

THE POLAR GROUPS OF PROTEIN AND AMINO ACID SURFACES IN LIQUIDS¹

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The most striking characteristic of the polar groups of protein molecules and of protein surfaces or films is the presence of comparatively large numbers of amino and carboxyl radicals. While many groups besides the COO^- and NH_3^+ groups serve to determine the general polarity of the molecule, these and other acidic and basic groups alone primarily determine the net charge of the protein under ordinary conditions.

A most sensitive indicator which gives special information regarding the number and orientation of the COO^- and NH_3^+ groups of protein surfaces is the electric mobility. Thus, it will be shown that, for the cases so far investigated, when

$$\sum \text{COO}^- = \sum \text{NH}_3^+$$

on each molecule over a time average, the electric mobility is zero, and the protein or the protein surface is called isoelectric. Near the isoelectric point the slope of the electric mobility-pH curve is greater than at regions far removed from the isoelectric point. An exception to this rule is found

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only in fairly alkaline solutions where the possibility of the instability of protein exists. On the other hand, in acid solutions not only does the difficulty of interpretation of hydrogen-ion activity measurements exist, but also the electric mobility curve of proteins flattens out so that the change in electric mobility, v , with pH becomes quite unimportant. In view of the fact that at or near the isoelectric point the value of dv/dpH is usually a maximum, experiments designed to investigate changes in the

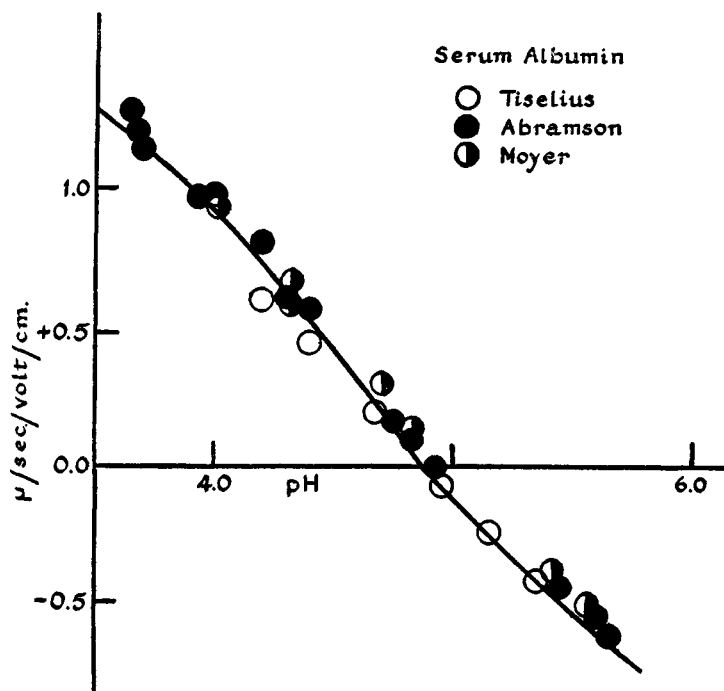


FIG. 1. The electric mobility of horse serum albumin as a function of pH, in 0.02 *M* acetate buffers at 20°C. There is no significant difference between the data for adsorbed (Abramson, Moyer) and dissolved (Tiselius) protein. The smooth curve was drawn free-hand through the points.

number and distribution of the polar groups of proteins and other organic ampholytes incidental to changes in state have always included measurements made at or near the isoelectric point. In this communication, systems will be described which summarize the changes in number and orientation of the amphoteric polar groups of proteins after adsorption at solid-liquid and liquid-liquid interfaces. In addition, data will be presented which will indicate the nature of the changes in the isoelectric point

occurring at the surfaces of organic ampholytes like amino acids incidental to crystallization.

On the basis of recent data, the changes in the polar groups of organic ampholytes incidental to surface formation may be classified into three groups:

Group I: This group deals primarily with proteins after adsorption by quartz and other particles. Within the limits of the method employed, little or no change occurs in the number and orientation of the acid and basic groups, as indicated by the constancy of the electric mobility and the isoelectric point.

Group II: This group, as group I, is composed of proteins. In group II slight changes in the number and orientation of polar groups occur, as evidenced by a change in the isoelectric point of the surface and the mobility-pH curves.

Group III: Here profound changes in the nature of the orientation and in the number of polar groups occur with surface formation. The best examples are the surfaces of amino acid crystals.

The first part of this paper will be concerned with these three groups. In the second part the relationship between titration curves and electric mobility of proteins will be discussed. Finally, the calculation of an "effective" radius for protein molecules from mobility data and its interpretation will be presented.

I. FILM FORMATION BY AMPHOLYTES

Group I

Although numerous experiments on adsorbed protein surfaces or particles of denatured protein had previously been performed by Pauli and Flecker (38), Walpole (49), Brossa and Freundlich (15), Loeb (22), Hitchcock (21), Reinders and Bendien (40), Freundlich and Abramson (19), Prideaux and Howitt (39), and Mattson (23), a direct comparison of the electric mobilities of dissolved proteins and of protein surfaces was not possible until Tiselius published his first group of moving boundary data in 1930.

The first direct comparison of the electric mobility of a dissolved protein with that of an adsorbed protein under precisely similar conditions was made by Abramson in 1932 (5). In figure 1 are given the data of Tiselius for the dissolved form of horse serum albumin and those of Abramson (5) and Moyer (29) for quartz and collodion particles, respectively, coated with this protein. All measurements presented were made in 0.02 *M* acetate buffers by methods described by Abramson (8), Abramson and Moyer (11), Abramson, Moyer and Voet (13), and Moyer (24, 28).

It may be seen from figure 1 that the points fall together on a smooth curve within the limits of experimental error, and therefore that the electric mobilities of dissolved serum albumin (in 0.02 *M* acetate buffer)

are the same as the electric mobilities of quartz and collodion particles covered with complete films of serum albumin. It is not known whether these films are monomolecular or polymolecular. The experiments were performed with a sufficient concentration of protein to yield at least complete films. Neither the size nor the nature of these particles covered with films of protein influenced the electric mobility of the protein-covered particles. Indeed, it was found in acetate buffers and in solutions containing other electrolytes that the mobility of a microscopically visible, protein-covered quartz particle having a diameter of 1 micron was exactly equal to the electric mobility of a particle similarly covered with protein but 10, 100, or even 1000 times larger (4, 8, 10). More striking, the ratio of the electrophoretic mobility of the particle to the electroosmotic mobility of the liquid along a *flat* surface covered with the same protein is equal to 1.0 (4, 6, 8, 16, 17, 27, 30, 33). In other words, protein films of this kind had mobilities independent of the size, shape, and orientation of the particle. All of these results may be visualized as follows: A single dissolved serum albumin molecule migrating in the same field with a quartz particle 1 micron in diameter, covered with a film of serum albumin, and a baseball covered with a film of this protein in the same electric field would migrate at the same rate. The physical reality of the phenomenon is approached by imagining that, rather than the particle itself in motion, we have the water streaming past the particle, with the result that the water has the same speed whether streaming past a single albumin molecule or passing along a wall coated with serum albumin.

The electric mobility, v , of a sphere or of other highly simplified models of charge density σ and radius r may, under certain circumstances, be represented by an expression of the form,

$$v = f(\sigma, r)$$

in a given medium (8). In order for the electric mobility to remain the same after adsorption of the protein on a surface, both the charge density and the radius must change in such a way that they exactly compensate one another, or else both must remain the same after adsorption as in the freely dissolved state. This maintenance of constant electric mobility for a single dissolved molecule and the adsorbed film could hardly be achieved by such a compensation mechanism. It is necessary, therefore, to conclude that in this instance there was no important change in the orientation or activity of the carboxyl and amino groups incidental to the adsorption of the protein and the formation of the complete film. Furthermore, a large degree of distortion in the molecules of the outermost layer could not have occurred. That is, the effective radii of curvature of the adsorbed protein molecules are essentially the same as the effective radii of curvature

of the dissolved molecules. The implications of this conclusion have been discussed by Abramson (5) and by Moyer (27, 28, 31).

An investigation by Gorin, on the other hand, has shown that with the charge remaining constant a simple elongation of the molecule, which would amount to a distortion, could occur without changing the mobility very much. However, it is unlikely that the net charge would remain constant upon distorting the molecule to any appreciable extent. This is supported both by the "salt-bridge" theory and by the work on egg albumin to be presented later in this paper.

Another protein which has since been investigated is pseudoglobulin. A comparison of the data on dissolved pseudoglobulin found by Tiselius

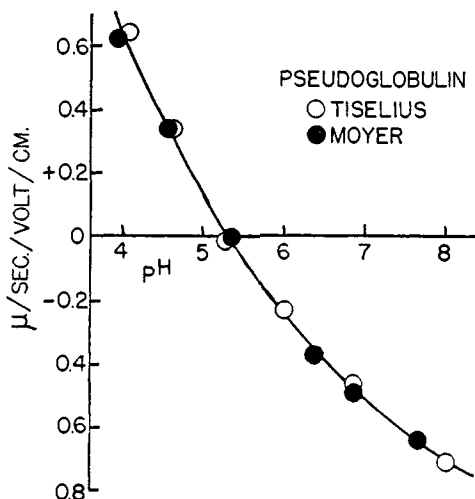


FIG. 2. The electric mobility curve of horse serum pseudoglobulin in buffers of a constant ionic strength of 0.1 at 20°C. Notice that a single curve fits the data for dissolved protein (Tiselius) as well as for adsorbed protein (Moyer).

(47) with the moving boundary method with those for adsorbed pseudoglobulin films on collodion particles by Moyer (29) reveals the same remarkable constancy of electric mobility from pH 4 to pH 8. This comparison is made in figure 2. This protein and serum albumin appear to be the only ones for which data are available for exact comparisons of the dissolved and adsorbed forms. Other data, however, give indirect evidence that similar cases may exist.²

² The data on the mobility of particles coated with gelatin and its relation to the titration curves of dissolved gelatin furnish indirect evidence that gelatin is another protein that displays this remarkable property. They will be presented in the section on titration curves and mobility.

Further experiments may be directed toward studying films of the various globulin fractions now made available by the more recent experiments of Tiselius (48) with the Toepler method of analysis of the moving boundary. The comparison of the films of these proteins, and of their mixtures, with dissolved molecules will be especially interesting, not only from the immunological point of view but also for a further analysis of the surface composition of living cells. In the case of systems where the protein itself can be traced by the behavior of protein-coated quartz particles, further elucidation of the problem of mutual adsorption of protein, or of selective adsorption of protein, may be sought. Experiments in this field have been begun by Moyer and Moyer (35). Earlier investigations of Abramson (2) on the behavior of quartz and paraffin oil droplets in whole serum are a case in point. Other biochemical and biological applications have been recently discussed by Abramson (8), Abramson and Moyer (11), and Moyer (25, 26, 31).

Group II

In the case of egg albumin the electrophoretic behavior of the adsorbed film differs slightly, but definitely, from that of the molecules in solution. The results of Moyer (28), Abramson (5), and Tiselius (46) are shown in figure 3. The solid line, labeled "adsorbed" in the figure, represents the smoothed data of Moyer for films of egg albumin on quartz, glass, oil droplets, collodion, and carbon. Earlier work of Abramson for films on quartz is represented by the closed circles. Data of Tiselius for dissolved egg albumin are shown by the open circles.

It may be observed that the smoothed curve for adsorbed egg albumin, found by Moyer and with which we agree, is essentially parallel to that of Tiselius over the range of pH investigated. However, it is definitely shifted to the right on the pH scale; the isoelectric point is at about pH 4.82 instead of pH 4.55. In the same figure is shown the smoothed curve by Moyer (broken line) for the mobilities of particles of surface-denatured egg albumin prepared by shaking. This curve lies still further to the right (isoelectric point at about pH 5.0) than that of the adsorbed normal egg albumin. The discrepancy between the points of Abramson and the smooth curve of Moyer is probably due to the fact that Abramson used quartz particles which had been in long contact with water. This is borne out by the fact that Moyer's curve for egg albumin films on silica gel particles is also anomalous and within the limits of error of Abramson's points.

It is evident that the electrokinetic properties of air-denatured egg albumin are considerably different from those of molecules of egg albumin in solution. The differences in the isoelectric point are to be correlated

with changes in the relative strengths of the acidic and basic groups incidental to denaturation. Alterations in the shape of the molecule may also occur. These would be expected to result in a curve of different shape, but would only indirectly affect the isoelectric point (28). No definite conclusions may be drawn however, for changes in the shape of the curve must also accompany changes in the number and relative strengths of the available acidic and basic groups.

The "salt-bridge" theory of Stearn and Eyring (43) is useful in considering changes in the relative strengths of acidic and basic groups that

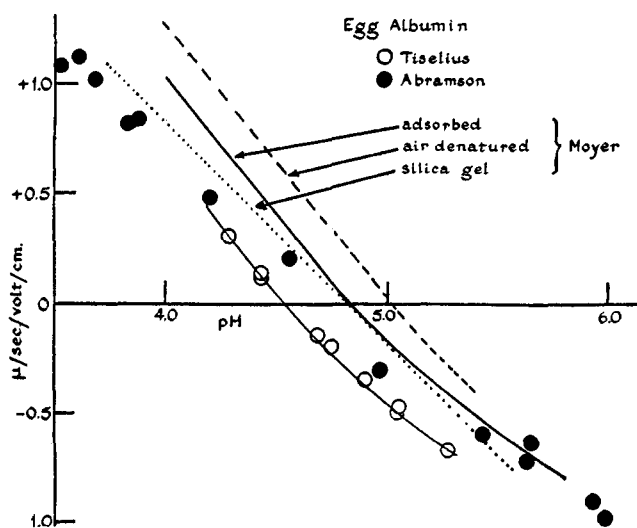


FIG. 3. Comparison of the data of Tiselius for dissolved egg albumin with the results of Abramson and of Moyer for adsorbed films of this protein. The smooth curve of Moyer represents the best curve drawn through data obtained with five preparations of egg albumin adsorbed on five different surfaces. For comparison, the smoothed curves for air-denatured egg albumin and egg albumin adsorbed on silica gel have been included. All data were obtained at $\mu = 0.02$ and $T = 20^\circ\text{C}$.

might occur upon denaturation. According to these authors, bonds between NH_3^+ and COO^- on opposite side chains are at least partially responsible for the maintenance of the folded state of the native protein. Upon denaturation, according to the widely accepted theories of Wu (51), Astbury (4), and others, the molecule unfolds and therefore these salt bridges are broken, say Stearn and Eyring. It is to be expected then, from this picture, that shifts in the relative strengths of the amino and carboxyl groups would occur upon denaturation, for important changes would take place in the position of some of these groups in the molecule.

Since the isoelectric point of the adsorbed films lies between that of the dissolved and denatured egg albumin, the molecules in these films may be considered to be "partially denatured." It is not to be inferred without further evidence that all the physical, chemical, and biological properties of the molecules in the adsorbed film lie between those of the dissolved and surface-denatured form. Rather, the term "partially denatured" is used to indicate that the difference between dissolved and adsorbed egg albumin may be due to the instability of this native protein toward surface denaturation.

Some difficulty may be experienced in classifying the electrokinetic behavior of insulin crystals. We have found no data in the literature for the electric mobility of insulin in the dissolved state, but data are

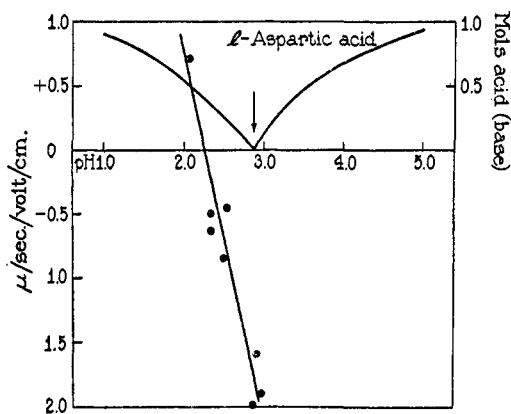


FIG. 4. A comparison of the electric mobilities of aspartic acid crystals in hydrochloric acid solutions and the titration curve of dissolved aspartic acid. The arrow indicates the position of the isoelectric point of dissolved aspartic acid. ●, hydrochloric acid. Smooth curve, titration.

available for the electrophoretic behavior of adsorbed insulin and amorphous particles of insulin suspended in buffers (50). If amorphous insulin, that is, insulin precipitated rapidly from solution at the isoelectric point, and films of insulin on quartz are compared directly in the same solution near the isoelectric point, the mobilities are, within the limits of error, identical. Crystals of insulin, however, studied under the same conditions, show a divergence from this behavior. The isoelectric point of one preparation of insulin crystals studied by Wintersteiner and Abramson differed from that of the adsorbed film of insulin by approximately 0.35 pH unit. The general shape of the mobility-pH curve of the insulin crystals is that of a typical ampholyte. It is not known in this instance

how much adsorbed insulin was present on the surface of the crystalline insulin, because some dissolved insulin is always present even at or near the isoelectric point. We may perhaps tentatively include insulin in the second group, because the difference between adsorbed and crystalline insulin is not marked. This difference might have been greater if no dissolved insulin at all had been present. On the basis of their state of aggregation, a better classification would perhaps include insulin crystals in the group of amphoteric crystals, the amino acids, which are now to be discussed.³

Group III

The recent work of Abramson and Moyer (12) on the electrokinetic properties of amino acid crystals furnishes data for a third type of behavior,—the very different isoelectric points and mobilities of dissolved and crystalline amino acids. It is offered, not because it is exceptional from a general point of view, but because it allows, by contrast, a better insight into the unique behavior of the protein surfaces included in the first group.

Crystals of aspartic acid, cystine, and tyrosine were suspended in hydrochloric acid, and, in the case of the last two, also in buffers at two ionic strengths. Their mobilities were determined in the flat microelectrophoresis instrument of Abramson (1, 3, 8, 9) over a wide range of pH. Results are given in figures 4 to 6 and are compared with those predicted for the dissolved molecules either from titration curves or solubility determinations. It is seen from these figures that the difference between the isoelectric points of dissolved and crystalline amino acids ranges from 0.5 pH unit in the case of aspartic acid to more than 3.0 units in the case of tyrosine, and that the isoelectric points of the crystals of the three substances are very close together (about pH 2.4), while those of the corresponding dissolved amino acids are far apart. The curves obtained for the crystals show no inflections at the isoelectric points of both the dissolved and the crystalline forms.

The effects of salts on the mobilities of cystine and tyrosine were investigated. It may be seen from figures 4 to 6 that increasing the ionic strength decreased the mobility at a given pH value. No data of this

³ One of us (H. A. A.) has measured the electric mobility of hemoglobin crystals at the isoelectric point of dissolved hemoglobin in a saturated solution of hemoglobin in phosphate buffers. Under these conditions the hemoglobin crystals were negatively charged. An insufficient number of experiments and the presence of impurities, even though the preparation had been recrystallized several times, prevent further comments from being made at this time, but the data illustrate that protein crystals, even in the presence of concentrated, saturated solutions of the protein, may not have the electrokinetic properties of the dissolved molecules.

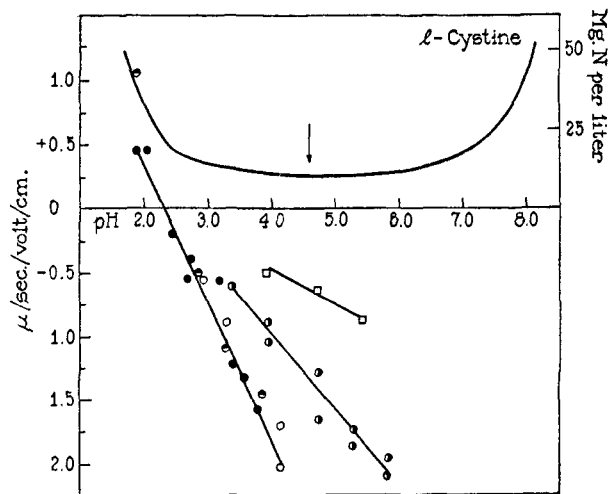


FIG. 5. The electric mobilities of cystine crystals suspended in hydrochloric acid and in sodium acetate buffers. The arrow points to the isoelectric point of dissolved cystine. ● ◐ ○, hydrochloric acid; ◐, $N/200$ sodium acetate; ◑, $N/200$ sodium acetate + $N/5$ sodium chloride. Smooth curve, solubility (Sano).

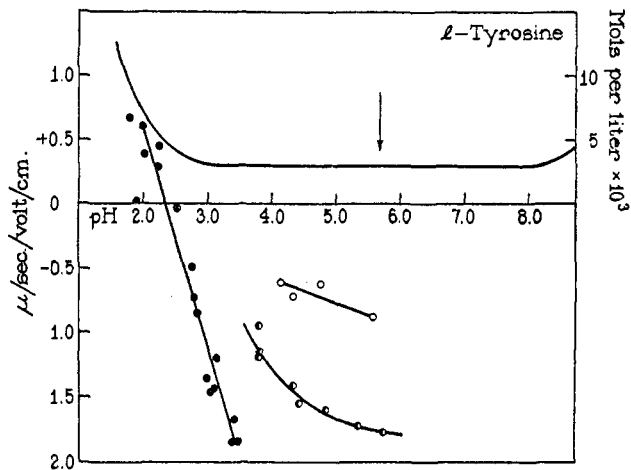


FIG. 6. The electric mobilities of tyrosine crystals suspended in hydrochloric acid and in sodium acetate buffers. The arrow points to the isoelectric point of dissolved tyrosine. ●, hydrochloric acid; ◐, $N/200$ sodium acetate; ○, $N/200$ sodium acetate + $N/8$ sodium chloride. Smooth curve, solubility (Hitchcock).

type were obtained on the positive side of the isoelectric point of the crystals, nor are there any data very close to the isoelectric point. Available measurements appear to extrapolate to the value of the isoelectric

point obtained for hydrochloric acid solutions. However, the possibility of the existence of bends in the curves at lower values of pH, with corresponding shifts in the isoelectric points, must be taken into consideration.

The tremendous shift in the isoelectric points of the surface of amino acid crystals is probably not to be explained merely by a corresponding change in the relative strengths of the acidic and basic groups. That

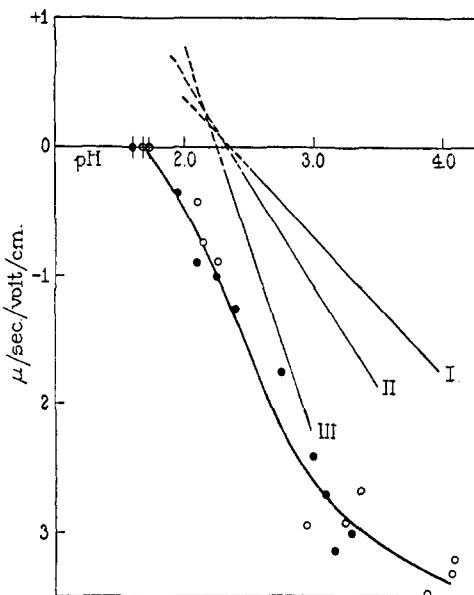


FIG. 7

FIG. 7. A comparison of the electric mobilities of emulsion droplets of simple alkylbenzenes with those of the surfaces of crystalline amino acids. O, *n*-propylbenzene; ●, ethylbenzene; I, cystine (crystal); II, tyrosine (crystal); III, aspartic acid (crystal).

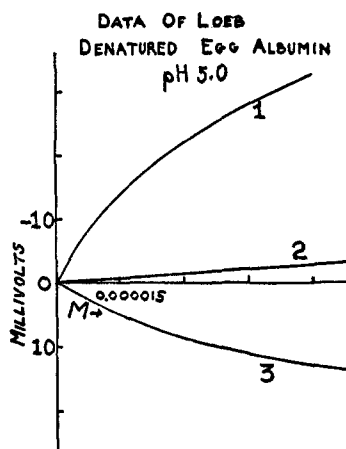


FIG. 8

FIG. 8. This figure illustrates the influence of polyvalent ions on the isoelectric point. By dividing the ordinate values by 13 the approximate electrical mobility may be obtained. The abscissa represents molarity of (1) sodium ferrocyanide, (2) sodium chloride, and (3) lanthanum chloride. Note that even at very low concentrations of polyvalent ions, the effect is much more than was observed at much higher concentrations of uni-univalent salts.

large changes in the relative strengths of the acidic and basic groups of the crystal surfaces, compared with those of the dissolved molecules, might occur is not to be denied. It is unlikely, however, that the charge at the surface of the amino acid crystals is solely determined by the amphoteric properties (ionogenic) of the surface. Negative ions may also be adsorbed at the surface. The net charge would then result from

a competition of hydrogen ions, or positively charged amino acid molecules, and the anions in the solutions, with a shift of the isoelectric point to lower pH values.

In the same paper Abramson and Moyer (12) reported investigations of the mobilities of droplets of *n*-propylbenzene and ethylbenzene in solutions of hydrochloric acid (pH 2 to 4). Their results are shown in figure 7 (taken from figure 6 of Abramson and Moyer) along with the corresponding results for cystine, tyrosine, and aspartic acid crystals already discussed. The non-amphoteric surfaces of *n*-propylbenzene and ethylbenzene show some similarities to those of the amino acid crystals. They differ primarily in the apparent absence of a reversal of charge at lower values of the pH. The mobilities are, in addition, greater at corresponding values of the pH.

II. TITRATION CURVES AND MOBILITY

The demonstration (5) that a direct proportionality exists between titration curves and electric mobility in simple systems furnished a basis for further applications of electrokinetic data to the investigation of the nature of the surfaces of protein molecules and of adsorbed films of proteins. For if, at constant ionic strength in simple systems, the average charge of the molecule is known to be almost completely determined by the average number of equivalents of hydrogen or hydroxide ions bound, it follows that changes in the isoelectric point incidental to surface film formation must be directly correlated with changes in the relative strengths of the amino and carboxyl groups. Furthermore, the application of modern theories of electrolytes to the interpretation of the entire mobility curve would be almost impossible if the average charge on the molecule were not known.

It must be emphasized that the preceding remarks are made specifically for protein solutions containing only simple electrolytes which do not form protein complexes (5, 7, 8). Loeb (22), for example, showed that particles of casein, denatured egg albumin, and gelatin were markedly influenced at the isoelectric point by polyvalent ions. Thus, a trace of sodium ferrocyanide ($M/65,000$) increased the mobility of isoelectric egg albumin particles to values unusually high for proteins at the pH investigated. Other data may be found among the investigations of Tiselius (46), who found that Ba^{++} shifted considerably the isoelectric point of dissolved egg albumin. The effect of citrate ion on the isoelectric point is well exemplified by the data of Ottenberg and Stenbuck (37), who found that the isoelectric point of typhoid agglutinin in solution was shifted about 1 pH unit by citrate buffers. The effects of other ions have been investigated by Smith (41, 42) with particles coated with egg albumin.

While data obtained on the electric mobility of proteins in the presence of polyvalent ions may be of significance in evaluating specific ion effects, considerable caution should be exercised before interpreting mobility data obtained in this way. At present it seems most pertinent for further progress to investigate the electric mobility and charging process of proteins in the presence of salts which do not shift the isoelectric point markedly. It might be of interest to investigate proteins in the presence

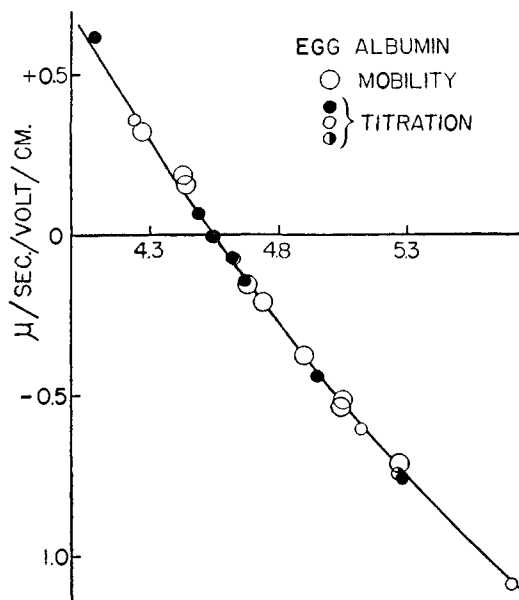


FIG. 9. Comparison of the titration data of Moyer and Abels (32) with the mobility data of Tiselius (46) for dissolved egg albumin at 25°C. The different kinds of circles represent different preparations of egg albumin. All determinations were made at a constant ionic strength of 0.02.

of perchlorate ion, because it, of all anions, has shown the least tendency to form complexes in other systems.

To demonstrate the proportionality between the combining power of proteins with acids and bases and their electric mobilities, various methods of plotting have been employed. The method employed here is that used by Abramson (5). In a plot with the number of equivalents of acid (base) bound per gram of protein as ordinate and the pH as abscissa, the zero point on the ordinate is found by shifting the coordinates vertically until the curve goes through zero at the isoelectric point as determined by electrophoresis. The mobility data are then compared with the titra-

tion data by taking any single point on the smoothed mobility curve and multiplying the mobility by a factor that will make the two curves correspond. If, when all the experimental points are multiplied by this same factor, they fall on the titration curve within the limits of error, this proportionality is to be considered as demonstrated. The most carefully worked out case is that of Moyer and Abels (32) for egg albumin. It is presented in figure 9, which was constructed as described above. In this instance, both sets of data had been measured at the same, constant ionic strength.

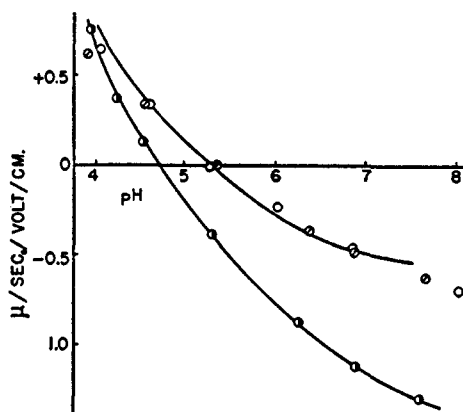


FIG. 10. Electric mobility curves of serum albumin (●) and pseudoglobulin (dissolved, ○; adsorbed, ⊙) in phosphate and acetate buffers at $\mu = 0.1$. The smooth curves are the titration curves for these proteins drawn to fit the mobility data. The scales for the titration curves are different. The ordinate gives equivalents of hydrogen ion bound when multiplied by 2×10^{-4} for pseudoglobulin and by 3×10^{-4} for serum albumin. This shows that over a wide pH range the electric mobility of these proteins is proportional to their combining power with hydrogen (or hydroxide) ions. (From Moyer and Abramson (34)).

There has been considerable confusion about the definition of the isoelectric point for ampholytes. Physically, this point refers to conditions under which the mobility of the ampholyte is zero, and therefore it can only be strictly defined in terms of the charge on the ampholyte and determined by electrokinetic or conductance measurements (8, 12). Thermodynamic definitions of the isoelectric point involve assumptions which may or may not be strictly valid but certainly cannot be taken for granted until demonstrated in each individual case. For proteins in simple systems containing only uni-univalent electrolytes, the assumptions are very nearly valid in cases so far investigated, and the thermodynamic definition may be considered applicable. It seems desirable, however, to define

the isoelectric point more generally, as suggested by one of us (8). According to this point of view, the isoelectric point of an ampholyte, whether dissolved or constituting a surface film, may be defined as a reference concentration (H^+ , for example) at which

$$\frac{1}{T} \int_0^T dt \left\{ \sum_1^s n_i z_i(e) + \sum_1^s n_i z_i(-e) \right\} = 0 \quad (1)$$

($T \gg \tau$)

Here e is the electronic charge and n_i is the number of ions of the i^{th} type, of valence z_i , at the surface during the time dt . T represents the time of observation and τ the life period of an ion at the surface. The conditions relating to the life period, τ , of the ion may be fulfilled by observing a large number of fluctuations during the period of measurement, i.e., a statistical sample.

Other cases in which the proportionality has been demonstrated between acid (base) bound and mobility are those of serum albumin and pseudoglobulin (34). They are shown in figure 10 and drawn as described above. Other cases referred to at the beginning of the section are those of gelatin and deaminized gelatin. There are no data available for the mobility of the dissolved forms, but it has been shown that a simple proportionality exists between the mobility of quartz particles coated with gelatin and the acid(or base)-binding of the dissolved form. It was thus shown indirectly that gelatin is another protein for which the mobilities of the dissolved and adsorbed forms must be very nearly identical.

III. INTERPRETATION OF ELECTRIC MOBILITIES

Calculation of protein radii

The potential at the surface of a particle of known net charge Q in solutions of electrolytes depends upon the size and shape of the particle, the charge distribution over its surface, and the nature of the electrical double layer surrounding the particle. For a rigid sphere, Debye and Hückel (18) derived the following expression for the surface potential, ζ ,

$$\zeta = \frac{Q}{Dr(\kappa r + 1)} \quad (2)$$

where D is the dielectric constant of the medium, r the radius of the molecule, Q the charge on the molecule, and $\kappa = 0.328 \times 10^8 \sqrt{C}$ for uni-univalent electrolytes at 25°C. The mobility, v , is related to ζ as follows,

$$v = \frac{D\zeta f(\kappa r)}{6\pi\eta} \quad (3)$$

where η is the viscosity of the medium (8, 27), and, according to Henry (20),⁴

$$f(\kappa r) = \left\{ 1 + \frac{(\kappa r)^2}{16} - \frac{5(\kappa r)^3}{48} - \frac{(\kappa r)^4}{96} + \frac{(\kappa r)^5}{96} - \left[\frac{12(\kappa r)^4}{96} - \frac{(\kappa r)^6}{96} \right] \int_{\infty}^{\kappa r} \frac{e^{-t}}{t} dt \right\}$$

The function, $f(\kappa r)$, varies between 1 and 1.5 as κr goes from zero to infinity. From equations 2 and 3 it follows that

$$v = \frac{Qf(\kappa r)}{6\pi\eta(\kappa r + 1)} \quad (4)$$

The assumptions involved in the Debye-Hückel development are: (1) Charge is uniformly distributed over the surface. (2) Interactions due to dipole and higher moments may be neglected; also, interactions due to van der Waals' forces are negligible. (3) Electrical interaction ($e\psi$) is small compared with the thermal energy of the ions in the ion atmosphere (kT). The assumption of uniform distribution of charge can only be justified by experimental verification of the theory. The remaining two assumptions are probably nearly valid for protein molecules under ordinary conditions.

Having demonstrated that the mobility under certain conditions is proportional to ϵ , the number of equivalents of H^+ or OH^- bound per mole of protein, it immediately follows that the charge Q on the protein molecule in such instances is given by

$$Q = e\epsilon$$

where e is the electronic charge. Since all quantities in equation 4 are known except r , it is possible to combine mobility and titration data and to calculate by means of equation 4 the "effective" radius, r , of the protein molecule (6, 32, 34).

This calculation has been made for three proteins,—egg albumin, serum albumin, and pseudoglobulin. Results are shown in table 1. Notice that the values calculated (third column) are in fair agreement with those calculated from the molecular volumes (fourth column), and that they are all larger than those obtained from the molecular volume.

Another way of comparing the experimental results with those predicted by the theory is to calculate the theoretical mobility of a sphere of given molecular volume and compare it with the actual mobility. The details of the calculation are omitted, since the equations involved follow directly from those already given. In the fifth column of table 1 is given

⁴ This equation differs from the one given by Henry (20), but follows directly from his method of derivation and corresponds to the function as plotted in his figure. It is concluded that the discrepancy between the printed equation and the one plotted in figure 1 was caused by a typographical error.

the ratio of the experimental to the theoretical mobility for the three proteins considered. It may be seen from the table that, while the experimental value of the mobility is less than the theoretical in all three cases, the agreement is good considering the type of model employed. It is now known that none of these proteins is spherical. However, egg albumin is probably more nearly so than the others, and the agreement, correspondingly, is best for this protein.

Finite size of the ions in the ion atmosphere and the hydration of the protein

In the derivation of equation 2 the ions in the ion atmosphere are implicitly assumed to be point charges. The effect of this assumption and of the hydration of the protein was estimated by one of us (M. H. G.). A short account of the investigation follows.

TABLE 1

Radii, in μ , calculated from titration and mobility data, at 20°C., using the Debye-Hückel theory for a sphere*

PROTEIN	MOLECULAR WEIGHT†	$r_{\text{calcd.}}$	$r_{M.V.}$	$(V_e/V_t)_{(2)}$	$(V_e/V_t)_{(2')}$
Egg albumin.....	42,200	3.26	2.32	0.592	0.615
Serum albumin.....	67,000	3.91	2.70	0.518	0.465
Pseudoglobulin.....	165,000	5.22	3.66	0.524	0.48

* Acetate buffers at constant ionic strength (0.02 *M* for egg albumin and 0.1 *M* for the other two proteins) were employed. η in equation 4 was corrected for buffer (1.055 η_0 for 0.1 *M* buffer and 1.011 η_0 for 0.02 *M* buffer).

† Molecular weights are taken from Svedberg (44).

The ζ -potential, as defined by equation 2, is the potential at the point of closest approach of the ions in the double layer. This point is not on the surface of the protein molecule, but on the surface of an imaginary sphere of radius $(r_p + r_i)$, where r_p is the radius of the protein and r_i is an "average" radius of the ions in the double layer. In general, r_p should be expected to be somewhat larger than $r_{M.V.}$; the radius of the protein calculated from the molecular volume due to hydration of the protein. In the development that follows it will be assumed that the protein molecule in solution is surrounded by a monolayer of firmly attached water molecules, through which the ions in the ion atmosphere do not penetrate, and the outside of which forms the surface of slippage between the molecule and the solution. On this basis, then, $r_p = r_{M.V.} + 0.15$, where 0.15 μ is the diameter of a water molecule. It is obvious, therefore, that the potential, as defined by equation 2, is not the potential required by equation 3, since it is not the potential at the point of slippage.

It is easily shown from the equations of Debye and Hückel that

$$\zeta = \frac{Q(1 + \kappa r_i)}{Dr_p[\kappa(r_p + r_i) + 1]} \quad (2')$$

Equation 2' differs most significantly from equation 2 in the numerator term, $(1 + \kappa r_i)$, which is independent of the size of the protein and is of greatest importance at high ionic strength.

It is of interest to compare the results given by equation 2' with those given by the empirical equation used by Abramson (7) and Moyer and Abels (32) for the determination of an equivalent radius by combining mobility data at two values of the ionic strength. Knowledge of the molecular weight is not necessary for this method. It can easily be shown that equation 2' gives for v_1/v_2 , the ratio of the mobilities at the ionic strengths corresponding to κ equals κ_1 and κ_2 , respectively,

$$v_1/v_2 = b = \frac{(\kappa_2 r + \kappa_2 r_i + 1)}{(\kappa_1 r + \kappa_1 r_i + 1)} \left(\frac{1 + r_i \kappa_1}{1 + r_i \kappa_2} \right) \quad (2'')$$

TABLE 2
Comparison of the theoretical and empirical equations

PROTEIN	RADIUS, IN $m\mu$, CALCULATED FROM		
	Equation 2''		Empirical equation $a = 2$
	$r_i = 0.2$	$r_i = 0.3$	
	$m\mu$	$m\mu$	$m\mu$
Egg albumin.....	1.6	2.0	2.2
Serum albumin.....	2.7	3.4	3.4
R-phycoerythrin.....	3.6	6.4	5.2

where r is the radius of the protein and r_i , as before, is the "average" radius of ions in the ion atmosphere. If v_1/v_2 is known, r can easily be calculated by means of equation 2''. In table 2 equation 2'' is compared with the empirical equation of Abramson (7) and Moyer and Abels (32)

$$v_1/v_2 = \left(\frac{\kappa_2 r + a}{\kappa_1 r + a} \right)$$

where a is an empirical factor, the best value of which is 2 (34). Data were taken from Moyer and Abramson (34, table 1 on page 400). It may be seen from table 2 that for $r_i = 0.3$, the values given by equation 2'' and those given by that of Moyer and Abels correspond very closely. The value, 3\AA ., for an average radius of the ions in solutions of ordinary electrolytes is a reasonable one. Therefore it may be concluded

that the empirical equation of Moyer and Abels may be represented by the theoretical one (equation 2'').

Equation 2' does not improve the agreement between the experimental and theoretical mobility, v_o/v_i . This fact is demonstrated in the last column of table 1, which is headed $(v_o/v_i)_{(2')}$, and is calculated by means of equation 2'. For these calculations r_i was taken as $0.2 \text{ m}\mu$, since they had been completed before it was realized that $0.3 \text{ m}\mu$ was a better value (see table 2).

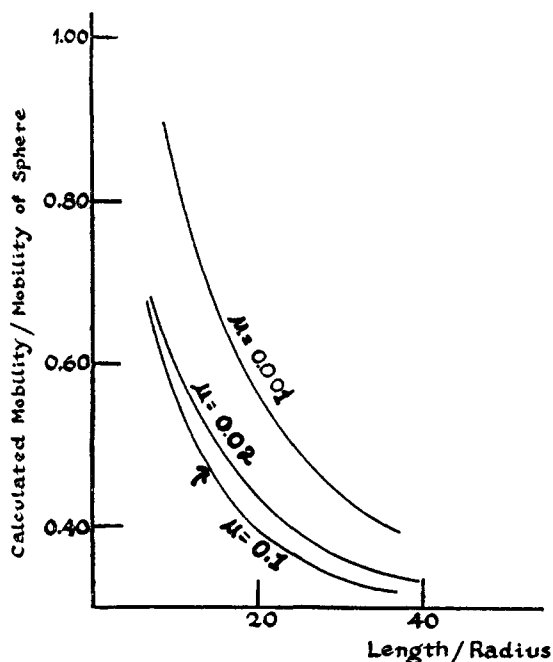


FIG. 11. Calculated mobility of a cylinder having the volume of a serum albumin molecule divided by the calculated mobility of a sphere of the same charge and volume (of the molecule) as a function of the elongation of the cylinder (length divided by the radius) for three values of the ionic strength ($\mu = 0.001, 0.02,$ and 0.10).

Calculation of the mobilities of long cylinders

Preliminary estimations of the electrokinetic potential of an aspherical protein molecule were made by using a highly simplified model. Since incomplete data are available for comparison with the theory, this work is offered as the first part of an investigation by one of us (M. H. G.) which may yield independent information about the shapes of protein molecules. Enough progress has been made to indicate that the discrepancy between theory and experiment in the electric mobilities of pro-

tein may be at least partially cleared up by taking their asymmetry into account.

For long cylinders, neglecting the ends, the ζ -potential as a function of the radius and ionic strength is

$$\zeta = \frac{2QK_0(\kappa a)}{Dl\kappa aK_1(\kappa a)} + \frac{2Q}{Dl} \ln \left(\frac{a + r_i}{a} \right) \quad (5)$$

where Q is the net charge, D is the dielectric constant of the medium, l is the length of the cylinder, and a is its radius. The functions K_0 and K_1 are special solutions of the modified Bessel's equation,⁵ of zero and first order, respectively,

$$\frac{d^2\psi}{dr^2} + \frac{1}{r} \frac{d\psi}{dr} - \left(1 + \frac{n^2}{r^2} \right) \psi = 0 \quad (6)$$

The Debye-Hückel approximations enumerated in the preceding section were used in this development. In applying equation 5, the quantity $a_{M.V.} + 0.15$ was used to take into account the hydration of the protein.

For a cylinder migrating parallel to the field, when κa is very large, the mobility is given by

$$v = \zeta D / 4\pi\eta \quad (7)$$

according to Henry (20). Henry, however, did not publish the complete analysis for the mobility as a function of κa . A graphical integration of Henry's equations yielded the following for v as a function of κa :

$$v = \zeta D / F\pi\eta$$

where F is given in the table below:

κa	F	κa	F
0.00	8.00	1.40	6.87
0.40	7.73	2.00	6.51
1.00	7.36	2.40	6.34
		3.00	6.10

The mobility therefore becomes

$$v = \frac{2QK_0(\kappa a)}{F\pi\eta l\kappa aK_1(\kappa a)} + \frac{2Q}{F\pi\eta l} \ln \left(\frac{a + r_i}{a} \right) \quad (9)$$

Equation 9 was applied to a molecule having the volume of serum albumin. In figure 11 the calculated mobilities, at three values of the ionic strength, of cylinders with various degrees of elongation are com-

⁵ The notation is that used by Whitaker and Watson in *Modern Analysis*, p. 373. Cambridge University Press, London (1927).

pared with those calculated for a sphere of the same volume. It may be seen from the figure that the lower the ionic strength the smaller is the difference between the mobility of a sphere and that of a cylinder. However, there is almost no difference between the curves for $\mu = 0.1$ and 0.02 . This fact would seem to explain the success of formulation for a sphere, equation 2'', in estimating v_1/v_2 for molecules that are definitely not spherical. In addition, it is of interest to note that there is a comparatively small difference between the mobility of spheres and that of highly elongated cylinders having the same net charge and molecular volume.

We may use the available data of Moyer and Abramson (34) for serum albumin at $\mu = 0.1$ to illustrate the use of the theory. The results for this protein require that the mobility be about 61.5 per cent (last column, table 1) of that of a sphere of the same molecular volume. This corresponds, as may be seen in figure 11, to a value of $l/r = 14$, or a ratio of

TABLE 3
Asymmetry from electrokinetic data for cylindrical model

PROTEIN	LENGTH/BREADTH	
	Electrokinetic data	Perrin's equation and Svedberg's data
Egg albumin.....	4.4	2.6
Serum albumin.....	6.3	5.7
"Globulin".....	7.3	7.6

length to breadth of about 7 for this molecule. When corrected for hydration, the ratio of length to breadth comes out to be 6.2 to 1. Svedberg (45) gives 1.29 for the dissymmetry constant of serum albumin. This, when substituted into Perrin's equation (see Neurath (36)), gives 5.7 for the ratio of length to breadth. The agreement is surprisingly good, considering the simplicity of the model employed. Equation 9 was also applied to the results for pseudoglobulin and egg albumin. The results, together with those for serum albumin, are shown in table 3. Agreement again is excellent.

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