ELECTRIC CONDUCTIVITY AND TRANSFERENCE OF ALKALI ALBUMINATES

BY W. J. H. M. MÖLLER, G. A. J. VAN OS AND J. TH. G. OVERBEEK *
Preclinical Institute, R.C. University, Nijmegen, The Netherlands
Dept. of Chemistry, University of Southern California, Los Angeles, U.S.A.

Received 30th May, 1960

The present experimental study of the conductance and transference of alkali salts of bovine serum albumin under varying conditions of charge and salt concentration aims at a quantitative interpretation of electrophoresis. Special attention is paid to the range of low and zero salt content. For salt-free albuminates at low protein charge, the contribution of the charge fluctuations of the protein to the current transport through the solution cannot be neglected. The electrophoretic mobility of the protein appears to be independent of the nature of the alkali ion and increases with the charge, whereas the counterion mobility decreases with increasing protein charge. This lowering increases in the order lithium, sodium, potassium, in agreement with theoretical expectations concerning the relaxation of the ion cloud. Dilution of salt-free albuminates strongly increases the equivalent conductance of both the protein and its counterions as a result of a gradual decrease of the electrical retardations, viz., the electrophoretic and relaxation effect. Albuminates, dialyzed against dilute salt solutions, reveal a pronounced dependence of the colloid mobility on the protein concentration. In albuminates of constant charge and alkali ion concentration, the protein mobility was measured for different ratios of the albuminate-ion and chloride-ion concentrations. In these mixtures the equivalent conductance of the protein was found to be constant. A theoretical treatment of these data will be given in the next paper.

The present study of conductance and electrophoresis has been made to obtain data that could be interpreted as rigorously as possible in terms of modern theories of electrophoresis.[1, 2] Alkali salts of bovine serum albumin in the absence, or presence, of the corresponding alkali chlorides served as the material for this investigation. This protein has a well-known molecular weight.[3] It can easily be obtained in the iso-ionic state and by addition of a known amount of alkali the iso-ionic protein is converted into the corresponding alkali-albuminate of known equivalent concentration. Several investigations point to the absence of specific interaction between the albumin and univalent cations such as sodium and potassium.[4-7] Doremus and Johnson,[8] however, using a method similar to that used in the present paper, have reported an appreciable absorption of sodium ions by albumin in the alkaline region. This question will be a point of discussion in this paper. It is further well known that in albuminate solutions also containing alkali chloride, binding of chloride ions occurs,[9, 10] which depends upon pH and chloride concentration and therefore must be taken into account under certain conditions. While there is still some doubt about the shape and size of the albumin molecule, it seems fairly well established that between pH 4·3 and 10·5, the molecule behaves as a compact, undeformable particle.[11-14]

The main object of this paper is to present conductance and transference data under various conditions of charge, protein concentration and salt concentration. The quantitative interpretation of these data will be given in the next paper.

* permanent address: van 't Hoff Laboratory, University of Utrecht, The Netherlands.

EXPERIMENTAL

MATERIALS

Bovine serum albumin (B.S.A.) of the Poviet Company, Amsterdam (lot no. 70.0 and 70.2) was dissolved in conductivity water; the protein was completely de-ionized by passing the solution at 2-4°C through two ion-exchange columns, each containing a 2 : 1 mixture of anionic and cationic resins, viz., Amberlite I.R. 120 in the hydrogen form and Amberlite I.R.A. 400 in the hydroxyl form respectively.[15] These ion exchangers also withdraw lipoid impurities from the solution.[16] The protein solution was collected with exclusion of carbon dioxide and stored at −15°C. The concentration of the protein was determined by lyophilizing or drying to constant weight at 105-110°C, both methods yielding the same value to within 0·2 %. No alkali or alkaline earth ions, citric acid or chloride could be detected in the protein. The residue after destruction and ashing was not weighable.

The specific conductance of the conductivity water, prepared by passing distilled water through an ion-exchange column similar to that used for the de-ionization of the protein was about 2×10^{-7} ohm^{-1} cm^{-1} (25°C), when free of CO_2. The conductances of the albumin solutions were corrected throughout by subtracting this value. The pH of a 2·5 % iso-ionic solution was 4·90 and κ_{spec} was $5{\cdot}0\times10^{-6}$ ohm^{-1} cm^{-1} (25°C), in rather good agreement with the data of other workers, e.g. Timasheff *et al.*[17] Alkali albuminates were prepared by adding calculated amounts of alkali hydroxide (freed from carbonate by passage over Amberlite I.R.A. 400) to definite volumes of an isoionic stock solution of known concentration. The so-called titration charge per molecule $\bar{z}e$ was calculated from the electroneutrality condition:

$$\bar{z}c_M + c_{OH} = c_{Me} + c_H, \tag{1}$$

where $\bar{z}$ = mean valency of the albumin ion (absolute value); c_M = molar albumin concentration; c_{Me} = equivalent concentration of added base; c_H and c_{OH} are the concentrations of H and OH ions. In most cases, c_H and c_{OH}, calculated from the pH of the solution, were so small, that they could be neglected. It should be noted that the titration charge $\bar{z}e$ as defined by eqn. (1) is in general not identical with the net charge. When there is uptake of Me-ions by the protein the net charge will be smaller than the titration charge. A molecular weight of 69,000 was accepted as the most reliable value.[3] Mijnlieff [18] determined the molecular weight of B.S.A. in a sample of our stock solution, using a Spinco ultracentrifuge, model E. His results were in agreement with the literature value within the limits of accuracy. Contrary to the results of Champagne, who studied the sedimentation of Armour B.S.A., our centrifuge data did not show the presence of material with a sedimentation constant higher than the value of the main component.

CONDUCTIVITY MEASUREMENTS

A precision conductivity bridge which has been described in some detail earlier [19] was used for the conductivity measurements. Cell constants were determined at 25·0°C by calibration with KCl solutions.[20] Polarization errors were eliminated by extrapolating the readings obtained at 500 and 2000 c/sec to infinite frequency ($\Delta R \sim \nu^{-\frac{1}{2}}$). The accuracy of the resistance measurements was in general better than 0·05 %. All measurements were made at 25°C. The temperature was kept constant within 0·003°C.

HITTORF MEASUREMENTS IN THE ABSENCE OF SALT

A cell of the type represented in fig. 1 was used. A silver electrode surrounded by a 20 % sucrose solution in 1 N KCl served as the anode, while the cathode was of the Ag-AgCl type. Before filling, a stream of CO_2-free air was passed through the apparatus. After passage of the current, the middle and cathode compartments were analyzed. The mobility u_{alb} of the protein was calculated from

$$u_{alb} = \frac{\Delta m_{alb}\,\kappa_{spec}}{c_g\,Q}, \tag{2}$$

where Δm_{alb} = gram albumin migrating from the cathode compartment during current passage assuming no displacement of water; κ_{spec} = specific conductance of the protein solution; c_g = gram albumin per cm^3 and Q = amount of electricity in coulombs transported through the cell. A current stabilizer kept the current constant to within 1 %.

The current was read at sufficiently frequent intervals to permit a calculation of Q to within 0·1 % by summation of $i\Delta t$ over the whole experiment. The temperature was 25·0±0·002°C. The water of the thermostat was electrically connected to one of the electrodes and current leakage was checked by measuring the potential difference over two equal precision resistances on both sides of the cell. This was usually found to be far below 0·1 %. Three different methods to obtain Δm_{alb} were used.

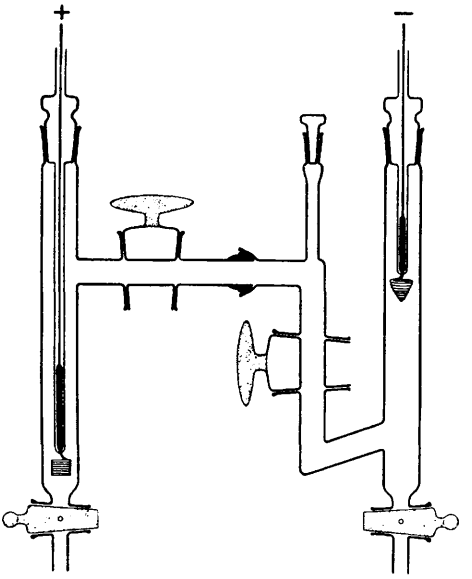


FIG. 1.—Hittorf apparatus.

(i) DRY WEIGHT DETERMINATION

For relatively high protein concentrations, i.e. 2-10 %, a 10 % concentration change in the cathode compartment could be computed with an accuracy of 1 %, provided the samples were air-dried (105-110°C) under identical circumstances. A correction was made for alkali chloride equivalent to the chloride liberated from the cathode.

(ii) U.-V. ABSORPTION AT 280 mμ

First portions of the original solution were exactly diluted to 95 %, 90 % and 80 % of the initial concentration. Next, the middle solution, the cathode solution and the " standard " solutions were all diluted in exactly the same proportion to a concentration in the range of about 1 mg protein per ml. This dilution served to attain a concentration range, where the extinction of the diluted 100 %, 95 % and 90 % samples, measured with reference to the diluted 80 % as blank were between 0·100 to 0·300. In this way the standards bracketed the unknown cathode solution in the analysis. A Unicam S.P. 500 spectrophotometer with the selector switch on position 0·1 was used for the differential analysis. The difference in albumin content of cathode and original solution could be determined with a relative accuracy of 2-5 % even for the most dilute solutions used.

(iii) INTERFEROMETER

The influence of salt and albumin concentration differences on the refractive index was determined separately and found to be additive in the concentration ranges used. The contribution of salt transport was taken into account. The accuracy of the albumin analysis depended on both the salt and the protein concentration and varied between 0·5-5 %. Measurements were made with a Zeiss interferometer.

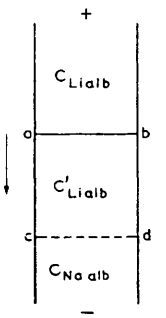
MOVING BOUNDARY METHOD

In the Hittorf experiments the mobility of the protein ions is found directly, while the counterion mobility is derived indirectly. In order to measure the counterion mobility

directly in salt-free sodium albuminates, the moving boundary method as described by MacInnes [21] was used. The albuminate ion was chosen as common ion, while sodium and lithium served as leading and indicator ion respectively (see fig. 2). The titration charge of the albumin, as calculated from eqn. (1), was chosen the same on both sides of the boundary. When the net charge is identical with the titration charge, i.e., when no uptake of cations by the protein is assumed, an identical anion throughout the solution would be obtained in this way. The adjusted concentration of lithium albuminate was calculated roughly from the equivalent conductances of sodium and lithium albuminates.[19]

FIG. 2.—Moving boundary situation.

ab original boundary;
cd boundary after current passage;
$c_{\text{Li alb}}$ = original Li alb concentration;
$c'_{\text{Li alb}}$ = adjusted Li alb concentration;
$c_{\text{Na alb}}$ = Na alb concentration.



If blurring of the boundary occurred, the original lithium albuminate concentration was apparently still too high and the lithium albuminate concentration was then lowered a few percent in the next experiment. Similarly, the concentration of the top solution was raised if a pronounced stationary boundary on the original starting point appeared. In this manner, an approximately adjusted value for $c_{\text{Li alb}}$ was found. The boundary was formed in a cylindrical tube with a cross-section of $0{\cdot}502 \pm 0{\cdot}001$ cm^2, and sharpened by suction through a fine capillary. The boundary motion was observed with a Philpot Svensson *Schlieren* optical system.

The equivalent conductance of the sodium ion was calculated from the observed boundary velocity v_{Na}:

$$\lambda_{\text{Na}} = v_{\text{Na}} F/E, \tag{3}$$

and

$$E = i/\kappa O, \tag{4}$$

where λ_{Na} = equivalent conductance of sodium; v_{Na} = boundary velocity in cm/sec; F = 96,500 coulombs; E = field strength in volt/cm in the sodium albuminate solution; i = current in ampères; κ = specific conductance of the sodium albuminate; O = cross section of the measuring tube in cm^2.

The corrections for the electrode reactions and concentration changes below the boundary were negligible throughout. For a more extensive description of the experimental details, see ref. (22).

HITTORF METHOD IN THE PRESENCE OF SALT

In Hittorf experiments, especially at a high salt content, the density near the cathode increases during current passage because accumulation of alkali chloride has a greater effect upon the density than the decrease of albuminate. In these cases, a Hittorf apparatus was used, different from that of fig. 1, in which the cathode could be placed near the bottom of the cell. The equivalent conductance λ_{Cl} of chloride was calculated from the equation,

$$\lambda_{\text{Cl}} = \frac{1000F(m_{\text{Cl}} - \Delta m_{\text{Cl}})\kappa_{\text{spec}}}{c_{\text{Cl}}\, Q}, \tag{5}$$

where m_{Cl} = number of equivalents of chloride, liberated from the AgCl electrode during current passage, which is equal to Q/F; Δm_{Cl} = increase of chloride content in the cathode compartment expressed in equivalents; c_{Cl} = chloride concentration in equiv./l. In this equation, $m_{\text{Cl}} - \Delta m_{\text{Cl}}$ represents the number of equivalents of chloride that leaves the cathode compartment as a result of current transport.

The increase Δm_{Cl} was again based on the solvent as a reference frame. Chloride analyses were performed by potentiometric titration with $AgNO_3$ of weighed samples of the original and of the cathode solution. Sharp end-point potentials were obtained by carrying out the titrations in 20 % acetic acid. The amount of $AgNO_3$, consumed by the presence of the SH groups in B.S.A., corresponded with 0·5 SH group per molecule albumin. Therefore, a small correction for this extra amount of $AgNO_3$ was made. Duplicate analyses were carried out to a precision of better than one part in a thousand. In the presence of salt, the albumin concentration was determined spectrophotometrically, since chloride does not interfere with this protein determination.

DIALYSIS

Iso-ionic albumin was brought to a pH of 8·8 by addition of a known amount of potassium hydroxide. Series of diluted potassium albuminate solutions in the concentration range of 0·1 to 5 % albumin, prepared from the stock solution, were dialyzed together against 10 l. of dilute KCl. A minute amount of KOH was added to this KCl solution, to bring the pH to a value close to that of the protein.

Previously, a certain amount of KCl was added to each protein solution in order to accelerate the attainment of equilibrium. After a dialyzing period of 7 to 10 days at 2°C equilibrium was attained, as proved by the fact that the e.m.f. between Ag/AgCl electrodes in the internal and external solutions was zero. Six series of such dialysis experiments were performed at salt concentrations of 0·5, 2, 5, 10, 20 and 50×10^{-3} equiv./l. The contents of the flask were magnetically stirred, CO_2 being excluded, while Nojax dialysis tube (Prins, Amsterdam) was used for the dialysis after a preliminary treatment as described by Klotz.[23]

The titration charge of the protein molecules was calculated from the electroneutrality condition, and was 20 ± 1 in all cases. In these salt-containing albuminates the net charge may differ from the titration charge for two reasons, a possible uptake of cations and a binding of chloride ions. At this high pH, however, binding of chloride ions is already so small [9, 10] that it may be neglected.

RESULTS AND DISCUSSION

SALT-FREE ALBUMINATES

At a constant concentration of 25 g serum albumin per l. and equivalent concentrations varying from 0-10·0 mequiv./l. (titration charge 0-30 elementary charges, pH 4·9 to 10·0) the equivalent conductance of the protein, $\lambda_{alb} = Fu_{alb}$ was derived from Hittorf experiments. The equivalent conductance of the alkali ion λ_{Me} (Me = Li, Na or K) was then calculated from the equation:

$$\kappa = \frac{c_{eq}}{1000}(\lambda_{Me}+\lambda_{alb})+\frac{c_H}{1000}\lambda_H^\circ+\frac{c_{OH}}{1000}\lambda_{OH}^\circ+\kappa_{water}. \qquad (6)$$

In this equation c_{eq} = concentration of Me-albuminate in equiv./l., c_H and c_{OH} are the concentrations of H^+ and OH^- ions as derived from the pH, λ_H° and λ_{OH}° are the corresponding limiting conductances and κ_{water} is the specific conductance of the CO_2-free water used in preparing the solutions. The last three terms are never more than small corrections (5 % in the most unfavourable case). It should be noted that, by using the equivalent concentration in eqn. (6), a charge is attributed to the albumin molecule which is equal to the titration charge. This means that the value for λ_{Me} found from this equation is a mean value in which not only are included the retardations of the cations due to the electrophoretic and relaxation effect, but also the influence of a possible binding of the cations by the protein, caused by specific or non-specific forces. The results are presented in fig. 3. First of all, it will be seen that at low equivalent concentration the equivalent conductances of the counterions exceed the limiting conductances. Since this effect appeared to be well beyond the limits of experimental errors, an explanation of this unexpected behaviour has been sought. A reasonable explanation can be found by taking into account the influence of the charge fluctuations of the protein, which occur because the molecules are continuously giving up and

taking on ions from their surroundings. A theoretical treatment of this effect has already been published elsewhere.[24] This treatment has shown that the fluctuations of the protein charge give rise to an increased transport of electricity compared to the situation in which all the macro-ions would carry identical charges. This current surplus leads to an additional term κ_{fluct} in the specific conductance, which should be added to the right-hand side of eqn. (6).

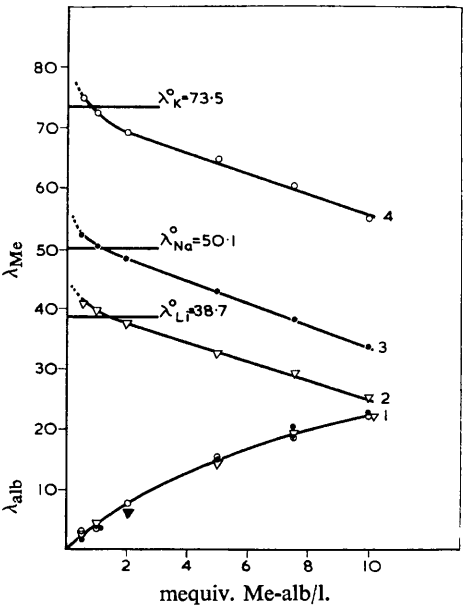


FIG. 3.—Effect of equivalent concentration on protein and counterion mobility at constant protein content (25 g B.S.A./l.); $t = 25°C$.

Curve 1: λ_{alb}; curve 2, 3, 4: λ_{Me}.
○ K alb; ● Na alb; ▽ Li alb.
Horizontal lines: λ°_{Me}.

It is clear that by omitting this correction term from eqn. (6) a too high value for λ_{Me} will be found. Moreover, when the other terms in eqn. (6) decrease (i.e. with decreasing equivalent concentration), the influence of κ_{fluct} on λ_{Me} will increase. The fluctuation conductance appears to be equal with good approximation to [24]

$$\kappa_{fluct} = \frac{Fc_M}{1000} \frac{du_{alb}}{d\bar{z}} \cdot (\overline{z^2} - \bar{z}^2), \tag{7}$$

in which $z^2 - \bar{z}^2$ is the difference between the mean square number of charges and the square of the mean number of charges. Assuming that in the low concentration region, the charge of the protein is entirely determined by the uptake of protons (i.e., the uptake of Me-ions can be neglected), the factor $\overline{z^2} - \bar{z}^2$ can be derived from a relation, first given by Linderstrøm-Lang:[25]

$$d\bar{z}/d(pH) = 2{\cdot}303(\overline{z^2} - \bar{z}^2). \tag{8}$$

Values of $d\bar{z}/d(pH)$ were derived from a salt-free titration curve of B.S.A. which was determined using a method similar to that of Tanford,[38] while values of $du_{alb}/d\bar{z}$ were computed from the λ_{alb} against $\bar{z}$ curve of fig 3. (For further details about the procedure followed and the values found, see ref. (22).) In this manner, κ_{fluct} and a new value of λ_{Me} were calculated. In fig. 4 we give corrected values of λ_{Me}.

It is of some interest to apply these ideas to isoionic serum albumin (conc. 25 g $l.^{-1}$). This solution has a pH of 4·9 and a specific conductance of $5{\cdot}2 \times 10^{-6}$ ohm^{-1} cm^{-1}. The pH would correspond to $c_H = 1{\cdot}26 \times 10^{-5}$ moles/l. if activity factors are neglected. With this amount of counterions the charge per albumin

molecule would be negative with about $\bar{z} = 0{\cdot}035$ and its equivalent conductance less than $\lambda_{alb} = 0{\cdot}1$. In order to compute κ_{fluct}, an estimate of the slope of the salt-free titration curve (2·5 %) at the very beginning of the negative charge interval was made, yielding $d\bar{z}/d(pH) = 2{\cdot}0 \pm 0{\cdot}4$. The value of $du/d\bar{z}$ was taken equal to the limiting mobility of albumin at $\bar{z} = 1$, which was calculated from Stokes' law:

$$u = \frac{1}{300}\frac{e}{6\pi\eta a}, \qquad (9)$$

in which e = the elementary charge = $4{\cdot}8 \times 10^{-10}$ e.s.u., η = the viscosity of water = 0·00894 poises at 25°C,[34] and a is the equivalent sphere radius of the protein, which was taken equal to 34·5 Å.[11, 13] An approximate value of

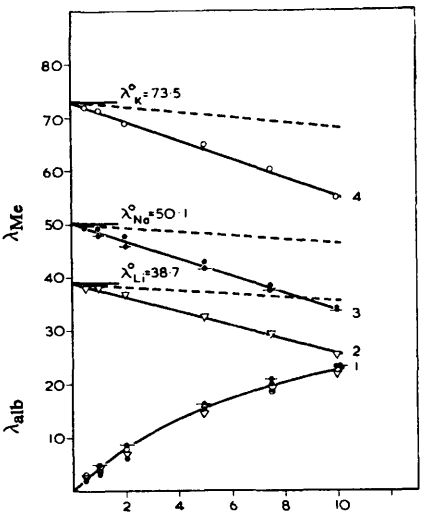


FIG. 4.—Effect of equivalent concentration on protein and counterion mobility after correction for charge fluctuation effect. Conc. albumin: 25 g B.S.A./l.; titration charge interval 0-30 elementary charges, pH 4·9 to 10·0; t = 25°C.

Curve 1: λ_{alb}; curve 2, 3, 4: λ_{Me}.
○ K alb, ● Na alb, ▽ Li alb—Hittorf method;
—●— Na alb—moving boundary method.
Dotted lines: λ_{Me} in MeCl solutions without protein (mequiv. MeCl/l.).

$F du/d\bar{z} = 2{\cdot}7$ was thus obtained. In this manner the fluctuation term was found to be $\kappa_{fluct} = 0{\cdot}85 \times 10^{-6}$. With $\kappa_{water} = 0{\cdot}2 \times 10^{-6}$ and assuming limiting mobility for the H-ions (350) at this low protein charge (cf. fig. 4) an adaptation of eqn. (6) can be used to calculate the concentration of H-ions, c_{eq}:

$$5{\cdot}2 \times 10^{-6} = \frac{c_{eq}}{1000}(350 + 0{\cdot}1) + 0{\cdot}2 \times 10^{-6} + 0{\cdot}85 \times 10^{-6}, \qquad (6')$$

leading to

$$c_{eq} = 1{\cdot}18 \times 10^{-5} \text{ moles/l.},$$

in agreement with the value calculated from the pH, as good as can be expected, considering the limited significance of the pH measurements in these media.[26] Inspection of the graphs in fig. 4 shows that by taking the charge fluctuations into account the equivalent conductances of the alkali ions can now be extrapolated correctly to the limiting conductances of these ions in water. Additional evidence for the validity of this explanation can be obtained by measuring the counterion mobility directly. To this end, moving-boundary measurements were made from which the value of λ_{Na} in sodium albuminate was derived directly. The results are also presented in fig. 4. It can be seen that these values are in good agreement with the corrected Hittorf values. The results of λ_{Na} as found from the moving boundary experiments can now be used to derive a value of λ_{alb}. Here again eqn. (6) with the correction term for the charge fluctuations, has to be used. The

values of λ_{alb} thus found (fig. 4) are also in good agreement with the directly measured Hittorf values.

It may be somewhat surprising that the value of λ_{Me}, when extrapolated to zero albumin charge equals the limiting conductance of the metal ion to within a few percent, because even at zero charge the solution still contains 2·5 % albumin and the viscosity of this solution is some 10 % higher than that of water.[27, 13] The counterions, however, do not move in a continuum of high viscosity, but in a liquid with the viscosity of water, in which a number of particles having much greater dimensions than the counterions are suspended. A closer analysis of the equivalent conductance of ions in the presence of insulating neutral spheres reveals two opposed effects.[33] The spheres being obstructions in the path of the current cause a decrease of the conductance, but most of this effect is offset by the increase of the concentration of ions, originating from the volume, occupied by the particles themselves. As a result of both effects, a lowering of the equivalent conductance of the alkali ions of about 1·0 % can be expected. The extrapolated values, however, are not accurate enough to detect this relatively small difference.

For comparison, the dotted curves in fig. 4 give the equivalent conductance of the alkali ions in the corresponding chlorides in water.[21] The slope of these curves is much less steep than those of the alkali albuminates. This effect has also been found in polyelectrolyte solutions by Wall and co-workers,[28-31] using a method similar to that described in this paper. They suppose that a fraction of the alkali-ions is bound to the polyelectrolyte and is thus forced to move with the polyelectrolyte molecules, while the rest of the alkali ions moves in the other direction with a mobility equal to that found in alkali chloride solutions of a concentration equal to that of the free alkali ions in the polyelectrolyte solution. This method has been used by Doremus and Johnson [8] to investigate the binding of sodium ions by B.S.A., and an appreciable absorption in the alkaline region was found. For instance, in a sodium albuminate solution of 11 g/l. (titration charge 27·2), they find a binding of 15·5 % of the counterions. Using the assumptions of Doremus and Johnson, we calculated from the data of fig. 4 at the same titration charge (albumin concentration 25 g/l.) a binding of 15·7, 18·3 and 17·6 % for K, Na and Li respectively. This, however, is in contrast to what has been found by Carr,[4] who, using activity measurements by means of permselective membranes, did not find any binding of sodium and potassium ions by B.S.A. on the alkaline side of the iso-ionic point, up to pH 8·8. Lewis and Saroff [5] also found, by means of a similar method, that B.S.A. does not bind potassium ions between pH 5 and 9. Only at pH 10·8, Carr finds a small binding of sodium and potassium, amounting to about 7 % of the counterions, but at this pH the albumin molecule probably is no longer a compact particle, since, as was suggested by Tanford *et al.*,[12] above pH 10·3 the molecule shows expansion. Moreover, as has already been pointed out by Martin and Van Winkle,[32] the assumption of Wall *et al.*, that the free counterions have the same mobility as in the corresponding alkali chloride solutions, should be questioned. They have found that this assumption may cause large errors in determining the degree of dissociation of polyelectrolytes. This is not surprising because the electrophoretic and relaxation retardations of the counterions of particles with a high charge can be much greater than in corresponding simple electrolytes. In view of the findings of Carr,[4] and Lewis and Saroff,[5] it seems, therefore, quite well possible that, at least between pH 5 and 9, the lowering of the mobility of the counterions in albuminates is not due to a binding of these ions in the sense as defined by Doremus and Johnson, but to non-specific electrostatic forces, resulting in high electrophoretic and relaxation retardations.

It will be shown in the next paper that, starting from this assumption and using the fact that the equivalent conductance of the protein is not perceptibly influenced by the nature of the counterions (see fig. 4), the relaxation retardation of the counterions and of the protein can be computed from the experimental mobilities of the counterions. The electrophoretic and relaxation forces also act on the

protein molecules and their influence could be demonstrated by varying the albumin concentration at constant titration charge. The 2·5 % alkali albuminate solution was simply diluted with water, except at the very lowest concentrations (<0·2 %) where it was diluted with very dilute alkali of the same pH as the original protein solution. In all cases, the titration charge, calculated from eqn. (1) remained constant upon dilution, because of the low concentrations of H^+ and OH^- ions. The results of Hittorf measurements made on thus diluted solutions are given in fig. 5 and fig. 6. From these curves it can be seen that dilution results in a strong increase of the equivalent conductance of the protein. This is as expected since dilution diminishes the screening effect of the double layer and therefore decreases the electrophoretic and relaxation retardations of the colloid.

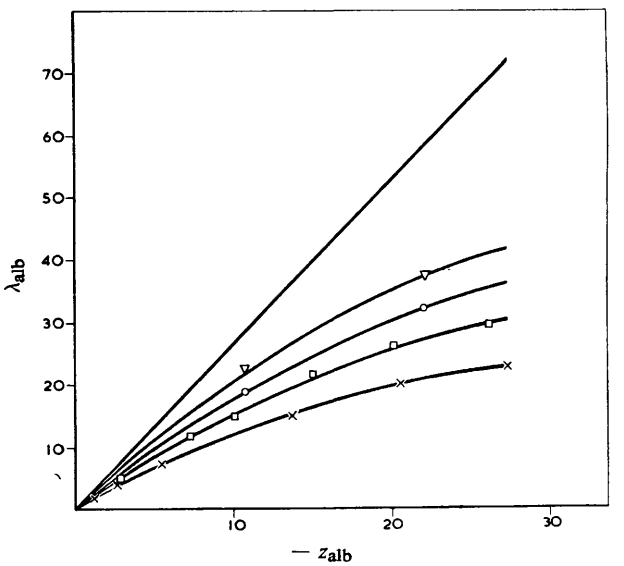


FIG. 5.—Influence of the titration charge on the protein mobility at different protein concentrations; Hittorf method; $t = 25°C$; upper line: λ°_{alb} (Stokes' law).

× 25 g B.S.A./l.; □ 10 g B.S.A./l.; ○ 2 g B.S.A./l.; ▽ 0·5 g B.S.A./l.

It should be noted that the increase in mobility of the protein cannot be explained from a possible binding of cations. In the numerical example given above, a binding of about 17 % of the counterions was found, while the increase in mobility of the protein upon dilution is very much greater. For comparison, values of λ_{alb} ($= Fu_{alb}$) at infinite dilution were computed from eqn. (9), in which the charge was taken 22·1 e instead of e.

In fig. 7 the equivalent conductances of the counterions potassium and lithium are given in albuminate solutions of a constant protein charge of 22·1 electron units. Inspection of the curves reveals that the retardations for potassium are higher than for lithium in substantial agreement with theoretical expectations concerning the relaxation effect. Since the charge fluctuation correction amounts to 0·5 % or less, this effect was neglected in this case.

ALKALI ALBUMINATES IN THE PRESENCE OF ADDED SALT

A series of Hittorf measurements was carried out using protein solutions of a constant titration charge of 20 ± 1, dialyzed together against different KCl concentrations. At the pH of these solutions (>8·5) binding of chloride ions is already so small [9, 10] that it may be neglected.

The influence of the protein concentration on λ_{alb} at different salt concentration is represented in fig. 8. The lowering influence of salt on λ_{alb} is obvious, while the variation of λ_{alb} with the protein concentration decreases steadily with increasing salt content. This dependence on the protein concentration and on the salt concentration will be discussed in more detail in the next paper.

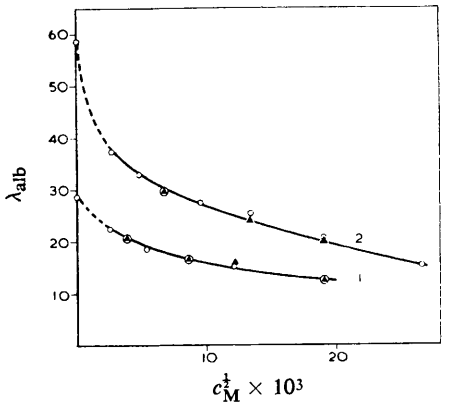


FIG. 6.—Effect of protein concentration on the protein mobility; Hittorf method; $t = 25°C$.

c_M = molar albumin concentration (range 0·05-5 % B.S.A.); curve 1: titration charge = 10·8 elementary charges per molecule, pH = 7·0 (25 g/l.); curve 2: titration charge = 22·1 elementary charges per molecule, pH = 8·5-8·9, depending on degree of dilution. ○ K alb; ▲ Li alb; extrapolated values: λ°_{alb} (Stokes' law).

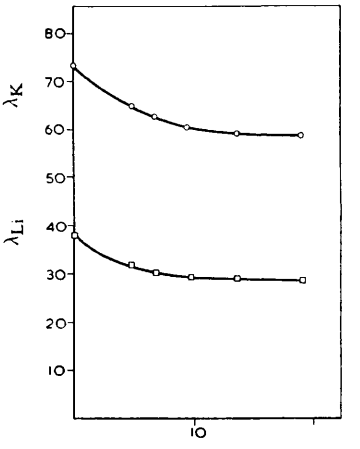


FIG. 7.—Influence of the protein concentration on the equivalent conductance of the counterions; titration charge = 22·1 elementary charges.

c_M = molar albumin concentration; extrapolated values: λ°_{Me} (water).
○ λ_K in K alb, □ λ_{Li} in Li alb.

In a second group of measurements the concentration of the alkali ions was fixed at 10^{-2} N and the [salt]/[protein] ratio varied, while the titration charge was kept at a constant value of 22·1 electron units. In these mixtures the protein ion was thus gradually replaced by chloride ions. Fig. 9 shows that in these cases λ_{alb} has a constant value of 20 ± 1 over the whole [salt]/[protein] range. In this connection it can be said that the Debye-Hückel theory,[35] as modified by counting the protein ion as z univalent ions in the " ionic strength " also predicts a constant value of λ_{alb}. As shown, there is no influence of the nature of the univalent counterion on λ_{alb}. Furthermore, the mean value of λ_{Cl} (73 ± 1) equals that of the solution of 0·01 N pure KCl (72·0 [36]) at least within the limits of accuracy. While it is known that chloride ions are bound by albumin at low protein charge,[9] this normal value of λ_{Cl} shows that chloride ions are not perceptibly bound at the relatively high net albumin charge of 22 electron units. Fig. 10 represents λ_{Me} as derived from the specific conductance with $\lambda_{alb} = 20$ and $\lambda_{Cl} = 72{\cdot}0$. The curves extrapolate correctly to the literature values of λ_{Me} in 0·01 N MeCl. In order to estimate the influence of the protein on the mobility of the cations, a formal separation of the conductance was made into contributions of the salt and of the albuminate which were assumed to be additive: [37]

$$\kappa_{Me\ alb} = \kappa_{spec} - \kappa_{MeCl}. \qquad (10)$$

First, κ_{MeCl} was calculated from the known chloride concentration, using literature values for the equivalent conductance of MeCl.[36] Then from eqn. (10) $\kappa_{Me\ alb}$

was found. $\kappa_{\text{Me alb}}$ can be written as

$$\kappa_{\text{Me alb}} = \frac{c_{\text{alb}}}{1000}(\lambda_{\text{alb}} + \lambda_{\text{Me(alb)}}), \tag{11}$$

where c_{alb} = equivalent concentration of albumin,
λ_{alb} = equivalent conductance of albumin,
$\lambda_{\text{Me(alb)}}$ = equivalent conductance of the alkali ions, "belonging" to the protein.

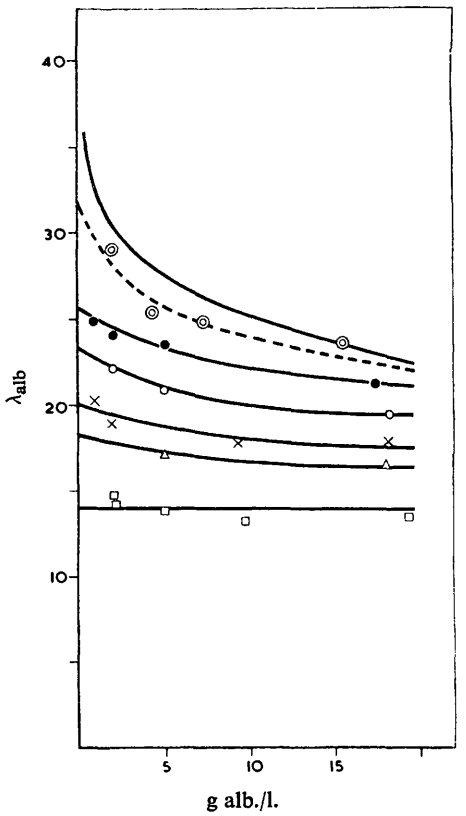


FIG. 8.—Influence of salt and protein concentration on the mobility of the protein ion in solutions of different protein concentration dialyzed together against KCl solutions of concentrations:

◎ 0·0005 N KCl, ● 0·002 N KCl,
○ 0·005 N KCl, × 0·010 N KCl,
▽ 0·020 N KCl, □ 0·050 N KCl.

Titration charge: 20 elementary charges; upper curve: salt-free albuminate; $t = 25°C$.

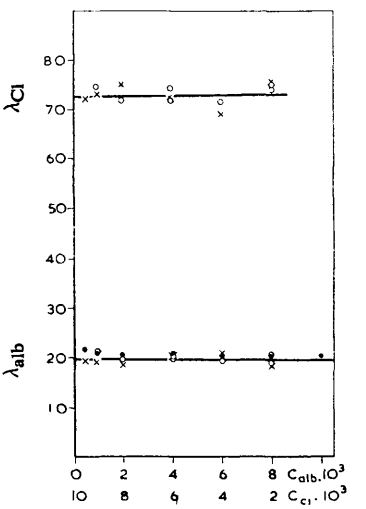


FIG 9.—λ_{Cl} and λ_{alb} at constant alkali ion concentration (0·01 N) and varying [chloride]/[protein] ratios; titration charge: 22 elementary charges; $C_{Me} + C_{alb} = 10^{-2}$ equiv./l.; $t = 25·0°C$.

○ K albuminate+KCl,
× Na albuminate+NaCl,
● Li albuminate+LiCl

From this equation, using the known values of c_{alb} and λ_{alb}, the corresponding values of $\lambda_{\text{Me(alb)}}$ were derived. The results are given in fig. 11. The low accuracy is due to the fact, that especially at lower protein concentration $\kappa_{\text{Me alb}}$ is but a few percent of the total conductance. Nevertheless, the trend is equal for the three alkali albuminates, viz., a strongly reduced rather constant $\lambda_{\text{Me(alb)}}$ value or perhaps a weak minimum over the [salt]/[protein] range investigated.

CHLORIDE BINDING TO ISO-IONIC ALBUMIN

In a recent study, Scatchard, Coleman and Shen [9] studied the chloride binding by means of e.m.f. measurements with permselective membranes, from which they

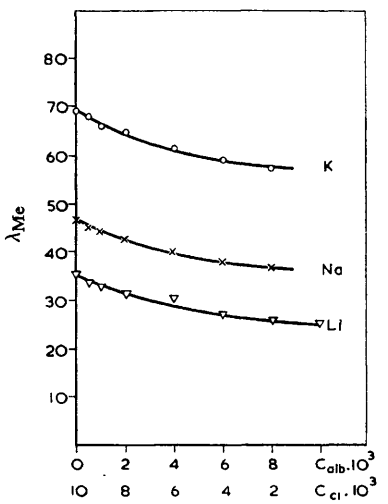


FIG. 10.—λ_{Me} in Me albuminates with $c_{Me} = c_{Cl} + c_{alb} = 10^{-2}$ equiv./l.

○ K albuminate+KCl,
× Na albuminate+NaCl,
▽ Li albuminate+LiCl.

Extrapolated values taken from literature (MacInnes); $t = 25°C$.

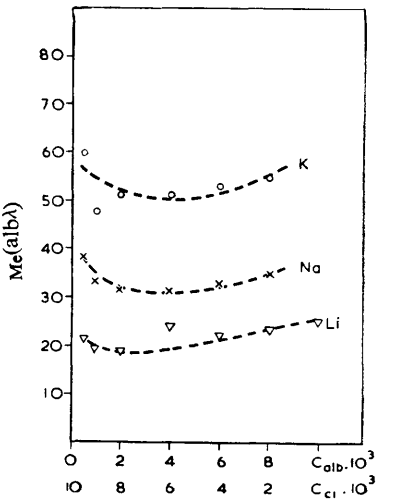


FIG. 11.—$\lambda_{Me(alb)}$ in chloride+protein mixtures with $c_{Me} = c_{Cl} + c_{alb} = 10^{-2}$ equiv./l.

○ K albuminate+KCl,
× Na albuminate+NaCl,
▽ Li albuminate+LiCl;
$t = 25°C$.

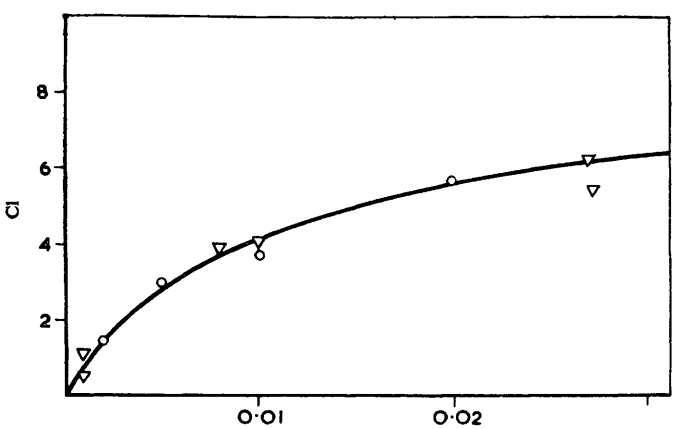


FIG. 12.—Comparison between activity and transport binding numbers of chloride in iso-ionic albumin.

▽ e.m.f. measurements with permselective membranes (Scatchard); ○ Hittorf measurements.

derived the number of chloride ions bound to the B.S.A. It is of interest to compare chloride binding numbers as derived from activity and transport measurements of iso-ionic albumin under identical circumstances. Therefore, we measured

λ_{Cl} and λ_{alb} in iso-ionic albumin solutions in the presence of added alkali chloride. The number ν_{Cl} of bound chloride ions per molecule was derived from the equations :

$$(c_{Cl}-\bar{z}c_M)\lambda_{Cl\,free}+\bar{z}c_M\lambda_{alb}=c_{Cl}\lambda_{Cl}, \tag{12}$$

and

$$\bar{z}=\nu_{Cl}, \tag{13}$$

in which $\lambda_{Cl\,free}$ = equivalent conductance of free chloride and c_{Cl}, λ_{Cl} and λ_{alb} are mean values in time (because of charge fluctuations). For this case, the purely electrical retardations experienced by the free ions will be almost negligible. Since the viscosity effect will also be small, we assumed that with good approximation the equivalent conductance of the free chloride ions is equal to that of the solution without protein. In fig. 12, the data of these Hittorf experiments are given. From this figure we see that activity and transport binding numbers agree within the limits of accuracy. The results indicate once more the potentialities of mobility measurements in detecting binding phenomena.

1 Overbeek, *Adv. Colloid Sci.*, 1950, **3**, 97.
2 Bier, *Electrophoresis* (Acad. Press, N.Y., 1959), chap. 1 and 8.
3 Harrington, Johnson and Ottewill, *Biochem. J.*, 1956, **62**, 569.
4 Carr, *Arch. Biochem. Biophys.*, 1956, **62**, 476.
5 Lewis and Saroff, *J. Amer. Chem. Soc.*, 1957, **79**, 2112.
6 Michaelis and Kawai, *Biochem. Z.*, 1925, **163**, 1.
7 Neuhausen and Marshall, *J. Biol. Chem.*, 1922, **53**, 365.
8 Doremus and Johnson, *J. Physic. Chem.*, 1958, **62**, 203.
9 Scatchard, Coleman and Shen, *J. Amer. Chem. Soc.*, 1957, **79**, 12.
10 Carr, *Arch. Biochem. Biophys.*, 1953, **46**, 417.
11 Champagne, *J. Chim. Physique*, 1957, **54**, 378.
12 Tanford, Swanson and Shore, *J. Amer. Chem. Soc.*, 1955, **77**, 6414.
13 Tanford and Buzzell, *J. Amer. Chem. Soc.*, 1956, **60**, 225.
14 Krause and O'Konski, *J. Amer. Chem. Soc.*, 1959, **81**, 5082.
15 Dintzis, *Doctoral Diss.* (Harvard University, 1952).
16 Goodman, *Science*, 1957, **125**, 1296.
17 Timasheff, Dintzis, Kirkwood and Coleman, *J. Amer. Chem. Soc.*, 1957, **79**, 782.
18 Mijnlieff, *Doctoral Diss.* (State University, Utrecht, 1958), p. 118.
19 van Os and Koopman van Eupen, *Rec. trav. chim.*, 1957, **76**, 390.
20 Jones and Bradshaw, *J. Amer. Chem. Soc.*, 1933, **55**, 1780.
21 MacInnes, *The Principles of Electrochemistry* (Reinhold Publishing Corporation, New York, 1939), p. 68.
22 Möller, *Doctoral Diss.* (State University, Utrecht, 1959).
23 Klotz, *Methods of Biochemical Analysis* (Interscience Publishers, New York, 1956, vol. III), p. 280.
24 van Os and Möller, *Proc. K. Acad. Wetensch. B*, 1959, **61**, 253.
25 Linderstrøm-Lang, *Proteins, Amino Acids and Peptides* (Reinhold Publishing Corporation, New York, 1943), p. 462.
26 Overbeek, *Progr. Biophysics*, 1956, **6**, 57.
27 Tanford, Buzzell, Rands and Swanson, *J. Amer. Chem. Soc.*, 1955, **77**, 6421.
28 Huizinga, Grieger and Wall, *J. Amer. Chem. Soc.*, 1950, **72**, 2636.
29 Wall and Doremus, *J. Amer. Chem. Soc.*, 1954, **76**, 1557.
30 Wall, Terayama and Techakumpuch, *J. Polymer Sci.*, 1956, **20**, 477.
31 Wall and Eitel, *J. Amer. Chem. Soc.*, 1957, **79**, 1550.
32 Martin and Van Winkle, *J. Physic. Chem.*, 1959, **63**, 1539.
33 Hamer, *The Structure of Electrolyte Solutions* (John Wiley, New York, 1959), chap. 20
34 Robinson and Stokes, *Electrolyte Solutions* (Butterworth, London, 1955), chap. 7.
35 Debye and Hückel, *Physik. Z.*, 1923, **24**, 305; 1924, **25**, 49.
36 MacInnes, *The Principles of Electrochemistry* (Reinhold Publishing Corporation, New York, 1939), p. 340.
37 van Geelen, *Doctoral Diss.* (State University, Utrecht, 1958), p. 13.
38 Tanford in Shedlovsky (ed.), *Electro-chemistry in Biology and Medicine* (John Wiley and Sons Inc., New York, N.Y., 1955).