ELECTROKINETIC PHENOMENA

X. ELECTRIC MOBILITY AND CHARGE OF PROTEINS IN ALCOHOL-WATER MIXTURES

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(Accepted for publication, October 10, 1932)

I

INTRODUCTION

The following experiments were undertaken with the object of studying the surface properties and ionization of adsorbed proteins in alcohol-water mixtures. When the charge on the protein as measured by titration curves is compared with the surface charge on the adsorbed protein as measured by the mobilities of protein-covered quartz particles, there is obtained information about the effect of the medium on the dissociation of the protein, the orientation of polar groups, and the relationship between charge and electrophoretic mobility.

Gelatin was used because it has been shown (1) that under the conditions maintained here, mobilities of gelatin and of deaminized gelatin (both adsorbed onto quartz) are in the same ratio as the respective amounts of acid (base) bound. This means that in aqueous media gelatin obeys the rule (1) that in solutions of the same ionic strength the electrophoretic mobility of the same protein at different hydrogen ion activities should be proportional to the number of hydrogen or hydroxyl ions bound by each molecule. It should be noted that this rule can apply to the adsorbed gelatin only if the active (dissociating) groups are free, that is oriented toward the liquid. Further, the rule will apply only if the protein salt is equally dissociated over the range of hydrogen ion activity considered.

1 It has been shown that in general the mobilities of freely dispersed proteins are equal to the mobilities of particles coated with the same proteins.
The rule just stated results from the Debye-Henry approximation (equation (8), reference (1)),

$$Q = 6 \pi \eta r \nu_m (\kappa r + 1),$$

where \( \nu_m \) = electrophoretic mobility = \( \mu \)/sec./volt/cm., \( r \) = radius, \( Q \) = charge, \( \eta \) = viscosity, \( \kappa = \sqrt{\frac{4 \pi \varepsilon^2}{D \kappa}} \sum \eta \omega^2 \). For a given molecule in a given medium this becomes \( Q = \nu_m (C' + C'') \), (equation (8a), reference (1)). But when the medium is altered (keeping the ionic strength constant), \( Q \) becomes a function of \( \nu_m \), \( \eta \), and \( D \). This provides a test of the question whether in equation (1) the viscosity and the dielectric constant of the medium can be used to predict changes in \( Q \). Since the medium was altered by the addition of ethyl alcohol it was not possible to vary the viscosity and the dielectric constant independently, but the combined effect was readily investigated.

The main part of the work was the study of the mobilities of gelatin in 35 per cent and in 60 per cent ethyl alcohol for comparison with each other and with titration curves in like concentrations of alcohol. In connection with this a shift of the isoelectric point of gelatin in the presence of ethyl alcohol was found and this shift was studied. But before any mobility measurements could be made it was necessary to study the change in electrophoretic velocity with change in field strength in alcohol solutions.

II

Methods

The pH measurements of solutions of alcohols other than ethyl alcohol were made with a hydrogen electrode referred to the pH of \( \sqrt{10} \) HCl as 1.07. The remaining pH measurements were made with a quinhydrone electrode. This can be used for ethyl alcohol since no reaction occurs between quinhydrone and alcohol-water, but a correction is necessary\(^2\) (2). The accuracy of the time measurements in electrophoresis obtained here is such that the average deviation is

\(^2\) The quinhydrone electrode was tested against a hydrogen electrode in a number of ethyl alcohol solutions; the two agreed to better than 0.05 pH.

The titration of gelatin in alcohol was checked with the glass electrode. The writer is indebted to Prof. Hans Clarke for permission to use the facilities of his laboratory.
usually less than 5 per cent. Exceptions occur when very low velocities are measured either very near the top or the bottom of the cell, or when a very low voltage is used. All other errors were well within these limits. The temperatures at which the experiments were done were always recorded, but the variations from 20° were not sufficient to make worth while corrections for temperature, except of course in the pH determinations.

The mobility measurements were made in the modified Northrop and Kunitz microelectrophoresis cell described by Abramson (3). The theory of von Smoluchowski (4) was followed. The field strength was found from the cross-section of the cell, the current flowing, and the conductivity of the solution, as follows:

\[
\text{Current in amperes} \div \text{Sp. conductance} \times \text{cross section in cm.}^2 = \text{volts/cm.} = X.
\]

The suspensions were made up in the following order: quartz, protein solution (in the case of gelatin sufficient to make one part in a thousand), acid or buffer, alcohol and water. Cooper's gelatin was used. The expression "per cent alcohol" is used to mean ml. of alcohol per 100 ml. of solution. The quartz used was a carefully purified suspension. The gelatin solutions were made by heating, but never above approximately 40° (5), to get rid of the air bubbles formed on mixing alcohol with water, and then cooled to room temperature.

The dielectric constants used are those given by Wyman (6) for pure alcohol-water mixtures, uncorrected for the salt and protein. The solutions contained one part in a thousand of gelatin; since 5 gm. of gelatin per 100 gm. of mixture lower the dielectric constant from 81 to 68 (7), the error here is negligible.

### III

#### Electrophoretic Velocity and Field Strength

The characterization of particles by the measurement of mobilities depends on the experimental fact that electrophoretic velocity is proportional to the field strength. Theoretically, the right hand side of the equation for the mobility of a small particle (10),

\[
v_m = \frac{v}{X} = \frac{D\zeta}{6\pi\eta}
\]

(where \(v = \) electrophoretic velocity, \(\zeta = \) electrokinetic potential, \(X = \) field strength), is constant when \(X\) is varied. If this were not so experimentally the mobility would have to be measured as a function of one or more variables and the interpretation would be greatly complicated. The linear relationship has been found generally (11), with few exceptions.
Recently, however, the results of Ettisch and Zwanzig (12) and of Martin and Gortner (13) have suggested that in alcoholic solutions $\zeta$ is a function of the velocity, while Köhler (14) has found that in non-alcoholic solutions the volume outflow in electroosmosis is not proportional to the field strength. Ettisch and Zwanzig studied streaming potentials. They used very dilute sodium chloride solutions and found that with the pure solution $\zeta$ was independent of pressure, but that in the presence of methyl alcohol $\zeta$ increased with increasing pressure. For equal pressures and varying alcohol concentration $\zeta$ went through a minimum. Propyl alcohol at high concentrations even reversed $\zeta$. Köhler used a palmitic acid diaphragm and measured the rate of outflow of electrolyte solutions under the influence of varying current. In none of these cases is there any definite independent proof of laminar flow.

Traube and Whang (15) and Traube and Dannenberg (16) showed that water flowing through a glass capillary coated with a polar substance (such as a fatty acid) flowed much faster than through the uncoated tube, when the angle at which the tube was tipped was small (about 3°); that is, when the pressure was low. Also the outflow speed of water solutions of surface active substances such as alcohols was greater than for pure water flowing through the same capillary, but again at a small angle only. At a greater angle (under greater pressure) both effects disappeared.

Because of these recent suggestions that $v/X$ may not always be constant and because of the flow anomalies (15) at low velocity gradients, it was considered necessary to demonstrate that under our conditions $v/X$ is constant.

As can be seen from Figs. 1 and 2, the field strength in these experiments with protein-covered quartz particles in various alcohol-water mixtures was varied from 2 to 20-fold. It is definitely shown that for field strengths of the order of magnitude of those used in the experiment, the mobility of these proteins when adsorbed on quartz in these alcohols is not a function of the field strength.

$^a$ By suitable experiment it was demonstrated that gliadin is adsorbed by quartz, forming a complete film.
FIG. 1. The electrophoretic velocities of quartz particles coated with various proteins in various alcohols are plotted against the field strength. For each protein in a given medium the velocity is proportional to the field strength.

FIG. 2. The electrophoretic velocities of gelatin-covered quartz particles in media containing various percentages of ethyl alcohol are plotted against the field strength. In each medium the velocity is proportional to the field strength.
IV

Electrophoresis and Electroosmosis

It has previously been found that inert particles covered with proteins have, in general, mobilities independent of the bulk radius of curvature of the particle (8). That is, the same electric mobility is obtained in aqueous media whether the inert particle is microscopic in size or is very large. That this is also true for protein surfaces in alcohols has been found here by studying the ratio of electrophoretic to electroosmotic mobility. Fig. 3 is for gelatin surfaces in 40 per cent ethyl alcohol. Similar results were obtained in several trials with gliadin. The dotted line is the curve on which the points should fall according to the theory of Debye (9), according to which electrophoretic and electroosmotic mobilities are not equal. Evidently, however, the electrophoretic and electroosmotic mobilities are equal, and in the experiments to be discussed the size of the quartz particles used does not influence the mobilities obtained.

Fig. 3. The relative velocities of gelatin-coated quartz particles in 40 per cent ethyl alcohol at different depths in the electrophoresis cell are plotted against depth in the cell. The full line is the parabola \( y = -30.4 x^2 + 30.4 x \). The fact that the points fall on a parabola going through the origin is evidence of the equality of the electrophoretic and electroosmotic velocities.
Electric Mobilities of Gelatin in Alcohol-Water Mixtures

Fig. 4 shows the mobilities of gelatin-coated quartz particles in N/150 sodium acetate buffer in 0 per cent, 35 per cent, and 60 per cent ethyl alcohol. It is clear that alcohol shifts the isoelectric point of the gelatin toward smaller hydrogen ion activities. It is also obvious that alcohol lowers very greatly the maximum mobilities. This lowering combined with the shift in isoelectric point causes the curves to intersect.

![Graph showing mobilities of gelatin in different alcohol concentrations.](image)

Fig. 4. The electrophoretic mobility of gelatin-covered quartz particles is plotted against the pH of the medium for media containing different percentages of ethyl alcohol. In the more acid regions NaCl-HCl mixtures were used in place of the acetate buffers.

The lowering of the mobilities by alcohol is not a simple phenomenon. Alcohol changes both the dielectric constant and the viscosity of the medium and may also be expected to alter the electrokinetic potential. According to equation (2) each of these three changes will alter the mobilities.

Further corrections for these changes are not entirely straightforward. The question has frequently been raised (17) whether the dielectric constant and viscosity of the medium or the quite different values which might be expected to obtain within the double layer should be substituted in equation (2). Here values for the medium in bulk will
be used. In the following section it will be possible to show that these values of $D$ and $\eta$ can be used in combination to predict changes in charge from mobilities.

At this point those differences in the mobilities which were due to altered viscosity were eliminated by calculating a quantity called here corrected mobility;

$$\text{corrected mobility} = \frac{v_m}{\eta/\eta_n}$$

where $\eta_n$ = viscosity of pure water. This quantity has the significance that the differences between curves of corrected mobilities should be due to changes in the dielectric constant alone, the values being in some ways more representative of the effect of the alcohol itself on $v_m$.

As mentioned previously, the data in Fig. 4 give the mobilities uncorrected for $\eta$. It would involve too many complications to discuss the change in $v_m/\eta_n/\eta_o$ except near the isoelectric point, where the curves are nearly linear over a small distance. In Table I there are compared values obtained from smoothed curves of $v_m$ and $v_m/\eta/\eta_n$ for equal charge (as determined by the amount of acid bound). The large differences in $v_n$ disappear almost completely when the correction for $\eta$ is applied, only a slight decrease taking place as the alcohol concentration increases. This result is similar to Walden's results for ions (18).

<table>
<thead>
<tr>
<th>$V_m$</th>
<th>$V_m/\eta/\eta_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 per cent alcohol</td>
<td>35 per cent</td>
</tr>
<tr>
<td>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>0.40</td>
<td>0.15</td>
</tr>
<tr>
<td>0.60</td>
<td>0.21</td>
</tr>
<tr>
<td>0.80</td>
<td>0.25</td>
</tr>
<tr>
<td>0 per cent</td>
<td>35 per cent</td>
</tr>
<tr>
<td>0.20</td>
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<tr>
<td>0.40</td>
<td>0.40</td>
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<tr>
<td>0.60</td>
<td>0.54</td>
</tr>
<tr>
<td>0.80</td>
<td>0.67</td>
</tr>
</tbody>
</table>

The figures in each horizontal row are for pH's of equal charge as determined by the titration curves.
Mobilities and Titration Curves

Before proceeding to a comparison of titration and mobility curves in solutions of different dielectric constant, the rule following from equation (1) was tested in 35 per cent alcohol and again tested in 60 per cent alcohol. If \( n, r, \) and \( \kappa \) are constant it follows from equation (1) that \( Q \propto \nu_m \) and also that \( Q \propto \text{corrected mobility} \). In any one concentration of alcohol and at constant ionic strength \( n, r \) (19), and \( \kappa \) will be constant. Since on the assumptions (1) underlying the rule given in Section I, \( Q \) is the number of hydrogen or hydroxyl ions bound by a molecule, \( Q \) is proportional to the mols of acid (base) bound by a gram of gelatin and is represented by the titration curve. Thus acid bound and corrected mobility will be proportional to one another if the rule is obeyed.

The titration curves were constructed on the assumption that no acid or base is bound at the isoelectric point. Fig. 5 shows \( \nu_m \), \( \eta/\eta_0 \) in 35 per cent alcohol plotted with the titration curve in 35 per cent alcohol in such a way that the two curves coincide at two points.
That is, the scales are arranged so that the two curves correspond at pH 4.30 as well as at the isoelectric point. Fig. 6 shows $v_m \eta_0/n_0$ and titration curve in 60 per cent alcohol similarly arranged with pH 2.94 chosen for correspondence. Figs. 5 and 6 show that in each medium considered separately the mobility is proportional to the combining power, and hence presumably to the charge, in 60 per cent alcohol and up to pH 4.0 in 35 per cent. The discrepancy of about 30 per cent in the very acid region in 35 per cent alcohol may be due to the fact that the mobilities were determined in solutions of constant, the titration curves of varying, ionic strength. As a result of the assumptions underlying the rule given in Section I the proportionality makes it possible to conclude that in alcohol-water mixtures as well as in the previously investigated water solutions gelatin is adsorbed with the polar groups oriented toward the liquid. It is interesting that in 60 per cent alcohol the portions of both the mobility and the titration curves which lie near the isoelectric point were determined with partly or completely precipitated gelatin, yet both curves are smooth.

![Graph](https://example.com/graph.png)
VII

Electric Mobility, Titration Curve, and Charge

By comparing the mobilities in the different media (differing in dielectric constant and viscosity) it is possible to test to some extent the applicability of the viscosity and the dielectric constant of the bulk of the medium to the electrophoresis equation for charge, equation (1). The simplest means of doing this is to calculate charge from mobility by means of equation (1), using the viscosity and dielectric constant of the bulk of the medium. If now the acid bound (measured directly), which on the assumptions referred to above is proportional to the charge on the protein, is, in different media, in the same ratio as the charge calculated from the mobility by equation (1), then within the limits of the experimental error equation (1) may be used to predict changes in charge, using the viscosity and dielectric constant of the bulk of the medium.

In the calculation of the charge from equation (1) certain complications arise. The use of the factor $6\pi$ in equation (2) is based on the fact that the mobilities of certain dispersed proteins and of protein-covered particles have been found to be equal (17). Under these circumstances the radius to be used will be not that of the quartz particle but that of the protein molecule. For gelatin $2 \times 10^{-7}$ cm. was used, from the molecular weight (20). However, had $1 \times 10^{-7}$ cm. or $3 \times 10^{-7}$ cm. been used, the final conclusions would not have been noticeably different, although the absolute values of the charges would have been altogether different.

Fig. 7 shows the agreement between $Q$ from equation (1) and titration curves in the middle pH region for 0 per cent alcohol and 35 per cent. This graph was made by drawing the 0 per cent alcohol titration curve and charge points to scales which made them coincide and then drawing the 35 per cent titration curve and charge points to the same scales. All the charge points calculated from mobilities determined in acetate buffer fall very well onto the titration curve. The charge points calculated from mobilities determined in NaCl-HCl do not agree so well. The first of these points is shown in Fig. 7. This point indicates the general divergence from theory. That there should
be irregularities connected with the change from acetate buffer to a NaCl-HCl mixture is not surprising, since the ionic type as well as the valence does exert an influence (21).

For the more acid regions of the 35 per cent curve, as just pointed out, and for the 60 per cent curve, the agreement is less complete (Fig. 8). Because of slight disagreement between theory and experiment except under optimal conditions with 35 per cent alcohol and acetate buffers (Fig. 7), all of the data for acetate buffers and NaCl-HCl mixtures from pH 2 to pH 7 have been plotted as in Fig. 8,

![Fig. 7. The full circles show the charge of gelatin calculated from the mobility of gelatin-covered quartz particles in acetate buffer. The open circles show the charge calculated from the mobility in acetate buffer and 35 per cent ethyl alcohol. The lines are titration curves of gelatin in 0 per cent and in 35 per cent ethyl alcohol. The figure is limited to a range fairly close to the isoelectric point.](image)

...titration curves in the upper half, mobility curves in the lower. The titration curves of gelatin in 0 per cent and 35 per cent alcohol have been compared from pH 2 to pH 10. The curves are very much of the same shape, the isoelectric point being shifted to a higher pH, the curves converging at the limits. Gelatin, therefore, becomes a weaker acid in alcohol.

The disagreement in the more acid region of the 35 per cent charge and titration curves when plotted to the same scales as the 0 per cent curves is probably due to several causes. First is the previously men-
tioned shift from acetate buffer to NaCl-HCl solution. Second is the fact that since the mutual action of the ions may be greater in the acid range, it is likely to be even more so in alcohol in the acid range. This would lead to the charge calculated from the mobilities being in the very acid region too low for the titration curve arranged as previously explained, and this seems to be the case.

Fig. 8. The upper curves are titration curves of gelatin in 0 per cent, 35 per cent, and 60 per cent ethyl alcohol. The lower curves are the charge curves calculated from the mobility of gelatin-covered quartz particles in 0 per cent, 35 per cent, and 60 per cent ethyl alcohol, the circles being experimental points.

Dehydration by the alcohol probably affects chiefly the results in 60 per cent alcohol (22–24). This conclusion is consistent with the fact that going from 0 per cent alcohol to 35 per cent, acid bound and charge calculated from mobility are proportional, while the proportionality does not extent to the gelatin in 60 per cent alcohol.

However, the agreement with theory in the case of the change from
0 per cent to 35 per cent alcohol points to the complete ionization of the protein salt in alcoholic solutions in the neighborhood of the isoelectric point (since it is completely ionized in aqueous solutions), and to the applicability of the ordinary dielectric constant and viscosity, and the usefulness of the Debye-Henry equation, in the prediction of changes of charge.

VIII

Isoelectric Point

The isoelectric point of the gelatin-covered particles was taken by interpolation from the smoothed pH mobility curves or determined from experiments arranged to show no motion in the electric field. In the presence of alcohol the isoelectric point of particles covered with gelatin films was found to be shifted toward smaller hydrogen ion activities as shown in Fig. 9. The shift is related apparently linearly to the volumes per cent of alcohol in the solution and also linearly to the dielectric constant (neglecting the salt) of the solution. The form of the relationship will be discussed later. The direction and order of magnitude of the shift must first be accounted for.

Michaelis and Mizutani (25) measured the pH of a very dilute mixture of equivalent amounts of amino acid and the sodium salt of the amino acid in alcohol solution (at various concentrations of alcohol). The hydrogen ion activity (referred to the normal hydrogen electrode in pure water and neglecting liquid junction potentials) of this solution they called $k_2$. The constant similarly measured in acid solution they called $k_1$. Making the assumption that the ratio of the activity coefficients of the protein anion and cation remains constant for any one concentration of alcohol, it follows that the hydrogen ion activity of the isoelectric point is $\sqrt{k_1 k_2}$. Then,

$$\text{pH of the isoelectric point} = -\frac{1}{2} \log k_1 k_2 = \frac{1}{2} p_{k_1} + \frac{1}{2} p_{k_2},$$

and

$$\Delta \text{pH of the isoelectric point in going from one alcohol concentration to another} = \frac{1}{2} \Delta p_{k_1} + \frac{1}{2} \Delta p_{k_2}.$$
since Michaelis and his coworkers found the effects of alcohol on various organic acids to be much the same, it is possible to estimate from

\[ \Delta \text{pH} = \frac{1}{2} \Delta \text{p}k_1 + \frac{1}{2} \Delta \text{p}k_2 = \text{(for glycerol going from 0 per cent to 60 per cent alcohol)} 0.42. \]
This is in the same direction and is of the same order of magnitude as the shift of 1.1 found experimentally for gelatin. Using the values for para-aminobenzoic acid

$$\Delta \text{pH (from 4 per cent to 60 per cent)} = 0.93.$$ 

The form of the shift, which is linear with the dielectric constant, is interesting. Abramson⁴ has found that if the isoelectric points in alcohol solutions are calculated from the dissociation constants obtained by Michaelis and Mizutani for a number of amino acids, the isoelectric point is in each case linearly related by a limiting law to the dielectric constant of the medium (if the salt is neglected). This is true both for glycocoll and for the aminobenzoic acids. The fact that similar changes are produced by alcohol for gelatin and for simple ampholytes is in harmony with our present notions of the simple amphoteric behavior of proteins.

**SUMMARY**

1. The electrophoretic velocities of gelatin-, egg-albumin-, and gliadin-covered quartz particles in various alcohol-water solutions are, within the limits employed in usual experimental procedures, proportional to the field strength.

2. The electrophoretic mobilities of small, irregularly shaped quartz particles covered with an adsorbed film of protein in alcohol-water solutions are equal to the electroosmotic mobilities of the liquid past similarly coated flat surfaces. Hence the size and shape of such particles do not influence their mobilities, which depend entirely on the protein film.

3. The corrected mobility and hence presumably the charge of gelatin-covered quartz particles in solutions containing 35 per cent ethyl alcohol is proportional to the combining power of the gelatin; therefore the gelatin is adsorbed with the active groups oriented toward the liquid. The same is true in 60 per cent alcohol.

4. The charge calculated by means of the Debye-Henry approximation from the mobility of gelatin in solutions containing up to 35 per cent ethyl alcohol is, in the neighborhood of the isoelectric point, pro-

⁴ Work not yet published.
portional to the combining power of the gelatin. Therefore the dielectric constant and the viscosity of the bulk of the medium may be used in the Debye-Henry approximation

\[ Q = 6 \pi \eta r_m (1 + \kappa r) \]

to predict changes in charge from mobility.

5. In the neighborhood of the isoelectric point gelatin is probably completely ionized in buffered ethyl alcohol-water mixtures up to 60 per cent alcohol.

6. In the presence of ethyl alcohol the isoelectric point of gelatin is shifted toward smaller hydrogen ion activities. This shift, like that caused by alcohol in the isoelectric points of certain amino acids, is approximately linearly related to the dielectric constant of the medium.

The writer is greatly indebted to Dr. Harold A. Abramson, who suggested this work and under whose direction it was carried out.

BIBLIOGRAPHY