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## Separation & Purification Reviews

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597294>

### The Influence of the size and Shape of Molecules and Particles on their Electrophoretic Mobility

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**To cite this Article** van Oss, Carel J.(1975) 'The Influence of the size and Shape of Molecules and Particles on their Electrophoretic Mobility', *Separation & Purification Reviews*, 4: 1, 167 — 188

**To link to this Article: DOI:** 10.1080/03602547508066038

**URL:** <http://dx.doi.org/10.1080/03602547508066038>

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THE INFLUENCE OF THE SIZE AND SHAPE  
OF MOLECULES AND PARTICLES ON THEIR  
ELECTROPHORETIC MOBILITY

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## I. INTRODUCTION

As a general rule the electrophoretic mobility of molecules and particles is solely governed by the potential at their surface of shear ( $\zeta$ -potential) and does not depend on their size or shape. There are however two sets of exceptional conditions under which the size or shape of molecules and particles may influence their electrophoretic mobility. These are:

1) Conditions under which the ratio between the dimensions of the molecules or particles and the thickness of their diffuse ionic double layer is such that the distortion of the electric field by the particles is neither at its minimum nor at its maximum.

2) Conditions under which the molecules or particles are migrating under the influence of an electric field inside a porous medium, the size of the pores of which is such that they impede the larger molecules or particles more than the smaller ones.

The influence of both these different sorts of conditions on electrophoresis will be treated here.<sup>1</sup> A few limitations<sup>2,3</sup>, of a practical order, will be imposed in advance:

1) Only rigid molecules or particles will be considered here. Very rarely, if ever, does one encounter molecules or particles that are sufficiently deformable under the influence of an electric field, to influence their electrophoretic mobility.<sup>4</sup>

2) Only non-conducting molecules or particles will be considered here. Even normally fairly conducting particles behave as insulated particles when subjected to electrophoresis. The electrical double layer surrounding the particles offers such a strong resistance to an electric current, that it transforms conducting into insulated particles.<sup>5</sup>

3) The particles' movement inside the liquid remains laminar. The electrophoretic mobilities that most particles can attain (a few  $\mu$  volt<sup>-1</sup> sec<sup>-1</sup> cm), cannot normally give rise to turbulence.<sup>1</sup>

4) Electroosmotic counter-flow of the bulk liquid along the walls of the vessel (which is governed by the  $\zeta$ -potential of the walls)<sup>6</sup>, although in practice frequently an important factor, will not be taken into account here, as it can always (at least theoretically) be dissociated from the real electrophoretic mobility of molecules or particles.

5) The influence of Brownian movement of the particles<sup>7</sup>, being quite slight<sup>5,14</sup>, will not be taken into account.

## II. THE TWO ELECTROPHORESIS EQUATIONS

### A. Thick Double Layer

The simplest situation that may be considered is the one in which one electrophoreses a small particle, with a very thick but unsubstantial electrical double layer, which offers no significant impediment to the electric field (see figure 1). Under these conditions the three forces<sup>8</sup> acting on the particle (see figure 2), ought, once a uniform velocity is attained, to cancel one another out:

$$F_1 + F_2 + F_3 = 0 \quad [1]$$

These forces are:

The attractive force of the electric field for the charged particle:

$$F_1 = + neX \quad [2]$$

(in which  $ne$  is the total effective electric charge of the molecule or particle and  $X$  the electric field strength);

The hydrodynamic friction force that the particle undergoes in its movement through the liquid:

$$F_2 = -6\pi\eta aU \quad [3]$$

(in which  $\eta$  is the viscosity of the medium,  $a$  the radius, or other expression for the dimension of the particle and  $U$  the electrophoretic velocity the particle attained);

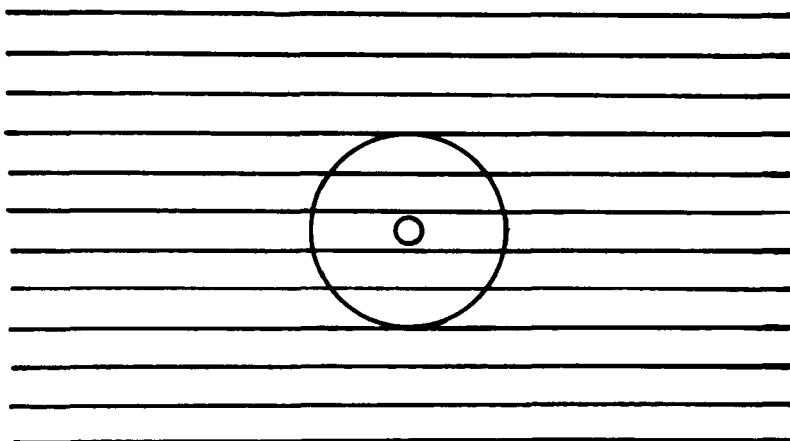


FIGURE 1

Small particle surrounded by a thick electrical double layer (small  $\kappa a$ ); the lines of force of the electric field are virtually unhindered by the particle or its double layer.

The electrophoretic friction force, caused by the movement in the direction opposed to that of the particle, of the oppositely charged ions:

$$F_3 = X(\epsilon\zeta a - ne) \quad [4]$$

(in which  $\epsilon$  is the dielectric constant of the medium and  $\zeta$  the potential of the particle at its surface of shear).

From [1, 2, 3, 4] it follows that:

$$u = \frac{\zeta\epsilon}{6\pi\eta} \quad [5]$$

$$\text{where } \frac{U}{X} = u \quad [6]$$

( $u$  being the electrophoretic mobility).

Thus, particles that are small compared to the thickness of their electric double layers, have an electrophoretic mobility  $u$  that is solely proportional to their electrokinetic  $\zeta$ -potential.

#### B. Thin Double Layer

The other situation that must be considered is the one in which one electrophoreses a somewhat larger particle (relatively

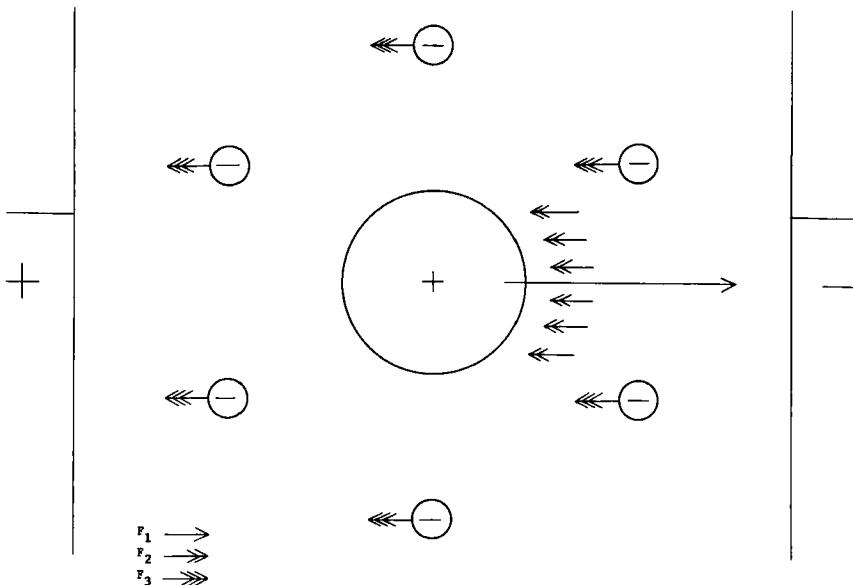


FIGURE 2

The three forces acting on particle in an electric field.  $F_1$  is the force the electric field exerts directly on the charge(s) of the particle.  $F_2$  is the hydrodynamic friction force the particle undergoes, caused by its movement through the liquid.  $F_3$  is the electrophoretic friction force, caused by the oppositely charged ions moving in the direction opposed to that of the particle.

speaking), with a thin but quite dense electrical double layer. The electric field will be considerably distorted, on account of both the particle size and the compactness of the double layer<sup>9</sup> (see figure 3). In this case the oppositely charged ions that are directly in the path of the moving particle will oppose little or no electrophoretic friction force to the particle. There remains an electrophoretic friction force by oppositely charged ions that are more tangential to the moving particle, but this

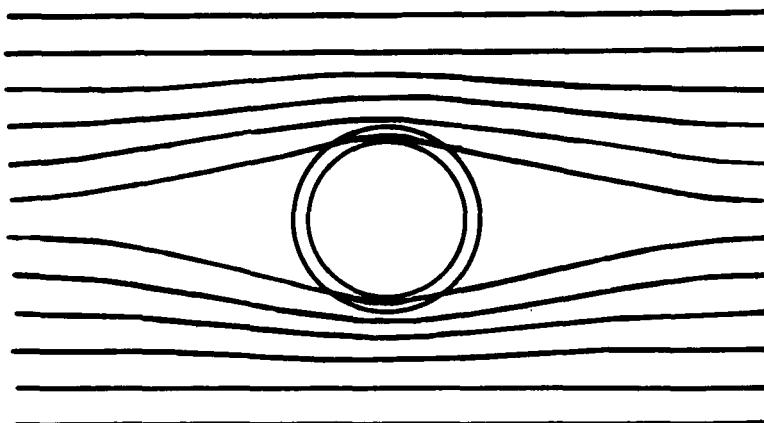


FIGURE 3

Large particle surrounded by a thin electrical double layer (large  $\kappa a$ ); the lines of force of the electric field are strongly deflected by the particle.

effect is fairly small. Thus the electrophoretic mobility of particles in this situation is greater than that of the particles considered earlier. Under these conditions, the equation for the electrophoretic mobility, (which results from the interaction between the flow of electricity and the flow of liquid in the double layer<sup>6</sup>), is derived with the help of Poisson's equation:

$$\frac{d}{dx} \left( \epsilon \frac{d\psi}{dx} \right) = -4\pi\rho \quad [7]$$

( $x$  is the distance of any given point from the surface of shear,  $\psi$  the electric potential of the double layer and  $\rho$  the space charge density), and integration by parts of the equation for the forces acting on all volume elements concerned. This yields<sup>2,9,10</sup>, (after equating  $\zeta$  with  $\psi$ , at the surface of shear):

$$u = \frac{U}{X} = \frac{\zeta e}{4\pi\eta} \quad [8]$$

## III. HENRY'S GENERAL EQUATION

The two discordant equations, that of Hückel<sup>8</sup> [5]:

$$u = \frac{6e}{6\pi\eta} \quad [5]$$

for the electrophoretic mobility of particles that are relatively small compared to the thickness of their electrical double layer and that of von Smoluchowski<sup>9</sup> [8]:

$$u = \frac{6e}{4\pi\eta} \quad [8]$$

for the electrophoretic mobility of particles that are large compared to the thickness of their double layers, were reconciled by Henry<sup>11</sup> into one equation, by taking both the radius  $a$  of the particle and the thickness of its electrical double layer, expressed as  $1/\kappa$ , into account;

$$u = \frac{6e}{6\pi\eta} f(\kappa a) \quad [9]$$

when  $\kappa a \rightarrow \infty$ , or, in practice, when  $\kappa a > 300$ ,  $f(\kappa a)$  becomes = 1.5, which makes equation [9] revert to von Smoluchowski's equation [8]. When  $\kappa a = 0$ , or, in practice, when  $\kappa a < 1$ ,  $f(\kappa a)$  becomes = 1.0, which makes equation [9] revert to Hückel's equation [5]. Figure 4 shows the variation of  $f(\kappa a)$  with  $\kappa a$  for spherical particles.<sup>6,12</sup> (For a long cylinder oriented perpendicular to the electric field,  $f(\kappa a)$  becomes = 0.75 at values of  $\kappa a$ , while at high values of  $\kappa a$  this function remains = 1.5. For a long cylinder, oriented parallel to the electric field,  $f(\kappa a)$  remains = 1.5 for all values of  $\kappa a$ ).

## IV. THE INFLUENCE OF RELAXATION

In all the situations treated above, it had been assumed, by Hückel<sup>8</sup>, by von Smoluchowski<sup>9</sup>, as well as by Henry<sup>11</sup>, that the initial symmetry of the electrical double layer surrounding the

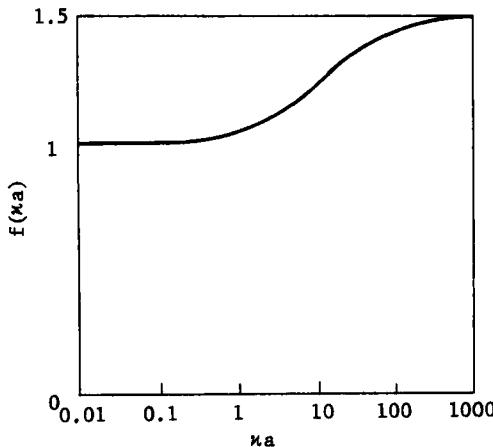


FIGURE 4

Graph of  $f(\kappa a)$  from Henry's equation [9] versus  $\kappa a$  (the latter is on a logarithmic scale), for spherical particles.

(Adapted from reference 6, p. 209).

particle remained unimpaired during the electrophoretic movement. However, this is not always the case: under certain quite common conditions the diffuse electrical double layer may be more impeded in its electrophoretic progress through the liquid than the particle it surrounds. This is called relaxation. Relaxation forces (see figure 5) can appreciably reduce the electrophoretic mobility. Overbeek<sup>2,5</sup> has indicated the conditions under which the relaxation effect may be neglected:

- 1) At  $\zeta < 25$  mV, for all values of  $\kappa a$ ;
- 2) At  $\kappa a \rightarrow 0$  for all values of  $\zeta$ , and
- 3) At  $\kappa a \rightarrow \infty$  for all values of  $\zeta$ .

In cases 2) and 3), in practice it generally suffices that  $\kappa a < 0.1$  or  $> 25$  for the relaxation effect to be quite small.

In other words: relaxation is of little import when:

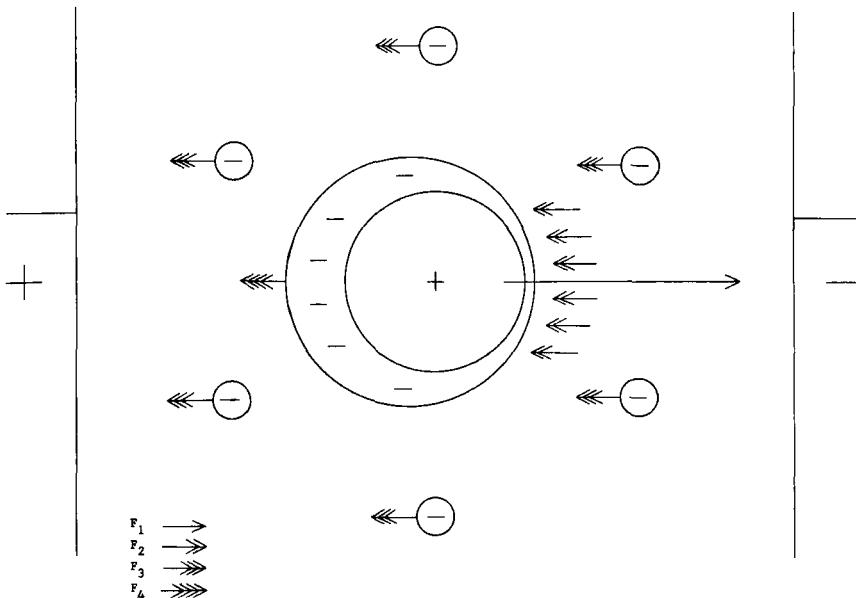


FIGURE 5

The same diagram as figure 3, with, in addition, the influence of the relaxation force  $F_4$ , which distorts the ionic double layer.

1)  $\zeta$  potentials and thus electrophoretic mobilities, are low, because the actual electrophoretic movement necessarily remains slow enough for double layer retardation to be minimal (at  $u < 2\mu \text{ volt}^{-1} \text{sec}^{-1} \text{cm}$ );

2) the electrical double layer is very thick but also very unsubstantial, and

3) the electrical double layer is very thin and dense.

In all other cases, i.e. when  $\zeta > 25 \text{ mV}$  and  $0.1 < \kappa a < 25$ , the relaxation effect seriously influences the electrophoretic mobility.<sup>2,5,13,14,15</sup> A number of complicated equations taking this effect into account have been given; as they are not readily

soluble without the help of a computer they are not repeated here. Figure 6 illustrates the effect of relaxation on the electrophoretic mobility at various values of  $\kappa_a$ , as a function of the  $\zeta$ -potential, in a 1-1 electrolyte.<sup>5</sup> For  $0.3 < \kappa_a < 20$  there appears to be a maximum in the attainable electrophoretic mobility, roughly between  $3.8$  and  $6 \mu \text{volt}^{-1} \text{sec}^{-1} \text{cm}$ , which agrees well with the "maximum" electrophoretic mobility of  $5 \mu \text{volt}^{-1} \text{sec}^{-1} \text{cm}$  found by von Hevesy.<sup>16</sup> The available aids for the calculation of  $\zeta$ -potentials from electrophoretic mobilities under a variety of conditions of  $\kappa_a$  are discussed below.

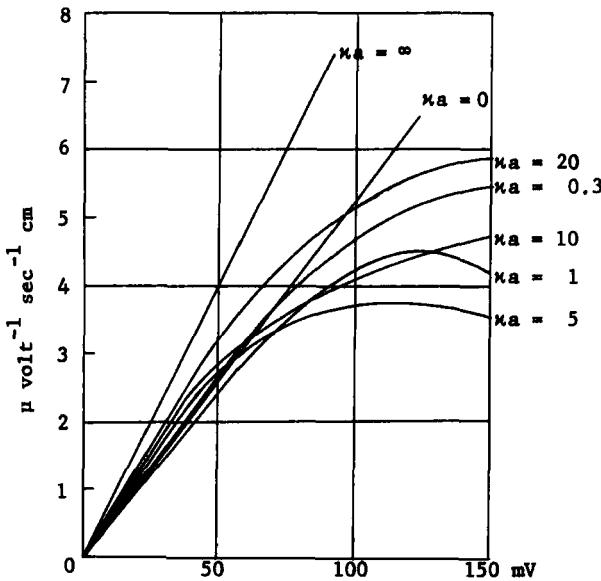


FIGURE 6

Graph of the electrophoretic mobility  $u$  (in  $\mu \text{volt}^{-1} \text{sec}^{-1} \text{cm}$ ) versus  $\zeta$  (in mV) for a number of values of  $\kappa_a$ . A maximum electrophoretic mobility between  $3.8$  and  $6 \mu \text{volt}^{-1} \text{sec}^{-1} \text{cm}$ , for  $0.3 < \kappa_a < 20$  is quite apparent.

(From reference 5).

V. DEMARCTION OF THE VALIDITY RANGES OF  
THE EQUATIONS ACCORDING TO THE ELECTRO-  
KINETIC CONDITIONS

A.  $\kappa a < 0.1$

When the ionic strength of the medium is very low and when the molecules or particles are quite small, Hückel's equation [5] is valid.

B.  $\kappa a > 300$

When the molecules or particles are large compared to the thickness of the electrical double layer, von Smoluchowski's equation [8] is valid. In table I, some minimum dimensions of  $a$  are given, for a number of values of  $1/\kappa$ , corresponding to a number of electrolyte solutions.<sup>11,17</sup>

Table I

Minimum dimensions of  $a$  for a number of values of  $1/\kappa$ , for a number of electrolytes

Solution	$1/\kappa$ in m $\mu$	Minimum value of $a$ , so that $\kappa a > 300$ (in m $\mu$ )
H <sub>2</sub> O	1,000	300,000
10 <sup>-5</sup> M NaCl	100	30,000
10 <sup>-3</sup> M NaCl	10	3,000
10 <sup>-1</sup> M NaCl	1	300
10 <sup>-5</sup> M Na <sub>2</sub> SO <sub>4</sub>	56	17,000
10 <sup>-3</sup> M Na <sub>2</sub> SO <sub>4</sub>	5.6	1,700
10 <sup>-1</sup> M Na <sub>2</sub> SO <sub>4</sub>	0.56	170
10 <sup>-5</sup> M MgSO <sub>4</sub>	48	14,000
10 <sup>-3</sup> M MgSO <sub>4</sub>	4.8	1,400
10 <sup>-1</sup> M MgSO <sub>4</sub>	0.48	140

C.  $0.1 < \kappa a < 300$

From table I it also becomes obvious that with most biological or biochemical substrates (e.g., proteins, nucleic acids, sub-

cellular particles), the dimensions (a) are such that at the usual concentrations of electrolytes  $\kappa a < 300$ .

In addition, with respect to the lower end of this range, with most buffers normally used for electrophoresis,  $1/\kappa < 10 \text{ m}\mu$ , so that  $a$  must be  $< 1 \text{ m}\mu$  for the ratio between the double layer and the particles' dimension to be small enough to use the unmodified Hückel equation [5]. Proteins and nucleic acids however generally have dimensions  $> 1 \text{ m}\mu$ , so that it must be concluded that for virtually all electrophoresis work with biological or biochemical substrates smaller than whole cells, the conditions are bound to be such that  $0.1 < \kappa a < 300$ . As in the entire range of  $0.1 < \kappa a < 300$  relaxation effects make themselves felt (at least at  $\zeta$ -potentials  $> 25 \text{ mV}$ ),<sup>5</sup> and as Hückel's equation [5] evolves into von Smoluchowski's [8] in the range of  $1 < \kappa a < 300$  (at all values for  $\zeta$ ), in this range electrophoretic mobilities can only be translated into  $\zeta$ -potentials after considerable computational effort. Fortunately however there now exist tables that facilitate that effort.

#### VI. CALCULATION OF $\zeta$ -POTENTIALS FROM ELECTROPHORETIC MOBILITY DATA

It is important to realize that the potentials alluded to in all equations used above are expressed in electrostatic units (esu) of potential difference.<sup>10</sup> In order to convert esu potential differences to volts,  $\zeta$  as well as  $X$  must be multiplied by a factor 300. Thus, for example in:

$$u = \frac{U}{X} = \frac{\zeta e}{4\pi\eta} \quad [8]$$

converted to:  $\zeta = \frac{4\pi\eta U}{Xe} = \frac{4\pi\eta u}{e} \quad [10]$

the value found for  $\frac{4\pi\eta u}{e}$  has to be multiplied by a factor 90,000, in order to express  $\zeta$  in volts (with  $u$  of course expressed in  $\text{cm}^2 \text{volt}^{-1} \text{sec}^{-1}$ ).

Table II gives the conversion to  $\zeta$ -potentials (in mV), for  $u = 1\mu \text{ volt}^{-1} \text{sec}^{-1} \text{cm}$ , at different temperatures, and thus at different values for  $\epsilon$  and  $\eta$  (for water), for both the Hückel and the von Smoluchowski equations.

Table II  
Conversion of  $u = 1\mu \text{ volt}^{-1} \text{sec}^{-1} \text{cm}$  to  $\zeta$ -potentials in millivolts \*  
at various temperatures

Temperature (in °C)	$\eta^{**}$ (in poise)	$\epsilon^{**}$	$\zeta$ in mV (after Hückel: $\zeta =$ $6\pi\eta u/\epsilon$ [5])	$\zeta$ in mV (after von Smoluchowski: $\zeta = 4\pi\eta u/\epsilon$ [8])
0	0.01787	87.90	34.49	22.99
5	0.01519	85.90	30.00	20.00
10	0.01307	83.95	26.42	17.61
15	0.01139	82.04	23.55	15.70
20	0.01002	80.18	21.20	14.13
25	0.008904	78.36	19.28	12.85
30	0.007975	76.58	17.67	11.78
35	0.007194	74.85	16.31	10.87

\*The units of  $\mu \text{ volt}^{-1} \text{sec}^{-1} \text{cm}$  have been used here because these units are most commonly given in the literature to express electrophoretic mobilities. The values of  $\zeta$  are given in mV, for the same reason of conformation to the most generally quoted unit for this entity. Thus, for the conversion of  $\mu$  to  $\zeta$  in this case, a multiplication factor of 9,000 has been employed.

\*\*From "Handbook of Chemistry and Physics", Chemical Rubber Co., 51st Edition, Cleveland, 1970.

For the range  $0.1 < \kappa a < 300$  it is best to consult the graphs and tables, giving the variation of the electrophoretic mobility with  $\kappa a$ , elaborated by Overbeek and Wiersema<sup>2</sup>, Wiersema, Loeb and Overbeek<sup>3</sup>, and Wiersema.<sup>14</sup> (See also Loeb, Wiersema and Overbeek).<sup>18</sup> These authors have first converted the principal parameters to dimensionless variables, in order to make the results

applicable to various temperatures, viscosities and other solvent properties. Thus, as a measure of the electrophoretic mobility, the entity  $E$  is introduced:<sup>3,14</sup>

$$E = \frac{6\pi\eta e}{\epsilon kT} \frac{U}{X} \quad [11]$$

where  $e$  is the elementary charge,  $k$  the Boltzmann constant and  $T$  the absolute temperature.

Instead of  $\zeta$ ,  $y_o$  is given:<sup>3,14</sup>

$$y_o = \frac{e\zeta}{kT} \quad [12]$$

Table III gives the values of  $E$  versus  $y_o$  for a number of values for  $\kappa a$ , for the electrophoresis of molecules or particles in a solution of 1 - 1 electrolytes in water.<sup>3,14</sup> Conversion factors for  $y_o$  to  $\zeta$  in mV and for  $u$  (in  $\mu$  volt<sup>-1</sup> sec<sup>-1</sup> cm) to  $E$ , at various temperatures, are given in table IV. For further refinement and for more complicated situations of ionic composition, etc., see Wiersema, Loeb and Overbeek<sup>3</sup> and Wiersema<sup>14</sup>, see also Figure 7.

For  $y_o < 1$ , the top row of table III may be used to obtain the  $E/y_o$  ratio for the appropriate value of  $\kappa a$ , as at these low values of  $y_o$  (and thus of  $\zeta$ ), relaxation plays no significant role.

## VII. CONFRONTATION OF THE EQUATIONS WITH EXPERIMENTAL DATA

### A. Large $\kappa a$

Micro-electrophoresis of a variety of particles of different sizes, coated with given proteins, has demonstrated conclusively that, at least in the large  $\kappa a$  range, the electrophoretic mobility is independent of particle size.<sup>4</sup>

### B. Small $\kappa a$

Few experimental data are available on the constancy or variability of the electrophoretic mobility of small molecules. Although in principle the verification of the independence of electrophoretic mobilities of  $a$  at low  $\kappa a$  seems quite feasible, no report of such a verification has been published to date. With

Table III  
Values of  $E$  as a function of  $y_0$  and of  $\kappa_a$ , for 1-1 electrolytes, from ref. 14; see also Figure 7.

$y_0 \backslash \kappa_a$	0	0.01	0.02	0.05	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000	$\infty$
1	1.00	1.00	1.00	1.00	0.99	1.01	1.04	1.13	1.22	1.33	1.42	1.45	1.47	1.49	1.50	1.50		
2	2.00	2.00	1.99	1.97	1.96	1.92	1.91	1.93	2.09	2.27	2.51	2.76	2.87	2.93	2.97	2.99	3.00	
3	3.00	2.99	2.98	2.96	2.90	2.85	2.71	2.63	2.60	2.74	3.06	3.43	3.92	4.20	4.34	4.45	4.48	
4	4.00	3.98	3.95	3.90	3.78	3.64	3.36	3.13	2.99	3.08	3.43	3.96	4.79	5.38	5.68	5.90	5.97	
5	5.00	4.97	4.92	4.80	4.59	4.31	3.80	3.44	3.20	3.17	3.47	4.10	5.25	6.22	6.88	7.33	7.46	
6	6.00	5.94	5.86	5.66	5.38	4.85	4.11	3.63	3.27	3.08	3.35	3.94	5.27	6.64	7.90	8.73	8.94	

a view to the separation of bio-polyelectrolytes according to size, gel electrophoresis techniques<sup>19</sup> have been developed (see below), using either substrates that differ only in size but have approximately the same charge (nucleic acids)<sup>20</sup> or substrates of differing sizes and charges such as proteins that can be given identical charges by the admixture of sodium dodecyl sulfate.<sup>21-23</sup> The same substrates might be used in unimpeded electrophoresis to accomplish this verification.

C The Transition  $0.1 < \kappa a < 300$

The oldest published work on the verification of the transition of  $\frac{6\epsilon}{6\pi\eta}$  to  $\frac{6\epsilon}{4\pi\eta}$  with increasing  $\kappa a$  was by Mooney<sup>24</sup>, who electrophoresed emulsified oil droplets of different sizes in water with a low electrolyte content. The electrophoretic mobility indeed gradually increased by 50% as the droplet size increased from 0.1  $\mu$  to 150  $\mu$ , to remain constant with further increases in

Table IV

Conversion of  $y_0$  to  $\zeta$  in mV and of  $u$  (in  $\mu$  volt $^{-1}$  sec $^{-1}$  cm) to  $E$ , at various temperatures\*

Temperature (in °C)	Multiplying factor for $u$ , to obtain $E$ (see table III)	Multiplying factor for $y_0$ , to obtain $\zeta$ (see table III)
0	1.464	23.55
5	1.251	23.98
10	1.082	24.42
15	0.948	24.85
20	0.839	25.28
25	0.750	25.71
30	0.676	26.14
35	0.614	26.57

\*The following values have been used:  $k = 1.3806 \times 10^{-16}$  erg deg.  $^{-1}$ ;  $e = 4.8032 \times 10^{-10}$  esu;  $T$  = temperature in °C + 273.15; for  $\eta$  and  $\epsilon$ , see table II. In both conversions, from  $y_0$  to  $\zeta$ , as well as from  $u$  to  $E$ , a factor 300 was used, for the transformation of esu into volts.

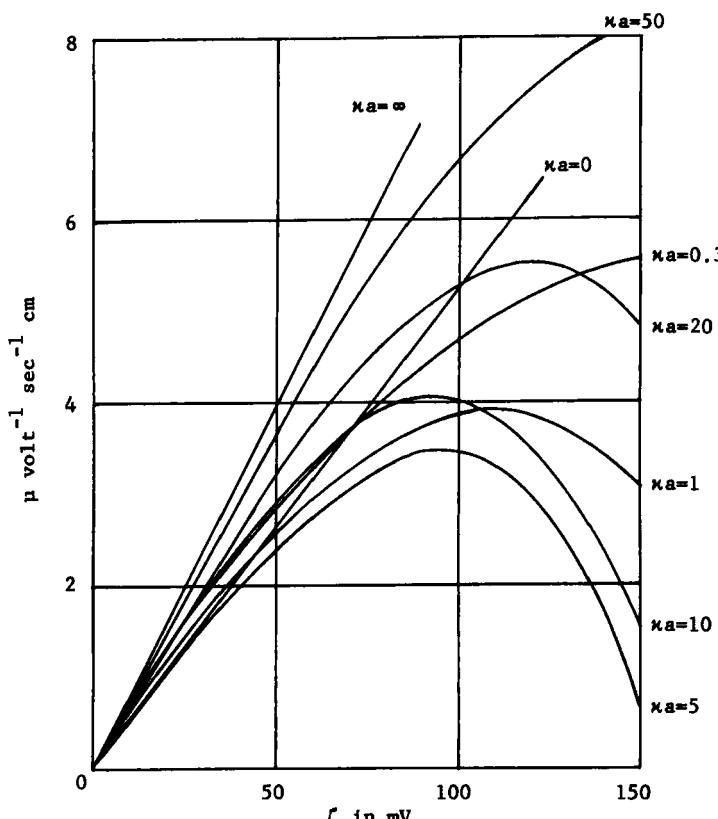


FIGURE 7

$E$  as a function of  $\kappa a$  for different values of  $y_0$  (see Table III).

(From reference 14).

droplet size. These and other verifications are discussed by Wiersema, Loeb and Overbeek.<sup>3</sup>

More recently, the availability of monodisperse polystyrene latex particles of various diameters<sup>25</sup>, has opened new possibilities for testing the degree of applicability of the electrophoresis equations. The remarkable uniformity of the particle

diameter of these latices permits hitherto unattainable experimental precision. And the fact that the particles are perfect and rigid spheres removes some of the objections raised to the work done with suspensions of liquid droplets, because with polystyrene particles, internal deformation and streaming<sup>3</sup> may be ruled out.

Shaw and Ottewill<sup>26</sup> were the first to use polystyrene latices of different diameters and at different ionic strengths for this purpose. Their mobilities conformed qualitatively to the theoretical curves, but they were in certain cases quantitatively higher than the calculated ones.<sup>2,3</sup>

The differences in electrophoretic mobility caused by differences in  $\kappa a$  in the range  $0.1 < \kappa a < 300$  has been put to use for the determination of latex particle size distributions by McCann et al.<sup>27</sup> and the phenomenon has been utilized for the electrophoretic separation of mixtures of polystyrene latex particles of  $0.8 \mu$  and  $0.2 \mu$  diameter, under conditions of 0 Gravity, during the flight of Apollo 16 to the Moon. Under these conditions, where  $1/\kappa \approx 10 \text{ m}u$  (the  $\kappa a$  of the larger and smaller particles thus being respectively  $\approx 80$  and  $\approx 20$ ), the larger particles migrated approximately 42% faster than the smaller ones.<sup>28</sup>

## VII. ELECTROPHORETIC MOBILITIES IN DENSE GELS

When electrophoresis is practiced in a gel with rather small pores<sup>19</sup>, the mobility of the particles that are being electrophoresed will be the slower, the nearer the size of the particles approaches the pore size of the gel. This phenomenon has first been proposed as a method of discrimination between polymers according to their molecular sizes by Synge and Tiselius.<sup>29</sup> Tiselius and Flodin<sup>30</sup> indicated that this effect is not limited to a gel: a concentrated colloidal sol (for instance a dextran solution) as the electrophoretic carrier medium will have the

same effect. This principle has been much used for the separation of proteins, with the help of starch gels and later, with polyacrylamide gels<sup>19</sup> ("disc-electrophoresis") and with agar gels, the latter two even permitting the separation of viruses according to size.

Nevertheless, any separation obtained by gel-electrophoresis leaves the question of whether two particles have been separated because they differ in electrical charge, or in size (or in both), entirely undecided. In this respect using gel-electrophoresis is analogous to attempting to solve one equation with two unknowns. The solution of this problem in electrophoresis is analogous to the solution of one equation with two unknowns, that is to isolate one of the unknowns and to make it a constant: when electrophoretic separations purely according to size are desired (and that is generally the reason why gel-electrophoresis is used), the charge of all the transported molecules or particles must be made constant. This is either already the case (e.g. with nucleic acid fractions)<sup>20</sup> or can be achieved by adsorbing a highly charged compound onto the material that is to be electrophoresed, (e.g. by electrophoresing proteins in a medium containing sodium dodecyl sulfate).<sup>21-23</sup>

An increase in the size of molecules or particles thus has the opposite effect in dense gel-electrophoresis as compared to electrophoresis in low viscosity liquids: in dense gel-electrophoresis the largest particles tend to be the slowest, while in normal liquids they may have electrophoretic mobilities up to 50% greater (for spherical particles) than small particles.

#### IX. SUMMARY

Although as a general rule the electrophoretic mobility of molecules and particles only depends on their  $\zeta$ -potential and not on their size or shape, there are certain conditions under which their size or shape may be of influence. Such conditions occur when the electrical double layer is of moderate thickness

as compared to the size of the molecules or particles and also when the electrophoresis takes place in a dense gel.

The Hückel equation (for thick electrical double layers) and the von Smoluchowski equation (for thin electrical double layers), which link electrophoretic mobilities to  $\zeta$ -potentials to a somewhat different extent, both are discussed, and reconciled by means of Henry's general equation. The influence of relaxation on the electrophoretic mobility is also treated and the validity ranges of the different equations are demarcated, as a function of the thickness of the double layer and the  $\zeta$ -potential. Methods for the calculation of  $\zeta$ -potentials from electrophoretic mobility data are given and the results predicted by these theories are compared with the available experimental data.

Under most conditions prevailing with biological materials, larger molecules or particles will, other factors being equal, have an up to 50% faster electrophoretic mobility than smaller ones. On the other hand, electrophoresis in dense gels endows larger molecules and particles with a slower electrophoretic mobility than the smaller ones.

#### X. ACKNOWLEDGMENTS

This work was supported in part by Public Health Service Research Grants CA 02357 and GM 16256 from the National Institutes of Health.

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### XII. SYMBOLS USED

E	$\frac{6\pi\eta e}{ekT} \frac{U}{X}$
F	force
T	absolute temperature
U	electrophoretic velocity
X	electric field strength
a	radius, or other expression for the dimension of the molecule or particle
e	unit charge ( $= 4.8032 \times 10^{-10}$ esu)
k	Boltzmann's constant ( $= 1.3806 \times 10^{-16}$ erg deg $^{-1}$ )
u	electrophoretic mobility
x	distance to surface of shear
y <sub>0</sub>	$\frac{e\zeta}{kT}$
ε	dielectric constant of medium
ζ	potential of the molecule or particle at the surface of shear of the diffuse double layer
η	viscosity of the medium
k	$1/(\text{thickness of the diffuse double layer})$
μ	$10^{-4}$ cm; $m\mu = 10^{-7}$ cm
p	space charge density
ψ	potential of the double layer of the molecule or particle