adsorbed macromolecules which protrude from the surface into the solution may use their free ends to attack other surfaces and to bind one cell to another. The outcome of such a double junction is the agglutination of cells and the formation of clumps (69). Agglutination sets in at a "critical potential" (indicated in Fig. 16) which is independent of the number of cells in suspension. In the present case, the critical potential is rather low and far removed from the point of zero mobility. Moreover, the higher the molecular weight of the polyelectrolyte, the lower the critical potential. The fact that agglutination takes place between negatively charged cells indicates that no close contacts between

the cells exist, but macromolecular bridges are formed between one cell surface and another.

The forces exerted by the macromolecular bridges are rather strong and profoundly affect the cell surface. Fig. 17 shows an electron micrograph of a section through a clump of erythrocytes agglutinated by a polylysine compound compared with a section of intact red blood cells (Fig. 18). It will be observed that the normal

FIGURE 17 Section of red blood cells agglutinated by polylysine of degree of polymerization 200. × 8000 (electronic × 2700). Note the marked deformation in shape of the red blood cells and the "bridges" in the narrow area of contact (69).

erythrocytes are entirely distorted and the cells have assumed a shape which allows maximum contact and highest utilization of the interaction energy.

A closer inspection of the seam of agglutination, which is clearly visible in Fig. 17, shows that the distance between the cells corresponds roughly to the length of the fully stretched macromolecules. This would be the expected behavior for oriented macromolecules joining two negatively charged and repellant surfaces. An addi-

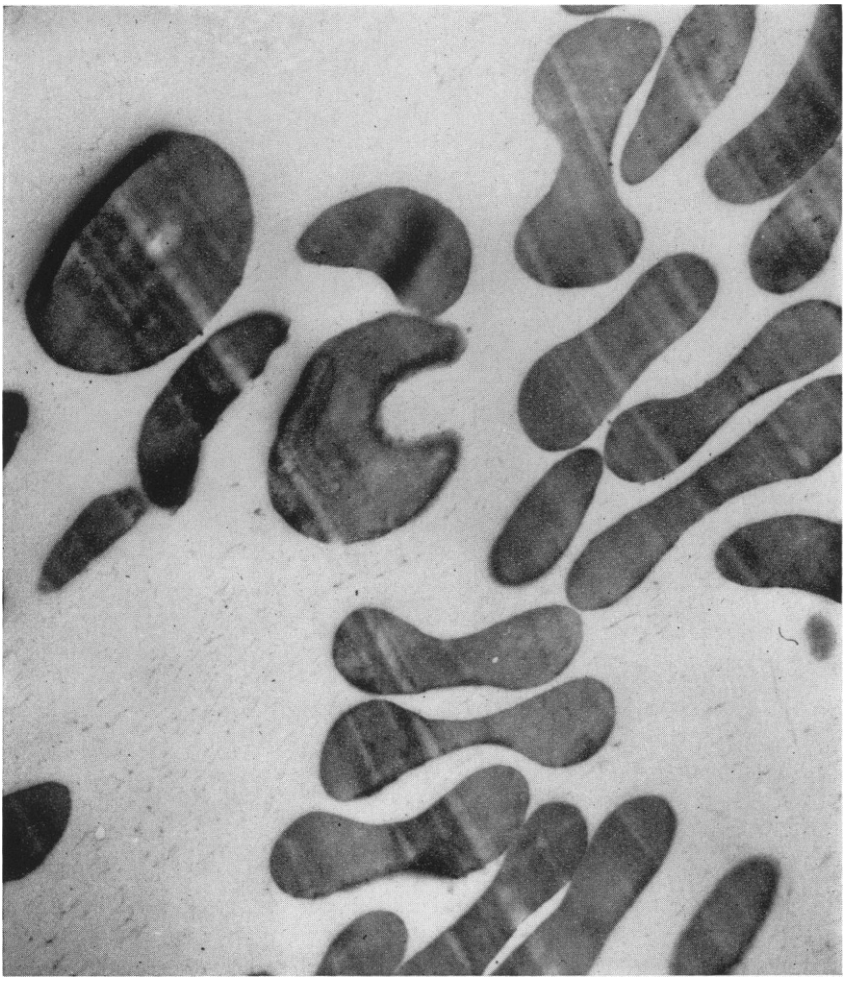


FIGURE 18 Section of a suspension of washed red blood cells. The bidiscoidal shape is well conserved; × 8000 (electronic × 2700) (69).

tional insight into the changes caused by the interaction of basic polyelectrolytes with biological surfaces may be obtained by observing their effect on erythrocyte ghosts. Fig. 19 is a section of an untreated red cell ghost at a magnification of 7000. Fig.

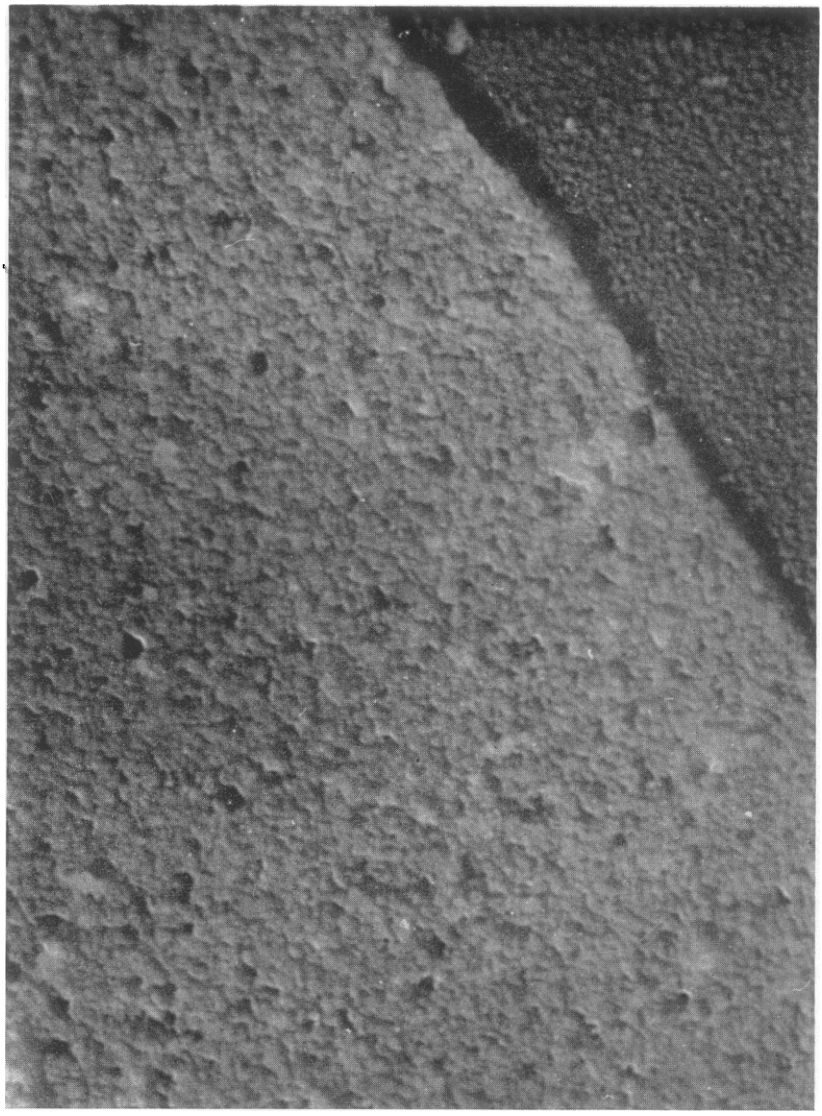


FIGURE 19 Part of an untreated red blood cell ghost; × 70,000 (electronic × 20,000).

20 is a corresponding picture of the point of contact of two ghosts agglutinated by polylysine. It will be observed that the polyelectrolyte glues the ghosts together so powerfully as to cause a full merging of the surfaces in the contact area. There is another noteworthy point about Fig. 20: the polybase located on the ghost may also join distant parts of the surface and open up larger holes in the cell membrane. These holes are the cause of the hemolysis observed in the treatment of erythrocytes with higher concentrations of polybases.

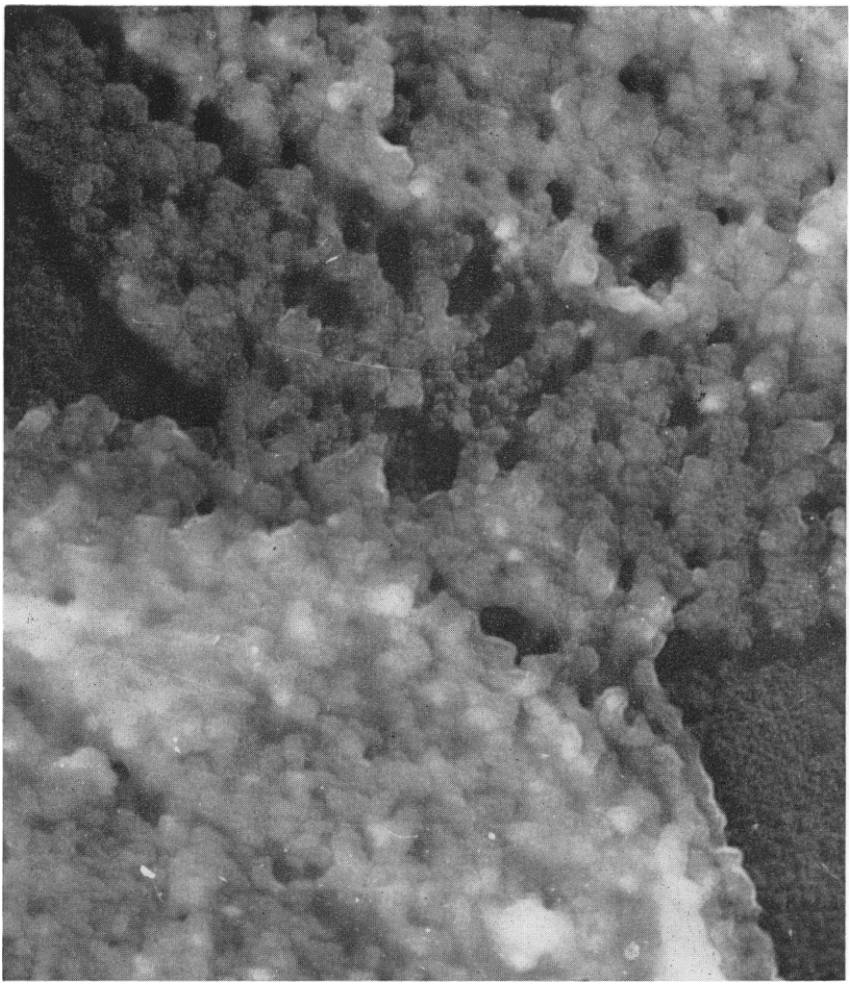


FIGURE 20 Area of contact between two ghosts agglutinated by polylysine of degree of polymerization 200. × 70,000 (electronic × 20,000) (69).

On concluding this paper it may be said that the electrical properties of polyelectrolytes endow them with powerful adhesive properties and with easy orientability which may play a role in the organization of biological structures. The time seems ripe for application of the physical chemical data to the study of charged biocolloids in an effort to understand the cooperative action of natural polyelectrolytes which combine single macromolecules into functional structures.

REFERENCES

1. OSTWALD, O., Die Welt der Vernachlassigten Dimensionen, Dresden, Steinkopff Verlag, 1927.

2. KERN, W., *Z. physik. Chem.*, 1938, **A181,** 240; 1939, **A184,** 207.
3. HERMANS, J. J., and OVERBEEK, J. T. G., *Rec. trav. chim. Pays-bas,* 1948, **67,** 761.
4. KATCHALSKY, A., and SPITNIK, P., *J. Polymer Sc.,* 1947, **2,** 432.
5. KUHN, W., KUNZLE, O., and KATCHALSKY, A., *Helv. Chim. Acta,* 1948, **31,** 1994.
6. KATCHALSKY, A., KUNZLE, O., and KUHN, W., *J. Polymer Sc.,* 1948, **5,** 283.
7. FUOSS, R. M., and STRAUSS, U. P., *J. Polymer Sc.,* 1948, **3,** 246.
8. GOLDSTEIN, L., Thesis submitted to the Hebrew University, 1963.
9. EISENBERG, H., *J. Polymer Sc.,* 1957, **23,** 579.
10. HERMANS, J., JR., and HERMANS, J. J., *K. Nederl. Akad. Wetensch., Proc., Series B,* 1958, **61,** 325.
11. KUHN, W., *Experientia,* 1949, **5,** 318. KATCHALSKY, A., *Experientia,* 1949, **5,** 319. BREITENBACH, J. W., and KARLINGER, H., *Monatsh. Chemie,* 1949, **80,** 311.
12. KUHN, W., and HARGITARY, B. M., *Experientia,* 1951, **7, 1**; *Z. Elektrochem.,* 1951, **55,** 490. KATCHALSKY, A., and ZWICK, M., *J. Polymer Sc.,* 1955, **16,** 221.
13. KUHN, W., RAMEL, A., and WALTERS, D. H., *in* Size and Shape of Contractile Polymers, (A. Wasserman, editor), London, Pergamon Press, 1960, 41.
14. KATCHALSKY, A., and MICHAELI, I., *J. Polymer Sc.,* 1950, **15,** 69.
15. KATCHALSKY, A., LIFSON, S., MICHAELI, I., and ZWICK, M., Size and Shape of Contractile Polymers, (A. Wasserman, editor), London, Pergamon Press, 1960, 1.
16. VERWEY, E. J. W., and OVERBEEK, J. T. G., Theory of the Stability of Lyophobic Colloids, Amsterdam, Elsevier Publishing Co., 1949.
17. WALL, F. T., and BERKOWITZ, J., *J. Chem. Physics,* 1957, **26,** 114.
18. LIFSON, S., *J. Chem. Physics,* 1957, **27,** 700.
19. FUOSS, R. M., KATCHALSKY, A., and LIFSON, S., *Proc. Nat. Acad. Sc.,* 1951, **37,** 579. LIFSON, S., and KATCHALSKY, A., *J. Polymer Sc.,* 1953, **13,** 43.
20. ALEXANDROWICZ, Z., *J. Polymer Sc.,* 1959, **40,** 91.
21. MOCK, R. A., and MARSHALL, C. A., *J. Polymer Sc.,* 1954, **13,** 263.
22. NAGASAWA, M., and KAGAWA, I., *J. Polymer Sc.,* 1959, **37,** 375.
23. ALEXANDROWICZ, Z., *J. Polymer Sc.,* 1959, **40,** 113; 1960, **43,** 337.
24. INAGAKI, H., and HIRAMI, M., *Z. Elektrochem.,* 1959, **63,** 419.
25. ALEXANDROWICZ, Z., and KATCHALSKY, A., *J. Polymer Sc.,* 1963, **1A,** 2093.
26. KAGAWA, I., and GREGOR, H. P., *J. Polymer Sc.,* 1956, **23,** 477.
27. KATCHALSKY, A., COOPER, R. E., UPADHYAY, J., and WASSERMAN, A., *J. Chem. Soc.,* 1961, 5198.
28. KLOTZ, I. M., *J. Am. Chem. Soc.,* 1948, **70,** 939.
29. HARRIS, F. E., and RICE, S. A., *J. Physic. Chem.,* 1954, **58,** 725, 733.
30. KOTLIAR, A. M., and MORAWETZ, H., *J. Am. Chem. Soc.,* 1955, **77,** 3692.
31. GREGOR, H. P., LUTTINGER, L. B., and LOEBEL, E. M., *J. Physic. Chem.,* 1955, **59,** 34.
32. WALL, F. T., and GILL, G. J., *J. Physic. Chem.,* 1954, **58,** 1128.
33. CARR, C. W., *Arch. Biochem.,* 1953, **43,** 147.
34. ROUX, W., *Arch. Entwcklngsmechn. Organ.,* 1894, **1,** 43.
35. HERBST, C., *Arch. Entwcklngsmechn. Organ.,* 1900, **9,** 424.
36. GINZBURG, B., *J. Exp. Bot.,* 1961, **12,** 85.
37. MONGAR, J. L., and WASSERMAN, A., *Nature,* 1947, **159,** 746; *J. Colloid Sc.,* 1952, 500. SPEISER, R., HILLS, C. H., and EDDY, C. R., *J. Physic. Chem.,* 1948, **49,** 328. SPEISER, R., COPLEY, M. J., and NUTTING, G. J., *J. Physic. and Colloid Chem.,* 1947, **51,** 117.
38. WALL, F. T., and DRENAN, J. W., *J. Polymer Sc.,* 1951, **7,** 83.
39. MICHAELI, I., *J. Polymer Sc.,* 1960, **48,** 291.
40. KATCHALSKY, A., and ZWICK, M., *J. Polymer Sc.,* 1955, **16,** 221.
41. MICHAELI, I., OVERBEEK, J. T. G., and VOORN, M. J., *J. Polymer Sc.,* 1957, **25,** 443.
42. MARVEL, C. S., SAMPLE, J. H., and ROY, M. F., *J. Am. Chem. Soc.,* 1939, **61,** 3241.
43. FLORY, P. J., *J. Am. Chem. Soc.,* 1942, **64,** 177. WALL, F. T., *J. Am. Chem. Soc.,* 1942, **64,** 269.

44. KOSSEL, A., *Deutsch. med. Woch.*, 1894, **147**; *Z. physiol. Chem.*, 1896, **22**, 56.
45. BUNGENBERG DE JONG, H. G., *in* Colloid Science, (A. R. Kruyt, editor), Elsevier Publishing Co., 1949, **2**, 232-258; 335-432.
46. TROSCHIN, A. S. Das Problem der Zell Permeabilitaet, Gustav Fischer Verlag, 1958.
47. FUOSS, R. M., and SADEK, H., *Science*, 1949, **110**, 552.
48. TARAYAMA, H., *J. Polymer Sc.*, 1952, **19**, 181.
49. HILL, T. L., *Discussions Faraday Soc.*, 1956, **21**, 31; *J. Physic. Chem.*, 1959, **63**, 501.
50. OVERBEEK, J. T. G., and VOORN, M. J., *J. Cell. and Comp. Physiol.*, 1957, **49**, suppl. 1, 7. VOORN, M. J., *Rec. trav. chim. Pays-bas*, 1956, **75**, 317, 405, 427, 1021.
51. SPITNIK, P., NEVO, A., and KATCHALSKY, A., *Bull. Research Council Israel*, 1954, **4**, No. 3. KATCHALSKY, A., and SPITNIK, P., *Colloq. Internat. Centre Nat. Rech. Sc. Strasbourg*, 1954.
52. FELSENFELD, G., *Biochim. et Biophysica Acta*, 1957, **26**, 457; 1958, **29**, 133.
53. BLOOM, W. L., WATSON, D. W., CROMARTIE, W. J., and FREED, M., *J. Infect. Dis.*, 1947, **80**, 41. BLOOM, W. L., and BLAKE, F. G., *J. Infect. Dis.*, 1948, **83**, 116.
54. REGELSON, W., TUNIS, M., and KUHAR, S., *Acta, Unio Internat. Contra Cancrum*, 1960, **16**, 729; *Clin. Pharmacol. and Therap.*, 1962, **3**, 330.
55. HUIZENGA, J. R., GRIEGER, P. F., and WALL, F. T., *J. Am. Chem. Soc.*, 1950, **72**, 2656, 4228.
56. EISENBERG, H., *J. Polymer Sc.*, 1958, **30**, 47.
57. KATCHALSKY, A., ALEXANDROWICZ, Z., and KEDEM, O., in preparation.
58. FRICKE, H., and SCHUMANN, *J. Physic. Chem.*, 1937, **41**, 729.
59. ONCLEY, J. L., *Chem. Rev.*, 1942, **30**, 433. ALLGEN, L. G., and ROSWALL, S., *J. Polymer Sc.*, 1957, **23**, 635. O'KONSKI, C. T., *J. Physic. Chem.*, 1960, **64**, 605.
60. HECKMANN, K., *Naturwissenschaften*, 1953, **40**, 478. SCHINDEWOLF, U., *Naturwissenchaften*, 1953, **40**, 435. JACOBSON, B., *Rev. Scient. Instr.*, 1953, **24**, 949.
61. EIGEN, M., and SCHWARTZ, C., *Z. physikal. Chem.*, 1955, **4**, 5/6, 380.
62. KATCHALSKI, E., and SELA, M., *Advances Protein Chem.*, 1958, **13**, 243.
63. DE VRIES, A., STEIN, Y., STEIN, O., FELDMAN, J., GUREVITCH, J., and KATCHALSKI, E., *Proc. 4th Internat. Congr., Soc. Hematol.*, Paris, 1954, VII-8, 385.
64. MACKOR, E. L., and VAN DER WAALS, J. M., *J. Colloid Sc.*, 1952, **7**, 535.
65. SAROLEA, L., *Bull. Cl. Sc., Acad. Roy Belg.*, 1954, **40**, 1131.
66. FRISCH, H. L., SIMHA, R., and EIRICH, F. R., *J. Chem. Physics*, 1953, **21**, 365.
67. SILBERBERG, A., *J. Physic. Chem.*, 1962, **66**, 1884.
68. NEVO, A., DE VRIES, A., and KATCHALSKY, A., *Biochim. et Biophysica Acta*, 1955, **17**, 536.
69. KATCHALSKY, A., DANON, D., NEVO, A., and DE VRIES, A., *Biochim. et Biophysica Acta*, 1959, **33**, 120.