

A Commentary on the Screened-Oseen, Counterion-Condensation Formalism of Polyion Electrophoresis

Stuart A. Allison* and Dirk Stigter†

*Department of Chemistry, Georgia State University, Atlanta, Georgia 30303 and †Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143, USA

ABSTRACT The use of linear theory, in particular, counterion condensation (CC) theory, in describing electrophoresis of polyelectrolyte chains, is criticized on several grounds. First, there are problems with CC theory in describing the equilibrium distribution of ions around polyelectrolytes. Second, CC theory is used to treat ion relaxation in a linear theory with respect to the polyion charge despite the fact that ion relaxation arises as a consequence of nonlinear charge effects. This nonlinearity has been well established by several investigators over the last 70 years for spherical, cylindrical, and arbitrarily shaped model polyions. Third, current use of CC theory ignores the electrophoretic hindrance as well as the ion relaxation for condensed counterions and only includes such interactions for uncondensed counterions. Because most of the condensed counterions lie outside the shear surface of the polyion (in the example of DNA), the assumption of ion condensation is artificial and unphysical. Fourth, the singular solution, based on a screened Oseen tensor, currently used in the above mentioned theories is simply wrong and fails to account for the incompressibility of the solvent. The actual singular solution, which has long been available, is discussed. In conclusion, it is pointed out that numerical alternatives based on classic electrophoresis theory (J.T.G. Overbeek, *Kolloid-Beih*, 1943, 54:287–364) are now available.

INTRODUCTION

Over the last few years, the electrophoretic mobility of flexible polyions have frequently been interpreted in terms of an approximate formalism (Manning, 1981; Barrat and Joanny, 1996) that combines bead hydrodynamics of the Oseen–Burgers–Kirkwood variety (Kirkwood, 1967) and counterion condensation theory (Manning, 1978). In the remainder of this work, this formalism shall be referred to as the screened-Oseen, counterion condensation (SOCC) formalism. A good example of the application of the SOCC approach is the recent analysis of the free solution mobility of short DNA fragments (Mohanty and Stellwagen, 1999). It is our firm belief that there are some serious problems with the SOCC formalism, and the primary purpose of the present work is to call some of these problems to public attention. A secondary objective is to point out that viable alternatives to the SOCC approach are available.

DISCUSSION

In modeling the transport of flexible polymers, it is common practice to model the polymer as a string of beads. Let \mathbf{y}_i denote the position of bead i , and \mathbf{F}_i the force acting on that bead. In the absence of external forces on the surrounding solvent, the fluid velocity at position \mathbf{x} is (Barrat and

Joanny, 1996; Mohanty and Stellwagen, 1999)

$$\mathbf{v}(\mathbf{x}) = \sum_i \mathbf{O}(\mathbf{x}, \mathbf{y}_i) \cdot \mathbf{F}_i, \quad (1)$$

where \mathbf{O} is the Oseen tensor given by

$$\mathbf{O}(\mathbf{x}, \mathbf{y}) = \frac{1}{8\pi\eta r} (\mathbf{I} + \mathbf{R}). \quad (2)$$

$\mathbf{r} = \mathbf{x} - \mathbf{y}$, $r = |\mathbf{r}|$, η is the solvent viscosity, \mathbf{I} is the 3 by 3 identity tensor, and \mathbf{R} is a second rank tensor with the i, j th component given by $(\mathbf{R})_{ij} = \mathbf{r}_i \mathbf{r}_j / r^2$. The SOCC formalism attempts to generalize this to the transport of polyions. Let $\mathbf{u}(\mathbf{y}_i)$ denote the velocity of bead i centered at \mathbf{y}_i , then, for the special case of electrophoresis (Barrat and Joanny, 1996; Mohanty and Stellwagen, 1999),

$$\mathbf{u}(\mathbf{y}_i) = \sum_j (\mathbf{T}(\mathbf{y}_i, \mathbf{y}_j) \cdot [\mathbf{F}_{j,\text{ext}} + q\mathbf{E}_{j,r}] + \mathbf{O}(\mathbf{y}_i, \mathbf{y}_j) \cdot [\mathbf{F}_{j,\text{pol}} + \mathbf{F}_{j,\text{rand}}]), \quad (3)$$

where $\mathbf{F}_{j,\text{ext}}$ is the external force on bead j (the product of the effective charge on a single bead, q , and the external field, \mathbf{E}), $\mathbf{F}_{j,\text{pol}}$ is the intramolecular force on bead j , $\mathbf{F}_{j,\text{rand}}$ is the random force on bead j due to diffusion, and $\mathbf{E}_{j,r}$ represents the electric field at bead location j due to the distortion of the ion atmosphere of the polyion from its equilibrium value. Also, $\mathbf{T}(\mathbf{x}, \mathbf{y})$ denotes a screened Oseen tensor given by (Manning, 1981)

$$\mathbf{T}(\mathbf{x}, \mathbf{y}) = e^{-\kappa r} \mathbf{O}(\mathbf{x}, \mathbf{y}), \quad (4)$$

where \mathbf{O} is again the Oseen tensor given by Eq. 2, and

$$\kappa^2 = \frac{8\pi e^2}{Dk_B T} I, \quad (5)$$

Received for publication June 4, 1999 and in final form October 8, 1999.

Address reprint requests to Stuart A. Allison, Department of Chemistry, Georgia State University, Atlanta, GA 30303. Tel.: 404-651-1986; Fax: 404-651-1416; E-mail: chesaa@panther.gsu.edu and stigter@laplace.ucsf.edu.

© 2000 by the Biophysical Society

0006-3495/00/01/121/04 \$2.00

where e is the protonic charge, D is the dielectric constant, k_B is Boltzmann's constant, T is absolute temperature, and I is the ionic strength of the solvent. The screening factor, $e^{-\kappa r}$, appearing in Eq. 4, is typical of solutions of the linearized Poisson–Boltzmann equation that are valid when the mean surface potential of the polyion, ζ , is low.

Here, we feel it worthwhile to provide some background on the electrophoresis of simple spherical polyions with uniform surface potential, ζ . This should serve as an aid in understanding the more complex issues associated with electrophoresis of arbitrary charged macromolecules, which the SOCC formalism is supposed to address. Hückel (1924) determined the velocity and pressure fields around a charged spherical polyion and derived a simple expression for its electrophoretic mobility. The assumption was made that the electric field in the vicinity of the sphere was the sum of the electric field due to the equilibrium charge distribution of the sphere and a uniform external field, \mathbf{E} . A similar model was formulated by Henry (1931), but account was taken of the difference in conductivity of solution and solid particle, and this difference distorts the electric field around the polyion in much the same way that a dielectric discontinuity between solid particle and fluid would. In both the Hückel and Henry models, the distortion of the ion atmosphere around the moving polyion, or ion relaxation, was ignored. Later, Overbeek (1943) accounted for ion relaxation and considered terms in the surface potential up to order ζ^3 . To first-order terms in ζ , Overbeek's results reduce to those of Henry, and it is observed that the effects of ion relaxation start with the higher, nonlinear terms that are proportional to ζ^2 and ζ^3 . Thus, Hückel and Henry were justified in using the equilibrium ionic atmospheres around the moving sphere in their work, because ion relaxation can be ignored provided the linearized Poisson–Boltzmann equation adequately describes the charge distribution around the spherical polyion. The criterion for this condition to be satisfied is that $|e\zeta/k_B T| \ll 1$. When $|e\zeta/k_B T|$ is not small, the linearized Poisson–Boltzmann equation does not adequately describe the charge distribution around a spherical polyion at rest, and also ion relaxation becomes a significant factor in electrophoresis. Finally, the effects of fluid flow and ion relaxation are not simply additive, but entangled with one another in the nonlinear terms. Booth (1950) subsequently confirmed Overbeek's results.

In all continuum theories of electrophoresis that account for ion relaxation and the coupled interactions of fluid, polyion, ion atmosphere, and external electric field, it is necessary to solve simultaneously the Navier–Stokes, Poisson, and ion transport equations. Provided the polyion is large relative to the mobile ions (co- and counterions), it is a good approximation to ignore the diffusion of the polyion relative to the small ions (Wiersema, 1964). Linearization of these equations with respect to the perturbing electric/flow fields is allowed provided the perturbing fields are weak. However, linearization with respect to the equilibrium po-

tential of the polyion itself is only valid if the polyion is weakly charged. This may indeed be valid in particular applications ($|e\zeta/k_B T| \ll 1$), but if this is so, it also follows that ion relaxation should also be negligible. In the formulation of Eq. 3, which is the fundamental equation of the SOCC approach, linearization of the various field equations is extensively carried out (Barrat and Joanny, 1996). In deriving an expression for the relaxation electric field around bead j , $\mathbf{E}_{j,r}$, ion densities derived from the linear Poisson–Boltzmann equation are first used in an approximate ion transport equation. (Incidentally, the ion transport equation used in Eq. B.2 of Barrat and Joanny (1996) is incomplete because it ignores solvent convection. However, given other problems that we regard as much more serious, we shall not consider this oversight further in the present work.). The ion transport equation is then again linearized with respect to the charge on each bead, q , which leads to Eqs. 7.1 and 7.2 of Barrat and Joanny (1996), and Eqs. 8 and 9 of Mohanty and Stellwagen (1999). As we have emphasized above, however, ion relaxation becomes important when the nonlinear terms in the electrostatic potential become important. Thus, there is a fundamental inconsistency in using terms linear in q to calculate relaxation electric fields. To be self-consistent, the SOCC may be valid if it is applied to polyelectrolytes where the monomer charges are low so that the condition $|e\zeta/k_B T| \ll 1$ is indeed satisfied, but, in that case, all terms related to ion relaxation should be thrown out. For the case of double-stranded high molecular-weight DNA, $|e\zeta/k_B T|$ is approximately 3.0 in monovalent salt at room temperature (Schellman and Stigter, 1977), which clearly falls well outside the range of validity of the linear PB equation.

It is at this point where counterion condensation theory enters the SOCC formalism (Manning, 1981). In counterion condensation theory (Manning, 1978) of a long linear polyion in the presence of one simple salt, there are two kinds of counterions—"condensed" (if the linear charge density of the polyion exceeds a certain threshold) and "free." The argument has been advanced that the condensed ions do not contribute to electrophoresis, but that the free ions do (Barrat and Joanny, 1996; Mohanty and Stellwagen, 1999). In the SOCC approach, the actual charge on each monomer is replaced by a much reduced effective charge, which accounts for the absorption of the condensed counterions by the line charge of the polyion. Only the uncondensed counterions and the effective charges of the polyion are assumed to contribute to electrophoresis. Then, if one accepts all of the assumptions, linearization of the fields may be allowed. Aided by its simplicity, the concept of counterion condensation has become firmly embedded in biochemistry. A few cautionary remarks are in order here to point out its non-physical origin and technical flaws.

Condensation of counterions on highly charged cylindrical polyions is assumed to avoid the infinite divergence of the phase integral involving counterions (Manning, 1969).

However, such divergence occurs only for the electrostatic potential field around a cylinder in the absence of counterions. Whenever counterions, and common salt at whatever low concentration, are present in the medium around the cylinder, the potential field is changed in such a way that the phase integral does not, in fact, diverge.

The distribution of the condensed counterions presents another problem. It has been argued (Manning, 1977) that condensed counterions are distributed uniformly in solution in the immediate vicinity of the polyion in a cylindrical volume, V . For example, for B-DNA, $V = 720$ ml/mole DNA phosphate. With an average radius of 10 \AA for the double helix, this corresponds to an outer radius of the condensation volume of 17 \AA . If the concentration of counterions in V is constant, then, following Boltzmann's law, the electrostatic potential in the uniformly charged volume, V , should also be constant. This, however, is contrary to Poisson's equation of electrostatics, which says that the potential cannot be constant in any charged region of the solution. This shows that the counterion distribution in condensation theory violates some fundamental physics. There are alternatives to counterion condensation theory that have been very successful in describing equilibrium properties of charged linear polyions (Anderson and Record, 1995).

There is also a kinetic puzzle in condensation theory. Outside a solid particle–solution interface, the local viscosity changes from a high value to the viscosity of the bulk solution. It is customary to contract this narrow region to a smooth surface enveloping the particle, called the hydrodynamic shear surface. Solute transport properties are often interpreted in terms of the location of the shear surface. A variety of experiments has shown that, for clay particles, and also for micelles of sodium dodecyl sulfate, the shear surface is located at $1 \pm 1 \text{ \AA}$ from the particle–water interface (Stigter, 1982). Modeling DNA as a cylinder, estimates of its hydrodynamic diameter vary from $25 \pm 1 \text{ \AA}$, as derived from sedimentation of high molecular weight DNA (Yamakawa and Fujii, 1973), to $20 \pm 1.5 \text{ \AA}$, as derived from analysis of rotational and translational diffusion constants of short DNA fragments (Eimer and Pecora, 1991). Comparison of a kinetic radius of about 11 \AA with the outer radius of 17 \AA of its condensation volume suggests that most of the condensed counterions lie well outside the shear surface of DNA. On physical grounds, one would therefore expect most of the condensed counterions to undergo ion relaxation. This is an inconsistency that is ignored in the SOCC approach.

In addition to these criticisms, we also feel that there is a serious problem with Eq. 4. This screened Oseen tensor should be equivalent to the singular solution of the velocity ($\mathbf{v}(\mathbf{x}, \mathbf{y})$) and pressure ($p(\mathbf{x}, \mathbf{y})$) fields for an incompressible fluid of viscosity η at field position \mathbf{x} due to a point charge of magnitude q at position \mathbf{y} in a uniform external electric field, \mathbf{E} . Except for the perturbation by the point charge, the

solvent is taken to be at rest. The assumption is also made that the point charge carries with it an equilibrium ion atmosphere characterized by the Debye–Hückel charge distribution,

$$\rho(\mathbf{x}) = -\frac{q\kappa^2}{4\pi r} e^{-\kappa r}. \quad (6)$$

It is also assumed that the perturbation of the solvent by the point charge is weak enough that the singular velocity/pressure fields are described by the linearized Navier–Stokes and solvent incompressibility equations,

$$-\eta \nabla^2 \mathbf{v} + \nabla p = q\mathbf{E}\delta(\mathbf{r}) + \rho(\mathbf{x})\mathbf{E} \quad (7a)$$

$$\nabla \cdot \mathbf{v} = 0. \quad (7b)$$

The differential operators in Eqs. 7 act on field position \mathbf{x} . It has been claimed that the solution of Eqs. 7 for $\mathbf{v}(\mathbf{x}, \mathbf{y})$ is (Barrat and Joanny, 1996; Mohanty and Stellwagen, 1999)

$$\mathbf{v}(\mathbf{x}, \mathbf{y}) = q\mathbf{T}(\mathbf{x}, \mathbf{y}) \cdot \mathbf{E}, \quad (8)$$

where \mathbf{T} is given by Eq. 4. In fact, this is not the case that can readily be demonstrated by simply applying Eq. 7b to Eq. 8, which yields $-q\kappa e^{-\kappa r} \mathbf{r} \cdot \mathbf{E}/4\pi\eta r^2$ instead of zero. Physically, Eq. 8 fails to account for the incompressibility of the solvent.

It is straightforward to deduce the actual singular solution of Eqs. 7a and 7b from the early work of Hückel (1924). Also, we have confirmed Hückel's results through an independent derivation, which yields

$$\mathbf{v}(\mathbf{r}) = q \mathbf{U}(\mathbf{x}, \mathbf{y}) \cdot \mathbf{E}, \quad (9a)$$

$$p(\mathbf{r}) = q \mathbf{P}(\mathbf{x}, \mathbf{y}) \cdot \mathbf{E}, \quad (9b)$$

where

$$\mathbf{U}(\mathbf{x}, \mathbf{y}) = \frac{1}{4\pi\eta r} \left\{ \left[e^{-\kappa r} \left(1 + \frac{1}{\kappa r} \right) - \frac{1}{\kappa^2 r^2} (1 - e^{-\kappa r}) \right] \mathbf{I} + \left[\frac{3}{\kappa^2 r^2} (1 - e^{-\kappa r}) - e^{-\kappa r} \left(1 + \frac{3}{\kappa r} \right) \right] \mathbf{R} \right\} \quad (10a)$$

$$\mathbf{P}(\mathbf{x}, \mathbf{y}) = \frac{e^{-\kappa r}}{4\pi r^3} (1 + \kappa r) \mathbf{r}. \quad (10b)$$

In the limit of zero salt, Eq. 10a reduces to the Oseen tensor, Eq. 2. It may, in fact, be possible to adapt the SOCC approach using the actual singular solutions given above, but that is beyond the scope of the present work.

It is the combination of a large number of monomeric charges and high average linear charge density that is responsible for the distinctive molecular, thermodynamic, and transport properties of linear polyelectrolytes. In a qualitative way, counterion condensation theory has been of considerable value in helping our understanding of many of these properties. Yet it remains an approximate device that

should not be used in serious quantitative theory. An alternative is based on the Poisson–Boltzmann ionic atmosphere around charged particles in salt solutions, the classic approach to electrophoresis (Hunter, 1981). Overbeek (1943) gave the general formulation of the coupled steady-state hydrodynamic and electrodynamic differential equations for the transport and force fields around a charged particle in electrophoresis with the appropriate boundary conditions. A number of investigators (Overbeek, 1943; Booth, 1950; Wiersema, 1964; Wiersema et al., 1966; O'Brien and White, 1978), solved these equations for charged spheres in salt solutions. This work was extended to long rods (Stigter, 1978a,b) and to particles of arbitrary shape (Allison, 1996). The same formal approach was also followed in an analysis of the electric polarizability of finite rods (Fixman and Jagannathan, 1981).

Mohanty and Stellwagen (1999) have recently considered how well the SOCC approach fits the experimental mobility of DNA fragments as a function of length. Figure 1 of their paper compares theoretical and experimental mobilities as a function of fragment length. The theoretical curve displays a much stronger length dependence than seen experimentally. Figure 2 of their paper presents what appears to be much better agreement after “scaling” the experimental data, but the authors do not explain what they mean by this. Classical analyses of the free solution electrophoretic mobility of hen eggwhite lysozyme as a function of pH (Allison et al., 1997) and of short DNA fragments (20–30 bp) (Allison and Mazur, 1998) have yielded good quantitative agreement between theory and experiment.

CONCLUSION

In summary, we feel that the SOCC formalism is, at best, a qualitative theory that has a flawed theoretical foundation for reasons discussed above. Numerical alternatives (O'Brien and White, 1978; Stigter, 1978a,b; Allison, 1996; Allison et al., 1997; Allison and Mazur, 1998) that are grounded in the formal transport theory of Overbeek (1943) are now available.

S.A.A. would like to acknowledge National Science Foundation grant MCB-9807541 for partial support of this work.

REFERENCES

- Allison, S. A. 1996. Modeling the electrophoresis of rigid polyions. Inclusion of ion relaxation. *Macromolecules*. 29:7391–7401.
- Allison, S. A., M. Potter, and J. A. McCammon. 1997. Modeling the electrophoresis of lysozyme. II. Inclusion of ion relaxation. *Biophys. J.* 73:133–140.
- Allison, S. A., and S. Mazur. 1998. Modeling the free solution electrophoretic mobility of short DNA fragments. *Biopolymers*. 46:359–373.
- Anderson, C. F., and M. T. Record. 1995. Salt–nucleic acid interactions. *Annu. Rev. Phys. Chem.* 46:657–700.
- Barrat, J.-L., and J.-F. Joanny. 1996. Theory of polyelectrolyte solutions. *Adv. Chem. Phys.* 94:45–66.
- Booth, F. 1950. The cataphoresis of spherical, solid non-conducting particles in a symmetric electrolyte. *Proc. R. Soc. London Ser. A*. 203: 514–533.
- Eimer, W., and R. Pecora. 1991. Rotational and translational diffusion of short rodlike molecules in solution: oligonucleotides. *J. Chem. Phys.* 94:2324–2329.
- Fixman, M., and S. Jagannathan. 1981. Electrical and convective polarization of cylindrical macroions. *J. Chem. Phys.* 75:4048–4059.
- Henry, D. C. 1931. The cataphoresis of suspended particles. Part I. The equation of cataphoresis. *Proc. R. Soc. London Ser. A*. 133:106–129.
- Huckel, E. 1924. Die Kataphorese der Kugel. *Physik. Zeitsch.* 25:204–210.
- Hunter, R. J. 1981. *Zeta Potential in Colloid Science*. Academic Press, New York.
- Kirkwood, J. G. 1967. Macromolecules, In John Gamble Kirkwood Collected Works, P. L. Auer, editor., Gordon and Breach, New York.
- Manning, G. S. 1969. Limiting laws and counterion condensation in polyelectrolyte solutions. I. Colligative properties. *J. Chem. Phys.* 51: 924–933.
- Manning, G. S. 1977. Limiting laws and counterion condensation in polyelectrolyte solutions. IV. The approach to the limit and the extraordinary stability of the charge fraction. *Biophys. Chem.* 7:95–102.
- Manning, G. S. 1978. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Quart. Rev. Biophys.* 11:179–246.
- Manning, G. S. 1981. Limiting laws and counterion condensation in polyelectrolyte solutions. 7. Electrophoretic mobility and conductance. *J. Phys. Chem.* 85:1506–1515.
- Mohanty, U., and N. C. Stellwagen. 1999. Free solution mobility of oligomeric DNA. *Biopolymers*. 49:209–214.
- O'Brien, R. W., and L. R. White. 1978. Electrophoretic mobility of a spherical colloid particle. *J. Chem. Soc. Faraday Trans. 2*. 74: 1607–1626.
- Overbeek, J. T. G. 1943. Theorie der Elektrophorese. Der Relaxationseffekt. *Kolloid-Beih.* 54:287–364.
- Schellman, J. A., and D. Stigter. 1977. Electrical double layer, zeta potential, and electrophoretic charge of double-stranded DNA. 16:415–1434.
- Stigter, D. 1978a. Electrophoresis of highly charged colloidal cylinders in univalent salt solutions. 1. Mobility in transverse field. *J. Phys. Chem.* 82:1417–1423.
- Stigter, D. 1978b. Electrophoresis of highly charged colloidal cylinders in univalent salt solutions. 2. Random orientation in external field and application to polyelectrolytes. *J. Phys. Chem.* 82:1424–1429.
- Stigter, D. 1982. Mobility of water near charged interfaces. *Adv. Colloid Interface Sci.* 16:253–265.
- Wiersema, P. H. 1964. On the theory of electrophoresis. Ph.D. thesis, Utrecht University, Utrecht, The Netherlands.
- Wiersema, P. H., A. L. Loeb, and J. T. G. Overbeek. 1966. Calculation of the electrophoretic mobility of a spherical colloid particle. *J. Colloid. Interface Sci.* 22:78–99.
- Yamakawa, H., and M. Fujii. 1973. Translational friction coefficient of wormlike chains. *Macromolecules*. 6:407–415.