INTERPRETATION OF THE CONDUCTANCE AND TRANSFERENCE OF BOVINE SERUM ALBUMIN SOLUTIONS

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It is shown how the relaxation effect can be determined from transference data on the same colloidal particle with different counterions. The electrophoretic retardation is calculated for spheres with overlapping double layers. The relation between charge and potential distribution is also developed for this case. The theory is applied to transference and electrophoresis data of solutions of bovine serum albumin. Agreement is very good for low charge of the albumin, but less good for charges of 10 or more elementary charges per molecule. The difference is (at least in part) due to the use of the Debye-Hückel approximation. In the range of charges (up to 27 elementary charges per molecule) and concentrations (up to 5 %) used, the electrophoretic retardation is much larger than the relaxation effect, which hardly surpasses 20 % of the total retardation.

1. Introduction

In a previous paper, experimental data on the equivalent conductance of bovine serum albumin (B.S.A.) and its counterions have been presented.[1] The experiments were performed under varying conditions of charge, salt and protein concentration. Many parallel experiments have been carried out where the only variation was in the nature of the cations (Li, Na or K). In this paper, a quantitative interpretation of these results is presented. Current theories of electrophoresis [2] which deal with a single particle immersed in a large volume of simple electrolyte, have been extended to include finite particle concentrations and overlapping double layers. An experimental estimate of the relaxation effect is derived from a mutual comparison of the mobilities of different counterions, assuming absence of specific interactions. Reasonable agreement between theory and experiments is obtained, when the B.S.A. molecule is assumed to be spherical with a hydrodynamic radius of 34·5 Å. A somewhat better agreement could perhaps be obtained with a prolate ellipsoid, but this can only be decided with certainty when the electrophoretic effect (§ 3) and the potential distribution in the double layer (§ 4) are also calculated for this model.

2. Relaxation effect as determined from the equivalent conductance of the counterions

1. Salt-free case

Consider a negatively charged particle of arbitrary shape, surrounded by an ionic atmosphere of univalent ions of opposite charge. On application of the external electric field, the situation is characterized by the following forces (see fig. 1):

(i) $X_1 = zeE$, where z = valency of the colloid (sign included); e = protonic charge and E = field strength in volt cm^{-1}.

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(ii) $X_2 = -fv$, where f = frictional constant of the particle (charge effects neglected) and v = velocity in stationary state.

(iii) $X_3 = ze\Delta E$, where ΔE = an averaged relaxation field of intensity ΔE acting in opposite direction to the original field of strength E.

(iv) $X_{4\,\mathrm{eff}}$ = effective electrophoretic force in the presence of relaxation.

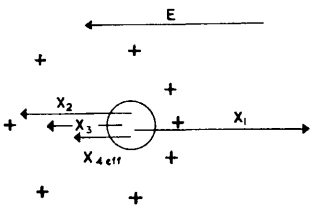

FIG. 1.—Forces on a negatively charged particle.

X_1 = force exerted by electric field X_2 = frictional force
X_3 = relaxation force $X_{4\,\mathrm{eff}}$ = electrophoretic force.

In the absence of deformation of the double layer, the electrophoretic force would be X_4. The actual deformation of the double layer, which gives rise to a relaxation field of strength ΔE, lowers the electrophoretic force to

$$X_{4\,\mathrm{eff}} = X_4(1+\Delta E/E). \tag{1}$$

Here the assumption is made that the average field strength responsible for the electrophoretic retardation is the same as that on the particle. In the stationary state all forces on the particle cancel:

$$X_1+X_2+X_3+X_{4\,\mathrm{eff}} = 0. \tag{2}$$

After substitution of the proper values for the different forces in eqn. (2), we obtain for the velocity of the particle at unit field strength,

$$u_{\mathrm{coll}} = (u^{\circ}_{\mathrm{coll}}+u'_{\mathrm{coll}})(1+\Delta E/E), \tag{3}$$

in which $u^{\circ}_{\mathrm{coll}} = ze/f$, the mobility of the colloid at infinite dilution, $u'_{\mathrm{coll}} = X_4/Ef$, the electrophoretic retardation in the absence of relaxation, and $(1+\Delta E/E)$ the fraction of the applied field which effectively operates on the particle. The latter is always smaller than one and gives the ratio of the mobilities with and without relaxation.

As the average relaxation field on the counterions must be the same as that on the particles, the mobility of the counterions can be written as

$$u_{\mathrm{Me}} = (u^{\circ}_{\mathrm{Me}}+u'_{\mathrm{Me}})(1+\Delta E/E), \tag{4}$$

where u°_{Me} = limiting mobility of the counterions; u'_{Me} = electrophoretic retardation in the absence of relaxation; u_{Me} = mean mobility of the counterions, electrophoretic and relaxation effect included. The relaxation field ΔE can be derived from a comparison of the mobilities of different types of counterions. As shown in the previous paper,[1] the protein mobility in salt-free alkali albuminates appeared to be independent of the nature of the alkali ion used within experimental error. When absence of specific interactions between counterions and protein is assumed, the ionic atmospheres will be practically identical for counterions of the same valence. Then, since the electrophoretic correction only depends on the distribution of charge in the atmosphere, the electrophoretic retardations u'_{coll} and u'_{Me} will be independent of the nature of the cation. Hence, it follows from eqn. (3) that $(1+\Delta E/E)$ must be independent of the nature of the alkali ion too. This is not surprising, since, although the relaxation effect is related to the mobility,

its sensitivity to it is only small. Confining attention to the alkali ions potassium and lithium, we can write

$$u_K = (u_K^\circ + u_K')(1+\Delta E/E), \tag{5}$$

$$u_{Li} = (u_{Li}^\circ + u_{Li}')(1+\Delta E/E), \tag{6}$$

and

$$u_K' = u_{Li}'. \tag{7}$$

Combination of (5), (6) and (7) gives

$$1+\frac{\Delta E}{E} = \frac{u_K - u_{Li}}{u_K^\circ - u_{Li}^\circ}. \tag{8}$$

From a measurement of u_{Me} for two different alkali ions in the corresponding salt-free albuminates, identical except for the choice of the alkali ions, the relaxation factor $(1+\Delta E/E)$ is thus easily derived.

Since $(1+\Delta E/E)$ also describes the relaxation of the colloid, the latter relaxation is known at the same time. Hence the problem of explaining the observed mobilities of the central particle is reduced to the proper treatment of the electrophoretic retardation u_{coll}' of the particle (see § 3).

2. Salt added

In the presence of salt, the situation is more complicated. Distant from the colloid, the equivalent conductance of the counterions may be expected to be equal to that found in a solution where the colloid is replaced by small co-ions (small ions with a charge of the same sign as that of the colloid), while in the regions close to the particle strong electrophoretic and relaxation effects may be expected. Since the co-ions are pushed away from the colloidal particles and since their mobility is not affected by the presence of serum albumin within the accuracy of our experiments, we make a formal separation of the counterions into " free " counterions and counterions belonging to the particle.[3] The " free " counterions are assumed to have normal mobility, while the counterions belonging to the particle just compensate its charge and are subject to electrophoretic retardations and to a relaxation field ΔE, which again is exactly equal to that on the colloid particle. The concentration of the " free " counterions is equal to the known concentration of the co-ions. Since the concentration of the counterions belonging to the particle is also known, the mobility of these ions can then be found from the observed mobility and the normal mobility of the " free " counterions. The relaxation field can then be calculated from a comparison between Li^+ and K^+ ions, just as for the salt-free case.

3. Electrophoretic effect in the presence of overlapping double layers

In the presence of overlapping double layers, the electrophoretic effect on one particle cannot strictly be calculated without taking the presence of other particles into account. In view of the mutual repulsion between particles, they will only rarely come close to one another. Therefore it seems reasonable to consider each particle to be surrounded by a spherical shell, containing just enough small ions to neutralize its charge, and having a size so that all spherical shells together just use up the total volume. This treatment has been used earlier by Katchalsky, Künzle and Kuhn,[6] and by Wall and Berkowitz,[7] for salt-free polyelectrolyte solutions.

When n_{coll} is the number of colloidal ions per cm^3, the outer radius of the spherical shell is given by

$$\tfrac{4}{3}\pi R^3 = 1/n_{coll}. \tag{9}$$

The electrophoretic mobility $u_{coll.\ el.}$ can now be derived following Onsager's reasoning[4] as applied to strong electrolytes. Here we assume a particle with a

hydrodynamic radius a and a charge of z elementary charges (sign included). Further the following conditions should be satisfied:

(i) The boundary condition $(\mathrm{d}\psi/\mathrm{d}r)_R = 0$ obtains, where $(\mathrm{d}\psi/\mathrm{d}r)_R$ is the potential gradient at the boundary of the sphere defined in eqn. (9).

(ii) The distortion of the applied electric field around the spherical particle is negligible, or the extension of the double layer is large with respect to the particle radius (see Henry [5]).

(iii) Viscosity η and dielectric constant ε are constant in the whole double layer.

(iv) Stokes' law obtains.

When the ionic atmosphere of the colloid is considered to be arranged in spherical shells, the electrophoretic retardation $\mathrm{d}u_{\mathrm{coll}}$ caused by a shell of thickness $\mathrm{d}r$ is given by

$$6\pi\eta r\mathrm{d}u'_{\mathrm{coll}} = \rho 4\pi r^2\mathrm{d}r, \tag{10}$$

in which r = distance from the centre of the particle ρ = charge density. Hence the total electrophoretic retardation u'_{coll} amounts to

$$u'_{\mathrm{coll}} = \frac{1}{6\pi\eta}\int_a^R 4\pi r\rho\mathrm{d}r. \tag{11}$$

From Poisson's relation we have

$$\rho = -\frac{\varepsilon}{4\pi}\frac{1}{r}\frac{\mathrm{d}^2(r\psi)}{\mathrm{d}r^2}, \tag{12}$$

and since

$$(\mathrm{d}\psi/\mathrm{d}r)_R = 0,$$

we obtain after integration,

$$u'_{\mathrm{coll}} = \frac{\varepsilon}{6\pi\eta}(\psi_a - \psi_R) + \frac{\varepsilon}{6\pi\eta}a\left(\frac{\mathrm{d}\psi}{\mathrm{d}r}\right)_a, \tag{13}$$

where ψ_a = potential at the particle surface and ψ_R = potential at the boundary of the large sphere.

The field strength $(\mathrm{d}\psi/\mathrm{d}r)_a$ at the particle surface is given by

$$(\mathrm{d}\psi/\mathrm{d}r)_a = -ze/\varepsilon a^2, \tag{14}$$

and the velocity of the particle according to Stokes' law can be written as

$$u^{\circ}_{\mathrm{coll}} = \frac{ze}{6\pi\eta a} = -\frac{\varepsilon}{6\pi\eta}a\left(\frac{\mathrm{d}\psi}{\mathrm{d}r}\right)_a. \tag{15}$$

The total electrophoretic velocity, relaxation excluded, is given by

$$u_{\mathrm{coll.el.}} = u^{\circ}_{\mathrm{coll}} + u'_{\mathrm{coll}} = \frac{\varepsilon}{6\pi\eta}(\psi_a - \psi_R), \tag{16}$$

a result identical to Hückel's [8] electrophoretic equation $v = \varepsilon\zeta/6\pi\eta$ if we define

$$\psi_a - \psi_R \equiv \zeta. \tag{17}$$

4. Potential distribution in the presence of overlapping double layers

Around a negatively charged particle the concentration of univalent counter-ions is, according to Maxwell-Boltzmann statistics,

$$n_+ = \bar{n}_+ \exp\{-e(\psi - \bar{\psi}_+)/kT\}, \tag{18}$$

and the concentration of small co-ions,

$$n_- = \bar{n}_- \exp\{+e(\psi - \bar{\psi}_-)/kT\}. \tag{19}$$

In these equations the reference concentrations have been identified with the stoichiometric concentrations of the small ions $\bar{n}_+$ and $\bar{n}_-$ (number per cm^3).

Consequently, $\bar{\psi}_+$ and $\bar{\psi}_-$ represent the potentials in those points between a and R, where the small ion concentrations just equal $\bar{n}_+$ and $\bar{n}_-$ respectively. The Poisson-Boltzmann equation for a spherical particle reads

$$\Delta\psi = \frac{1}{r}\frac{d^2(r\psi)}{dr^2} = -\frac{4\pi}{\varepsilon}[\bar{n}_+ e \exp\{-e(\psi-\bar{\psi}_+)/kT\} - \bar{n}_- e \exp\{+e(\psi-\bar{\psi}_-)/kT\}]. \quad (20)$$

It is convenient to define the following dimensionless variables,

$$\Phi = \frac{e\psi}{kT}; \quad \Phi_+ = \frac{e\bar{\psi}_+}{kT}; \quad \Phi_- = \frac{e\bar{\psi}_-}{kT}, \quad (21)$$

and the important quantity,

$$\kappa = \left\{\frac{4\pi e^2}{\varepsilon kT}(\bar{n}_+ + \bar{n}_-)\right\}^{\frac{1}{2}}. \quad (22)$$

Eqn. (20) cannot be solved analytically. An approximate solution, valid in the range $\Phi-\Phi_+$ and $\Phi-\Phi_-\ll 1$ (corresponding to 25·6 mV at 25°C) can be obtained by using the Debye-Hückel approximation. After linearizing the exponential terms, and using the electroneutrality condition

$$\bar{n}_+ - \bar{n}_- + n_{coll} z = 0$$

(where n_{coll} = number of colloidal ions per cm^3 and z their valence, sign included) one obtains

$$\frac{d^2(r\Phi)}{dr^2} = \kappa^2 r\left(\Phi + \frac{n_{coll} r - \bar{n}_+\Phi_+ - \bar{n}_-\Phi_-}{\bar{n}_+ + \bar{n}_-}\right) = \kappa^2 r(\Phi + b). \quad (23)$$

In eqn. (20) the choice of the reference levels was immaterial. The calculated change of the potential with the distance is strictly independent of this choice. This is no longer exactly true for the linearized eqn. (23). The reference levels should, therefore, be chosen in such a way as to keep $\Phi-\Phi_+$ and $\Phi-\Phi_-$ as small as possible. This is obtained by choosing the reference levels between $r = a$ and $r = R$ as we have done.

Eqn. (23) has the general solution,

$$\Phi = A\frac{\exp(-\kappa r)}{r} + B\frac{\exp(+\kappa r)}{r} - b. \quad (24)$$

The integration constants A and B can be found by means of the boundary conditions,

$$(d\Phi/dr)_R = 0, \quad (25)$$

and

$$(d\Phi/dr)_a = -ze^2/\varepsilon a^2 kT. \quad (26)$$

After reconverting Φ into ψ, the solution of (23), satisfying the two boundary conditions then reads:

$$\psi_r = \frac{ez}{\varepsilon r}\left[\frac{\exp\{-\kappa a\}}{\frac{\kappa R-1}{\kappa R+1}(\kappa a+1)\exp\{2\kappa(R-a)\} - \kappa a + 1}\right] \left[\frac{\kappa R-1}{\kappa R+1}\exp\{\kappa(2R-r)\} + \exp\{\kappa r\}\right] - \frac{\frac{kT}{e}n_{coll} z - \bar{n}_+\bar{\psi}_+ - \bar{n}_-\bar{\psi}_-}{\bar{n}_+ + \bar{n}_-}, \quad (27)$$

from which the potential difference $\psi_a - \psi_R$ can be directly computed.

Two limiting cases are of special interest.

(i) " SALT-FREE " COLLOIDAL SYSTEMS

In the absence of co-ions $\bar{n}_- = 0$ and κ becomes

$$\kappa = \left\{\frac{4\pi e^2}{\varepsilon kT}\bar{n}_+\right\}^{\frac{1}{2}}. \tag{28}$$

For the salt-free case, the potential equation therefore reads:

$$\psi_r = \frac{ez}{\varepsilon r}\left[\frac{\exp\{-\kappa a\}}{\frac{\kappa R-1}{\kappa R+1}(\kappa a+1)\exp\{2\kappa(R-a)\}-\kappa a+1}\right]$$
$$\left[\frac{\kappa R-1}{\kappa R+1}\exp\{\kappa(2R-r)\}+\exp\{\kappa r\}\right]+\frac{kT}{e}+\bar{\psi}_+, \tag{29}$$

differing from eqn. (27) only in the physical unimportant constant term.

(ii) HIGH SALT AND LOW COLLOID CONCENTRATION

It can be shown that for large values of κ and R, the potential differences in the double layer become

$$\psi_r-\psi_R = \frac{ez}{\varepsilon r}\frac{\exp\{\kappa(a-r)\}}{1+\kappa a}, \tag{30}$$

a result which is identical with the familiar Debye-Hückel expression.[9]

5. APPLICATION TO DATA ON BOVINE SERUM ALBUMIN

1. NO SALT ADDED

Our theory so far has only been worked out for spherical particles. However, uncertainty still exists with respect to the shape and size of the B.S.A. molecule. Champagne,[10] as well as Tanford and Buzzell,[11] were unable to give a definite conclusion about the shape of the particle, although they suggest that it cannot be very far from a sphere. These authors found from viscosity and diffusion measurements, that if the molecule is assumed to be a sphere, its radius is between 33 and 36 Å. We shall, therefore, first consider the B.S.A. molecule to be a sphere with a radius of 34·5 Å. This assumption has no influence on our estimate of the relaxation effect from eqn. (8), since the derivation of this equation is independent of the shape of the particle. The eqn. (16) and (17) for the relation between ζ-potential and electrophoretic velocity (relaxation neglected) will probably not depend too much on the particle form.

Eqn. (27) is used to calculate $\zeta = \psi_a - \psi_R$; when no uptake of cations is assumed the charge ze can be taken equal to the titration charge (see previous paper [1]). The molecular weight of B.S.A. was taken as 69,000. Using eqn. (8) to calculate the relaxation effect from the transference data of the counterions, and combining the eqn. (3), (16) and (17), the equivalent conductance of the B.S.A. can be found from

$$\lambda_{\text{alb.el.}} = F\varepsilon\zeta/6\pi\eta, \tag{31}$$

and

$$\lambda_{\text{alb.comp.}} = \lambda_{\text{alb.el.}}(1+\Delta E/E), \tag{32}$$

where $\lambda_{\text{alb.el.}}$ = equivalent conductance without relaxation correction and $\lambda_{\text{alb.comp.}}$ = equivalent conductance including relaxation correction. The following numerical values were used: $\eta = 0{\cdot}008937$ poise (25°C); $\varepsilon = 78{\cdot}54$ (25°C); $F = 96{,}490$ coulombs, while various other constants were taken from the literature.[12]

Fig. 2 illustrates the validity of the theory for salt-free alkali albuminates of varying charge and constant protein content. For comparison, values of λ°_{alb} are given as derived from Stokes' law (using 34·5 Å for the radius). From these curves it is clear that the electrophoretic effect is by far the most important retardation, the relaxation being of secondary importance. Nevertheless, the

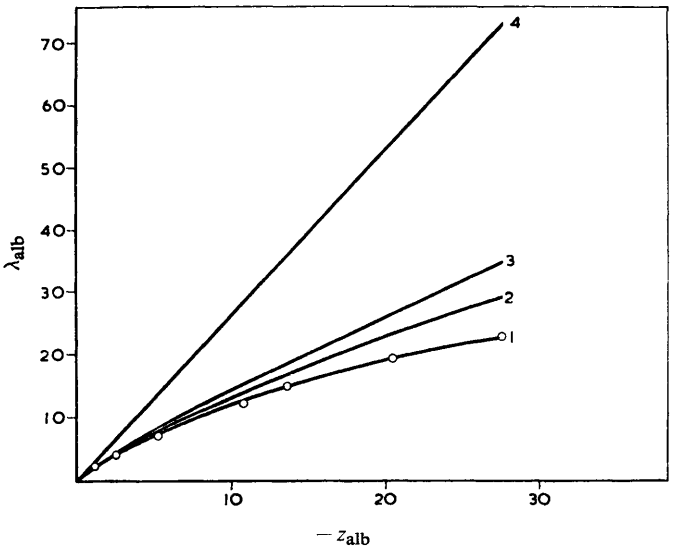

FIG. 2.—Equivalent conductance (25·0°C) of B.S.A. in salt-free alkali albuminates, 2·5 %, as a function of the albumin charge.

Curve 1: as found from experiment; curve 2: if calculated from (32) electrophoretic and relaxation effect included; curve 3: if calculated from (31) (electrophoretic retardation included); curve 4: if calculated from Stokes' law (a = 34·5 Å).

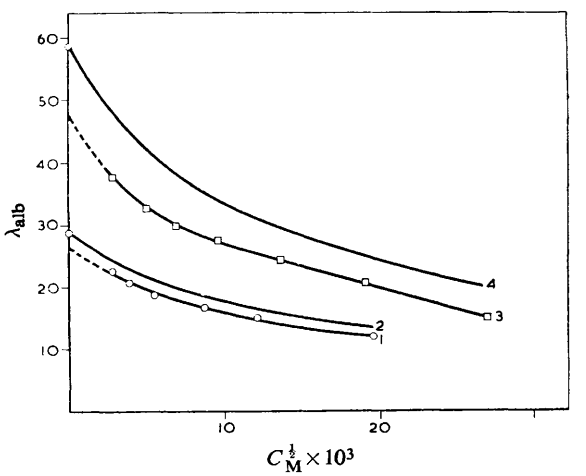

FIG. 3.—Equivalent conductance (25·0°C) of B.S.A. in salt-free alkali-albuminates of constant charge and varying protein concentration.

Curve 1: experimental values, charge 10·8 elementary charges; curve 2: calculated with (32), charge 10·8 elementary charges; curve 3: experimental values, charge 22·1 elementary charges; curve 4: calculated with (32), charge 22·1 elementary charges.

experimental values of the equivalent conductance of the protein are still somewhat lower than the theoretical ones and the difference increases systematically with the protein charge.

The influence of the protein concentration is clearly demonstrated in fig. 3. The difference between observed and computed values of λ_{alb} is again most pronounced for the highest protein charge and in the experimental range this difference appears to be rather insensitive to the concentration of the B.S.A. molecule. Possible causes of this difference will be discussed below. The precise numerical data of the two types of experiments are given in table 1. The ratio between the observed and computed value of the equivalent conductance of the B.S.A. molecule is plotted as a function of the charge and the protein concentration in fig. 4 and fig. 5.

TABLE 1.—COMPARISON BETWEEN VALUES OF $\lambda^{\circ}_{alb.}$, $\lambda_{alb.\,el.}$, $\lambda_{alb.\,comp.}$ AND $\lambda_{alb.\,obs.}$ FOR SALT-FREE ALBUMINATES OF VARYING CHARGE AND PROTEIN CONTENT; TEMP. 25·0°C

A. INFLUENCE OF THE ALBUMIN CHARGE AT FIXED CONCENTRATIONS OF 25·00 g B.S.A. $l.^{-1}$

$a = 34{\cdot}5$ Å; $R = 102$ Å; pH = 9·7 ($z = 27{\cdot}6$) to 5·5 ($z = 2{\cdot}77$)

$-z$	κ in 10^6 cm^{-1}	κa	$1+\Delta E/E$	$\lvert\psi_a-\psi_R\rvert$ in mV	$\lambda^{\circ}_{alb.}$	$\lambda_{alb.\,el.}$	$\lambda_{alb.\,comp.}$	$\lambda_{alb.\,obs.}$	$\frac{\lambda_{alb.\,obs.}}{\lambda_{alb.\,comp.}}$
					in Ω^{-1} cm^2 equiv.$^{-1}$				
27·6	2·31	0·797	0·85	69·20	73·4	34·60	29·30	22·5	0·77
22·1	2·07	0·713	0·88	56·58	58·7	28·29	24·90	20·6	0·83
20·6	2·00	0·690	0·87	53·04	54·8	26·52	23·18	19·0	0·82
13·8	1·63	0·562	0·90	36·61	36·7	18·30	16·42	14·8	0·90
10·8	1·45	0·499	0·93	29·10	28·8	14·55	13·53	12·3	0·91
5·52	1·03	0·356	0·93	15·21	14·7	7·60	7·10	6·5	0·92
2·77	0·728	0·251	0·95	7·68	7·73	3·84	3·64	3·8	1·04

B. INFLUENCE OF THE PROTEIN CONCENTRATION AT FIXED TITRATION CHARGE OF $z = 10{\cdot}83$ e.u.

$a = 34{\cdot}5$ Å; $\lambda^{\circ}_{alb} = 28{\cdot}8$ Ω^{-1} cm^2 equiv.$^{-1}$; C_M = molar albumin concentration; pH = 7·0 (25 g/l.)

g B.S.A./l.	$C_M^{\frac{1}{2}}$ in 10^{-3} mole$^{\frac{1}{2}}$ $l.^{-\frac{1}{2}}$	R in Å	κ in 10^6 cm^{-1}	κa	$1+\Delta E/E$	$\lvert\psi_a-\psi_R\rvert$ in mV	$\lambda_{alb.\,el.}$	$\lambda_{alb.\,comp.}$	$\lambda_{alb.\,obs.}$	$\frac{\lambda_{alb.\,obs.}}{\lambda_{alb.\,comp.}}$
							in Ω^{-1} cm^2 equiv.$^{-1}$			
25·00	19·05	102	1·45	0·499	0·93	29·10	$14{\cdot}5_5$	$13{\cdot}5_3$	12·3	0·91
10·00	12·04	138	0·912	0·315	0·94	35·87	$14{\cdot}8_4$	$17{\cdot}7_7$	15·2	0·90
5·00	8·57	174	0·648	0·223	0·95	39·40	$19{\cdot}6_4$	$18{\cdot}7_1$	16·8	0·90
2·00	5·39	237	0·409	0·141	0·96 *	44·06	$22{\cdot}0_3$	$21{\cdot}1_5$	18·9	0·89
1·00	3·80	298	0·290	0·0997	0·97 *	46·75	$23{\cdot}3_7$	$22{\cdot}6_7$	20·7	0·91
0·50	2·70	376	0·205	0·0707	0·98 *	51·07	$25{\cdot}5_4$	$25{\cdot}0_3$	22·5	0·90

* assumed

C. INFLUENCE OF THE PROTEIN CONCENTRATION AT FIXED TITRATION CHARGE OF $z = 22{\cdot}1$ e.u.

$a = 34{\cdot}5$ Å; $\lambda_{alb} = 58{\cdot}7$ Ω^{-1} cm^2 equiv.$^{-1}$; C_M = molar albumin concentration; pH = 8·5 (25 g/l.)

g B.S.A./l	$C_M^{\frac{1}{2}}$ in 10^{-3} mole$^{\frac{1}{2}}$ $l.^{-\frac{1}{2}}$	R in Å	κ in 10^6 cm^{-1}	κa	$1+\Delta E/E$	$\lvert\psi_a-\psi_R\rvert$ in mV	$\lambda_{alb.\,el.}$	$\lambda_{alb.\,comp.}$	$\lambda_{alb.\,obs.}$	$\frac{\lambda_{alb.\,obs.}}{\lambda_{alb.\,comp.}}$
							in Ω^{-1} cm^2 equiv.$^{-1}$			
50·00	26·9	81	2·93	1·009	0·88	45·59	$22{\cdot}8_0$	$20{\cdot}0_6$	15·4	0·77
25·00	19·1	102	2·07	0·713	0·88	56·58	$28{\cdot}2_4$	$24{\cdot}9_0$	20·6	0·83
12·50	13·5	129	1·46	0·504	0·88	66·94	$33{\cdot}4_7$	$29{\cdot}4_5$	24·2	0·82
6·25	9·53	162	1·03	0·356	0·89	75·88	$37{\cdot}9_4$	$33{\cdot}7_7$	27·6	0·82
3·13	6·73	204	0·731	0·252	0·90	84·12	$42{\cdot}0_6$	$37{\cdot}8_5$	29·7	0·78
1·56	4·77	257	0·517	0·178	0·93	90·65	$45{\cdot}3_2$	$42{\cdot}1_5$	32·9	0·78
0·50	2·70	376	0·293	0·101	0·96	99·07	$49{\cdot}5_4$	$47{\cdot}5_6$	37·5	0·79

Fig. 4 shows the results for a 2·5 % B.S.A. solution of varying charge. Although the accuracy of the experimental points is rather low, it can be seen that the ratio varies roughly linearly with the protein charge, a value of one being closely approached as z approaches zero. On the other hand, fig. 5 shows that even a hundred-fold dilution of a salt-free albuminate of constant charge has only little

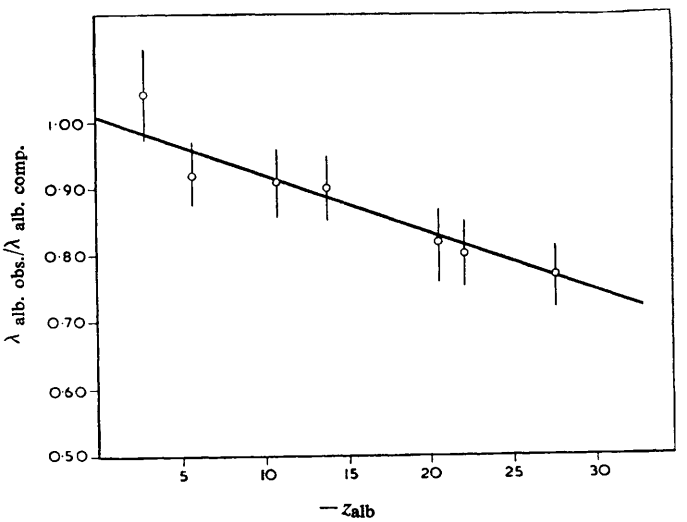

FIG. 4.—Equivalent conductance ratio $\lambda_{\text{alb. obs.}}/\lambda_{\text{alb. comp.}}$ of B.S.A. in 2·5 % salt-free alkali albuminates as a function of the albumin charge.

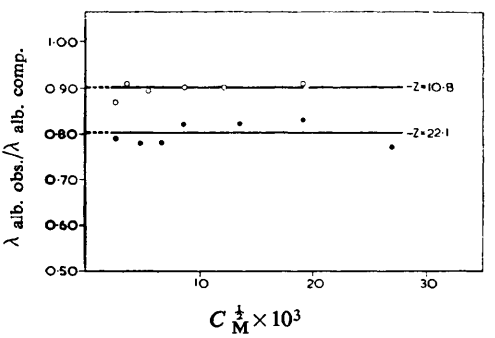

FIG. 5.—Equivalent conductance ratio $\lambda_{\text{alb. obs.}}/\lambda_{\text{alb. comp.}}$ of B.S.A. in salt-free alkali albuminates of constant charge and varying protein concentration.

○ albumin charge 10·8 elementary charges; pH = 7·0 (25 g/l.).

● albumin charge 22·1 elementary charges; pH = 8·5 (25 g/l.).

influence on the equivalent conductance ratio. At lower charge of the protein, the corresponding counterion concentration decreases steadily, which implies a gradually more and more diffuse double layer. Therefore in the limiting case of vanishing protein charge the equivalent conductance ratio will be expected to approach

$$\lambda_{\text{alb.obs.}}/\lambda_{\text{alb.comp.}} = f_{34\cdot5}/f_{\text{obs.}}, \tag{33}$$

where $f_{34\cdot5}$ is the computed frictional constant of B.S.A. corresponding to a sphere of radius 34·5 Å and $f_{\text{obs.}}$ the observed frictional constant of B.S.A. Now it follows from the extrapolation in fig. 4 that at zero protein charge the frictional

constant as found from our electrophoretic experiments equals that of a sphere of radius $34{\cdot}5 \pm 1{\cdot}0$ Å ($f_{34{\cdot}5}/f_{obs.} = 1{\cdot}00 \pm 0{\cdot}03$). In this manner we confirm electrophoretically the frictional constant which was used among other data by Champagne [10] in her estimate of the equivalent sphere radius of the B.S.A. molecule.

The deviations at non-zero charge may be due to the following defects in the theory:

(i) The Debye-Hückel approximation was used to solve the Poisson-Boltzmann equation. While an exact solution is independent of the choice of the reference concentrations, this is no longer true after linearization, so that a choice, different from that based on *mean* concentrations, influences the numerical value of κ and thereby the computed double-layer potentials $\psi_a - \psi_R$ slightly. An illustrative example is given in table 2. In the range of 25-50 mV, a doubling of the reference

TABLE 2.—THEORETICAL VALUES OF $|\psi_a - \psi_R|$ FOR TWO DIFFERENT REFERENCE CONDITIONS, AS COMPUTED FROM EQN. (27). SALT-FREE ALKALI ALBUMINATES; CHARGE 10·8; (1) REF. CONC.: $\bar{n}_+$; (2) REF. CONC.: $2\bar{n}_+$

g B.S.A./l.	κ (1) in 10^6 cm^{-1}	κ (2) in 10^6 cm^{-1}	$\lvert\psi_a - \psi_R\rvert$ (1) in mV	$\lvert\psi_a - \psi_R\rvert$ (2) in mV
25·00	1·45	2·05	29·1	27·7
10·00	0·91	1·29	35·9	34·3
5·00	0·65	0·92	39·4	38·6
2·00	0·41	0·58	44·1	43·3
1·00	0·29	0·41	46·8	46·3

concentration (which is an excessively large change) shifts the value of $\psi_a - \psi_R$ only by a few percent at most. More direct information regarding this question can be obtained by solving the Poisson-Boltzmann equation completely. Recently, Wall and Berkowitz [7] presented a numerical solution, which fully retained the exponentials for polyelectrolytes. Unfortunately, only for two single cases a comparison between both methods could be made, the results of which are given in table 3.

TABLE 3.—COMPARISON BETWEEN $|\psi_a - \psi_R|$ AS DERIVED FROM SOLUTIONS OF THE COMPLETE AND THE LINEARIZED POISSON-BOLTZMANN EQUATION FOR ALKALI ALBUMINATES IN THE ABSENCE OF ADDED ELECTROLYTE

g B.S.A./l.	$\bar{z}_{alb}$	κa	approximated $\lvert\psi_a - \psi_R\rvert$ in mV	complete $\lvert\psi_a - \psi_R\rvert$ in mV
65	21·77	0·837	38·6	37·5
35	41·10	1·084	117·9	97·7

Since in our experiments the potential $\psi_a - \psi_R$ in general was much less than 100 mV, it can be seen that our computed values of $\psi_a - \psi_R$ are over-estimated about 10 % in the most unfavourable case.

(ii) In our treatment of the electrophoretic effect, we disregarded the distortion of the electric lines of force around the particle. Henry [5] showed that this distortion increases the mobility of particles with non-overlapping double layers by less than 3 % for values of κa less than one. There is no reason to expect a much larger effect in our case.

(iii) Binding of counterions.

In the literature [13-16] evidence is found for the absence of binding of alkali ions to serum albumin between pH 5 and 9. However, Doremus and Johnson [17] concluded that there was an appreciable binding of sodium ions. This point has been discussed in some detail in the previous paper [1] and arguments were given for an interpretation of our experiments, which started from the assumption

that B.S.A. does not bind alkali ions. From the fact that the mobility of B.S.A. is practically independent of the type of alkali ions used, it seems reasonable to suppose that, when there is some binding of counterions, this binding is not specific. There might be some non-specific electrostatic binding of counterions in the hydration water of the protein molecule. The hydrodynamic radius of 34·5 Å corresponds to about 0·8 cm^3 of hydration water per g protein. If all this hydration water contains the highest concentration of counterions that is present in the diffuse double layer, one can easily calculate that this would amount at most to about 10 %, and in by far the most cases to less than 2 % binding of counterions.

(iv) The correction of Gorin,[18] taking into account the finite dimensions of the small ions, was neglected in view of the uncertainties of the structure of the protein surface and the actual radius of the alkali ions, which should be used. The correction increases the theoretical λ_{alb} values anyway by not more than a few percent at most.

(v) Deviations from the spherical form.

Small deviations from sphericity (e.g. up to an axial ratio of 2) are not expected to affect the results very much, since the extension of the double layer is always large in our case. As mentioned above, large axial ratios would demand extension of the theory before anything can be said with certainty about their influence. Finally, from sedimentation and diffusion measurements of numerous authors,[19-26] frictional constants of B.S.A. can be computed lying between $f = 6{\cdot}6$ and $7{\cdot}0 \times 10^{-8}$ in substantial agreement with the friction constant of $6{\cdot}6 \times 10^{-8}$ experienced by a sphere of 34·5 Å. On the other hand, Krause and O'Konski [27] concluded from electric birefringence measurements that a prolate ellipsoid of $v = 130{\cdot}000$ Å^3 and axial ratio 1 : 7, corresponding to a frictional constant of $f = 8{\cdot}2 \times 10^{-8}$ was their best estimate of the size and shape of the B.S.A. molecule.

In addition, Champagne, Luzzatti and Nicolaieff [28] using small-angle scattering arrive at a prolate ellipsoid of $v = 130{\cdot}000$ Å^3 and axial ratio 1 : 10, implying a frictional constant even higher than $f = 8{\cdot}2 \times 10^{-8}$. The larger value of f would improve agreement at large z but this worsens at $z \to 0$.

Summarizing, reasonable agreement between theory and experiment is obtained at low charge. At higher charge, however, perceptible deviations start to occur resulting into an over-estimation of the equivalent conductance by about 25 % at most. Only part of this percentage can be ascribed to imperfections in the theory: the use of the Debye-Hückel approximation may explain about 10 % in the most unfavourable case, while the various other factors partly cancel each other and affect λ_{alb} therefore, by a few percent at most. Nevertheless, as long as the theory is not worked out for higher potentials and non-spherical forms, it seems somewhat premature to decide whether at higher protein charge the theory as such or the protein model fails.

2. In the presence of salt

Fig. 6 represents a comparison between theory and experiment for a series of protein solutions with added electrolyte. For this series, the concentration of alkali ions was fixed at 10^{-2} N and the protein ion gradually replaced by chloride ions, while the titration charge was kept at a constant value of 22·1 elementary charges. The experimental points are averages between data obtained with Li, Na and K ions. The relaxation factor $(1 + \Delta E/E)$ was computed as described in § 2.2, by using the experimental data of the corresponding alkali-ion mobilities of potassium and lithium albuminate (see fig. 11 of the previous paper [1]). In this manner a rather constant value of $1 + \Delta E/E = 0{\cdot}86 \pm 0{\cdot}04$ was obtained, or a relaxation effect of 14 %.

Inspection of the lines of fig. 6 shows that the retarding effects strongly reduce the equivalent conductance from 60 (Stokes) to a value of 24 ± 1. The experimental values are again slightly lower, viz., 20 ± 1, implying a difference of about

20 % with the theory. Before deciding in how much this is due to an incorrect choice of form or friction factor, it would be necessary to improve the theory by the straightforward numerical solution of the potential equation where the exponentials of the Poisson-Boltzmann equation are fully maintained.

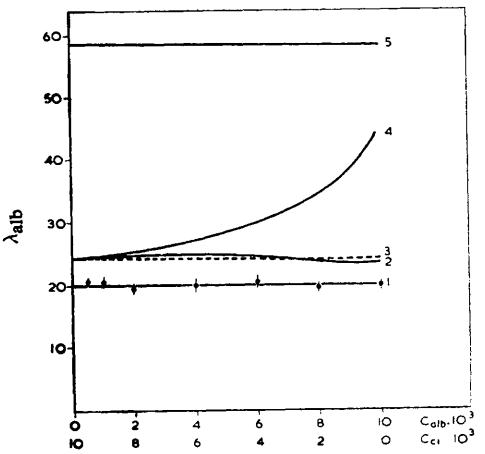

FIG. 6.—Equivalent conductance of albumin (25·0°C) in alkali albuminates with $C_{Me} = C_{alb}+C_{Cl} = 10^{-2}$ equiv. $l.^{-1}$; charge 22.1 elementary charges; pH = 8·5.

Curve 1: observed; curve 2: computed from (32) and (27); curve 3: computed from (32), (34) and (35); curve 4: computed from (32), (34) and (36); curve 5: calculated from Stokes' law (a = 34·5 Å).

6. A USEFUL APPROXIMATION

It seemed worthwhile to find out if the rather complicated eqn. (27) could be replaced by a simpler one. We tried to estimate $\psi_a-\psi_R$ by simply counting the protein as a 1-1 electrolyte in the " ionic strength " and by applying the following variant of the Debye-Hückel theory to our case:

$$\psi_a-\psi_R = \frac{ze}{\varepsilon a}\frac{1}{1+\kappa_{11}a}, \tag{34}$$

with

$$\kappa_{11} = \left\{\frac{4\pi e^2}{\varepsilon kT}2\bar{n}_K\right\}^{\frac{1}{2}}, \tag{35}$$

where $\bar{n}_K$ is the stoichiometric concentration (number per cm^3) of the counterions. The results of this calculation for solutions containing potassium albuminate and potassium chloride are also given in fig. 6. Counting the albumin of charge z as z-univalent ions in the ionic strength and using eqn. (34) and (35), we get an almost identical result to that with the more correct treatment based upon eqn. (27). However, if we neglect the albumin contribution to the " ionic strength " altogether —the value of κ then becomes

$$\kappa = \left\{\frac{4\pi e^2}{\varepsilon kT}2\bar{n}_{Cl}\right\}^{\frac{1}{2}}; \tag{36}$$

the theory overestimates λ_{alb} strongly as shown by the curved line of fig. 6. In this procedure, the counterions belonging to the protein are insufficiently accounted for in the Debye-Hückel factor. As expected, these counterions play a decisive part in the electrical screening of the macro-ion.

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