Biophysical Chemistry 5 (1976) 243–251
© North-Holland Publishing Company

SEDIMENTATION IN THE ULTRACENTRIFUGE AND DIFFUSION OF MACROMOLECULES CARRYING ELECTRICAL CHARGES

Henryk EISENBERG
Polymer Department, The Weizmann Institute of Science, Rehovot, Israel

The calculation of Alexandrowicz and Daniel of the charge effect in the sedimentation of macromolecules carrying electrical charges was reformulated by introducing buoyant molecular weights and concentrations of electroneutral components only (rather than those of the ionized species) in the final equations. Some interesting limiting cases are discussed. A similar calculation was performed for the case of isothermal diffusion. It is also shown how the present discussion relates to more elaborate treatments of the sedimentation and diffusion of polyelectrolytes by the methods of irreversible thermodynamics.

1. Introduction

An early detailed theoretical attempt to characterize sedimentation of systems of charged macromolecules is due to Pedersen [1]. He observes that if charged macromolecules are sedimented in the ultracentrifuge, the higher sedimentation tendency of the macromolecules, as contrasted to that of the slower sedimenting counterions, results in a microscopic separation of charge between the bottom of the cell and the macromolecular boundary. This separation establishes an electric field $E = \mathrm{d}\psi/\mathrm{d}r$ in the intervening column of solution, which slows down the macromolecular ion and speeds up the counterions (ψ is the potential and r is the distance from the center of rotation). Both ions move with equal intermediate velocity. This is called the primary *charge effect*. In diffusion, on the other hand, the small counterions diffuse faster than the large macroions and pull the latter after them. Thus, whereas in sedimentation the charge effect slows down the large ions (as opposed to an equivalent non-charged particle) in diffusion the contrary is observed. We shall see below that a much debated point concerns the question whether or not the primary charge effect vanishes at infinite dilution of macromolecules.

With increasing concentration of simple salt the charges on the macromolecules are screened, the sedimentation coefficient increases and the diffusion coefficient decreases. This is known as the *primary salt effect*. Do these coefficients ever approach the values for uncharged macromolecules when simple salt concentration increases "to infinity"? We must distinguish here between almost rigid (globular or multihelical) undeformable macromolecules, and (mostly chain-like) macromolecules whose conformations are sensibly affected by ionic strength. In the first case one might indeed approach (by extrapolation to high ionic strength) the sedimentation and diffusion coefficients of the uncharged species – in the second case we are faced with an inextricable mixture of charge and conformation effects.

The *secondary salt effect* arises from unequal mobilities and sedimentation coefficients of the ions of the simple electrolyte in mixed polyelectrolyte-electrolyte solutions. It is less fundamental and can be minimized or eliminated by choosing a supporting electrolyte such as NaCl, for instance, in which both cation and anion have nearly identical sedimentation coefficients*.

Although the charge effects in the sedimentation and diffusion of multicomponent polyelectrolyte systems

---

* Sometimes though the specific nature of an experiment may preclude such a choice (consider a sedimentation study in a density gradient for instance, in which CsCl has been used for considerations relating to the formation of the density gradient) and in such an instance the secondary salt effect must be taken into consideration in the interpretation of the experimental results.

have been extensively investigated both on theoretical and on experimental grounds in the last two decades, the problem cannot be considered solved in a satisfactory way. Transport properties in these complex systems have not been treated with the rigor with which the equilibrium properties have been interpreted. A simple question for instance which has not been answered in unequivocal terms, refers to the more limited problem whether combination of the sedimentation and diffusion coefficients (in the manner in which this is undertaken for binary systems in the Svedberg equation) leads to a well defined value for the molecular weight, at all macromolecular concentrations, or only in the limit of infinite dilution.

Pedersen [1] calculated the effect by which the sedimentation of the macromolecular species is retarded by the electrostatic field set up between the meniscus and the bottom of the ultracentrifuge cell (under non-equilibrium conditions) by the unequal mobilities of the ions constituting the various electroneutral components. Electroneutrality requires the ions to move together (in electroneutral combinations) and therefore no net current flows in the ultracentrifuge cell*. Pedersen explicitly introduced the electrophoretic mobilities of the ions to account for the retardation of the macroion in the electrostatic field associated with the sedimentation velocity experiment of charged species, but apparently could account only for part of the effect. Alexandrowicz and Daniel [4,5] argued that because of electrostatic interactions between the macroions and the small counterions the retardation effect becomes smaller still, yet the failure of Pedersen to account for the retardation effect is attributed to the fact that introduction of explicit electrophoretic mobilities is not justified in velocity sedimentation. Thus, whereas in electrophoresis the two oppositely charged ions move in opposite directions, in sedimentation motion of both ions is directed by the buoyancy and the direction of the field. Alexandrowicz and Daniel eliminate the actual mobilities from the equations of motion.

A more systematic approach that deals with the combined problem of sedimentation and diffusion of charged species uses the methods of irreversible thermodynamics. A rather formidable analysis by this approach is due to Mijnlieff and Overbeek [6] and Mijnlieff [7]; we owe Mijnlieff [8] a comprehensible summary of this work. More recently Varoqui and Schmitt [9] have reformulated this problem in simpler terms and have also calculated friction coefficients of charged species for the rod-like polyelectrolyte model. The earlier authors [1,4] claim that charge effects due to the (in principle measurable) electrostatic potential between the meniscus and bottom of the ultracentrifuge cell disappears at vanishing concentration of the sedimenting charged species. Varoqui and Schmitt, on the other hand, calculate the effect of charge on the friction coefficients, which persists at all reasonable dilutions at which measurements with polyelectrolyte systems have meaning. This limiting charge effect was not considered by Pedersen, who assumed that at "infinite" dilution the counterions to the macroion are spread over the whole volume of solution, and was absorbed by Alexandrowicz and Daniel into an "effective charge" parameter. Unfortunately, many problems remain. Frictional coefficients depend both on the radius of the ionic atmosphere and on the shape of the macroion coils (cf. Nagasawa and Eguchi [10]) and macromolecules of perfectly well known and well defined rigid shape are not readily available. We must also consider the problem of the coupling between the motion of the macroion and the ions composing the atmosphere, for which no complete solution has been provided. In sedimentation the motion of the sedimenting species is at zero concentration gradient. The macroion sediments because of its large size; the counterions in the atmosphere are much smaller and would not be "dragged" along were it not for the electroneutrality requirement. If the coupling between macroion and atmosphere is tight, then the frictional resistance of the ions in the atmosphere must be considered (Alexandrowicz and Daniel assumed that the "bound" counterions do not contribute to the frictional resistance at all). On the other hand, in the case of less tight coupling we can visualize the macroion as moving through the homogeneous solution, leaving some of its counterions behind and picking up the complement required for electroneutrality from the macroions sedimenting from the slice of solution just ahead in the field. Reduced contribution to the frictional resistance

---

* Under conditions of sedimentation equilibrium readjustment of the concentrations of the solutes leads to vanishing of the electrostatic potential [2]. Sedimentation equilibrium can therefore be considered exclusively for the electroneutral components without reference to the electrostatic field [3].

by the counterions would be expected in this case. In diffusion the analysis classically considers a process occurring in a concentration gradient, yet diffusion also occurs, and is measurable, in homogeneous solution. Another difficulty concerns the evaluation of the hydrodynamic properties of macromolecular coils with excluded volume and the extent to which these coils are freely draining or shield the motion of small ions or solvent molecules; for space filling globular macromolecules, hydrodynamic undertainties may arise from the odd shape of the protein, for instance, or ill defined hydration layers.

The difficulties enumerated above need not lead to an abandonment of the use of sedimentation and diffusion for the characterization of charged species. At high enough concentrations of salt, the radius κ_{DH}^{-1} of the Debye–Huckel ionic atmosphere is small when compared to the dimensions of the bulky macromolecules, and with judicious choice of simple salt systems (to avoid secondary charge phenomena) the effects of charge in sedimentation and diffusion may be reduced to a small contribution. The quantitative discussion to follow should be helpful towards the design of critical experimental procedures.

2. Reformulation of the Alexandrowicz–Daniel calculation

Following the basic concept of Svedberg and Pedersen [11], Alexandrowicz and Daniel [4,5] "regard the actual velocity v of each ion in sedimentation as composed of two virtual velocities, namely a sedimentation velocity which would be obtained under the isolated action of the external centrifugal field $\omega^2 r$ (ω is the angular velocity) and an electrophoretic velocity which would be obtained under the isolated action of the internally created field $d\psi/dr$ [11]. The hypothetical velocities are expressed in their explicit form as forces divided by frictions, and not as electrophoretic mobilities, as was proposed by Pedersen. For the system water (component 1), PX_Z (component 2), XY (component 3), where P is a Z-valent negative ion, X and Y are positive counter- and negative co-ions, respectively,

$$v_P = \frac{\overline{M}^*_P \omega^2 r}{f_P} + \frac{iZe}{f_P}\frac{d\psi}{dr}, \tag{1}$$

$$v_X = \frac{\overline{M}_X \omega^2 r}{f_X} - \frac{e}{f_X}\frac{d\psi}{dr}, \tag{2}$$

$$v_Y = \frac{\overline{M}_Y \omega^2 r}{f_Y} + \frac{e}{f_Y}\frac{d\psi}{dr}, \tag{3}$$

where

$$\overline{M}_j \equiv M_j(1-\bar{v}_j\rho), \qquad \overline{M}^*_P \equiv \overline{M}_P + (1-i)Z\overline{M}_X,$$

i is an effective charge parameter ($0 \leqslant i \leqslant 1$) which may at this point be considered as a formal device and need not be defined precisely presently; f_P, f_X and f_Y are the frictional coefficients per mole of the ionic species P*, X and Y[†]; the M_j are molecular weights and the $\bar{v}_j$ partial specific volumes of the ionic species, and ρ is the density of the solvent medium[††].

Eqs. (1) to (3) are modified by multiplying both sides of the equations for v_P, v_X and v_Y by the corresponding charge and concentrations C_j (in moles of ions per liter), i.e., by $-iZeC_P$, eC_X and $-eC_Y$, respectively. The sum of the left-hand sides of these modified equations yields

$$eC_X v_X - eC_Y v_Y - iZeC_P v_P = 0, \tag{4}$$

because of the assumption of zero current flow. From the sum of the right-hand side of these equations we find (electroneutrality is observed at all points, $C_X = C_Y + iZC_P$)

$$\frac{d\psi}{dr} = \frac{\omega^2 r}{e}\,\frac{\left(\frac{\overline{M}_X}{f_X} - \frac{\overline{M}_Y}{f_Y}\right) C_Y + \left(\frac{\overline{M}_X}{f_X} - \frac{\overline{M}^*_P}{f_P}\right) C_P iZ}{\left(\frac{1}{f_X} + \frac{1}{f_Y}\right) C_Y + \left(\frac{1}{f_X} + \frac{iZ}{f_P}\right) C_P iZ}, \tag{5}$$

for $d\psi/dr$; this expression for $d\psi/dr$ is now substituted into eq. (1) and the sedimentation coefficient

$$s_P \equiv v_P/\omega^2 r$$

evaluated. *We also introduce the buoyant molecular weights and the concentrations of the electroneutral species*

[†] These frictional coefficients represent the average frictional resistance of P*, X and Y with respect to the solvent mixture; proper frictional cross-coefficient between the various species in solution will be considered in the formulation of the problem by the methods of irreversible thermodynamics.

[††] We assume the process of the formation of the hypothetical species M^*_P to occur without change of volume.

$$\bar{M}_2 = \bar{M}_P^* + iZ\bar{M}_X = M_2(1-\bar{v}_2\rho),$$

$$\bar{M}_3 = \bar{M}_X + \bar{M}_Y = M_3(1-\bar{v}_3\rho),$$

$$M_u = M_2/Z, \qquad M_u = \bar{M}_2/Z,$$

$$C_2 = C_P = C_u/Z, \qquad C_3 = C_Y,$$

to find

$$\frac{1}{s_P} = \frac{\dfrac{f_P}{\bar{M}_2} + \dfrac{i^2}{\bar{M}_u}\dfrac{C_u}{\left(\dfrac{1}{f_X}+\dfrac{1}{f_Y}\right)C_3 + \dfrac{i}{f_X}C_u}}{1 - \dfrac{\bar{M}_3}{\bar{M}_u}\dfrac{i}{f_Y}\dfrac{C_3}{\left(\dfrac{1}{f_X}+\dfrac{1}{f_Y}\right)C_3 + \dfrac{i}{f_X}C_u}}, \tag{6}$$

Eq. (6), which is significantly different from the expressions derived by Alexandrowicz and Daniel, assumes some interesting and simple limiting forms.[‡]

Consider first the case $\bar{M}_3 = 0$ discussed by Ziccardi and Schumaker [12]; this case arises if the component 3 is chosen such that $(1-\bar{v}_3\rho)$ effectively vanishes. Tetramethyl-amonium chloride is suggested [12] for this purpose. If $\bar{M}_3$ vanishes then the denominator of eq. (6) becomes unity and the expression reduces to

$$\bar{M}_3 = 0. \quad \frac{1}{s_P} = \frac{f_P}{\bar{M}_2} + \frac{i^2}{\bar{M}_u}\frac{C_u}{\left(\dfrac{1}{f_X}+\dfrac{1}{f_Y}\right)C_3 + \dfrac{i}{f_X}C_u}. \tag{7}$$

Upon extrapolation to vanishing concentration C_u (equivalents of charged groups per liter) the limiting value of the sedimentation coefficient is given in terms of the properties of the macromolecular component only.

To determine the limiting value s_P^0 of s_P, as C_u vanishes, in the general case, we develop eq. (6) exactly through the linear term in C_u, to give

$$\frac{1}{s_P} = \frac{f_P}{\bar{M}_2 - iZ\bar{M}_3\left(1+\dfrac{f_Y}{f_X}\right)^{-1}} + \frac{C_u}{C_3}\frac{i^2 f_Y}{\bar{M}_u\left(1+\dfrac{f_Y}{f_X}\right) - i\bar{M}_3}\left\{1 - \frac{\bar{M}_3 f_P/f_X}{\bar{M}_2\left(1+\dfrac{f_Y}{f_X}\right) - iZ\bar{M}_3}\right\} + \ldots . \tag{8}$$

We need not worry about the complicated form of the term linear in C_u. The interesting term is the limiting value of s_P^{-1} which assumes an even more significant form when component 3 is chosen such that $f_Y = f_X \equiv f_{X,Y}$ and the secondary charge effect disappears. We then obtain from eq. (8)

$$\frac{1}{s_P} = \frac{f_P}{\bar{M}_2 - \frac{1}{2}iZ\bar{M}_3} + \frac{C_u i^2}{C_3(2\bar{M}_u - i\bar{M}_3)}\left(f_{X,Y} - \frac{\bar{M}_3 f_P}{2\bar{M}_2 - iZ\bar{M}_3}\right). \tag{9}$$

We recall that i was introduced at the beginning of this discussion as a nonspecified effective charge parameter. The only other assumption in this calculation was that of zero net current and electroneutrality. If $i/2$ here be identified [3] with the thermodynamic Donnan distribution coefficient Γ then, after some elementary transformations [3], the following extremely interesting result is obtained for s_P^0,

$$s_P^0 = \frac{\bar{M}_2 - \Gamma Z\bar{M}_3}{f_P} = \frac{M_2}{f_P}\left(\frac{\partial\rho}{\partial c_2}\right)_\mu, \tag{10}$$

where $\Gamma \equiv (\partial m_3/\partial m_u)_\mu$ and $(\partial\rho/\partial c_2)_\mu$ is the density increment, both at constant chemical potential μ of components diffusible through a semipermeable membrane (m_3 and $m_2 = m_u/Z$ are the molalities of components 3 and 2, respectively). This result is identical with an equation previously derived [13] for non-ionic systems by the methods of irreversible thermodynamics, on the assumption that the total chemical potential of component 3 is uniform over the whole centrifuge cell.[‡‡]

The equivalence of nonequivalence of the formal parameter i from various transport experiments (sedimentation, diffusion, conductance and electrophoresis) and its identification with the quantity derived from equilibrium studies should be submitted to further experimental scrutiny.

[‡] In all the derivations presented here, the classical non-ionic concentration dependence [19] of s_P, of the form $(1-kC)$, has been disregarded.

[‡‡] We disagree with the limiting form of s_P obtained by Alexandrowicz and Daniel who find $s_P^0 = \bar{M}_P^*/f_P$, where $\bar{M}_P^* = (1-\bar{v}_P^*\rho)$ is the buoyant weight of the charged species, rather than the buoyant weight of neutral components only, as found here.

From eq. (9) we learn that the concentration dependence of s_P (due to charge effects) diminishes with increasing C_3. On the other hand i^2 (or Γ^2) may significantly increase with increasing C_3 (cf. Cohen and Eisenberg [14] for the behavior of mixed DNA–simple salt solutions) and s_P may therefore actually decrease with increasing C_3; additional changes of s_P may be due to changes in the value of the frictional coefficient f_P (resulting from changes in molecular size and shape) under these conditions.

A further test of eq. (6) concerns the salt-free case when C_3 goes to zero. We derive the classical result

$$s_P = \frac{\bar{M}_2}{f_P + iZf_X}, \tag{11}$$

which indicates that in this case the frictional resistance of the macromolecular component is the sum of the frictional resistance f_P of the polyion and iZ times the frictional resistance of the counterions; s_P in this case, as is well known, is rather low. The measurement of the sedimentation (as well as diffusion) coefficient in salt-free polyelectrolyte solutions is extremely precarious because of boundary instabilities and errors due to convection. It is also extremely difficult to render a polyelectrolyte solution completely salt-free. Some of these difficulties have been pointed out by Auer and Alexandrowicz [15] in their study of the sedimentation, diffusion and osmotic pressure of sodium DNA in salt-free solutions and by Nagasawa and Fujita [16] who investigated the diffusion of polystyrene-sulfonic acid and its sodium salt in the absence of added salt.

We now consider the problem of isothermal diffusion for the same system PX_Z, XY discussed above. For this process we write for the velocities v_i of the ionic species [in analogy to eq. (1) to (3)]

$$v_P = -f_P^{-1}(\mathrm{d}\mu_P^*/\mathrm{d}r) + iZef_P^{-1}(\mathrm{d}\psi/\mathrm{d}r), \tag{12}$$

$$v_X = -f_X^{-1}(\mathrm{d}\mu_X/\mathrm{d}r) - ef_X^{-1}(\mathrm{d}\psi/\mathrm{d}r), \tag{13}$$

$$v_Y = -f_Y^{-1}(\mathrm{d}\mu_Y/\mathrm{d}r) + ef_Y^{-1}(\mathrm{d}\psi/\mathrm{d}r), \tag{14}$$

where we assume

$$\mu_P^* = \mu_P + (1-i)Z\mu_X .$$

As before, we multiply eqs. (12) to (14) by $-iZeC_P$, eC_X and $-eC_Y$ respectively, sum, and set the sum of the left-hand-side terms equal to zero; we also introduce electroneutrality, $C_X = C_Y + iZC_P$. The effective charge parameter i in diffusion could conceivably be different from the parameter used in velocity sedimentation, but we are not concerned with this aspect now.

Performing these operations we find

$$e\frac{\mathrm{d}\psi}{\mathrm{d}r} = \frac{\left(\dfrac{\mathrm{d}\mu_Y/\mathrm{d}r}{f_Y} - \dfrac{\mathrm{d}\mu_X/\mathrm{d}r}{f_X}\right)C_Y + \left(\dfrac{\mathrm{d}\mu_P^*/\mathrm{d}r}{f_P} - \dfrac{\mathrm{d}\mu_X/\mathrm{d}r}{f_X}\right)iZC_P}{(f_X^{-1} + f_Y^{-1})C_Y + (iZf_P^{-1} + f_X^{-1})\,iZC_P}. \tag{15}$$

We introduce $\mathrm{d}\psi/\mathrm{d}r$ from the above expression into eq. (12) for v_P, consider

$$\mathrm{d}\mu_2 = \mathrm{d}\mu_P^* + iZ\,\mathrm{d}\mu_X ,$$

and

$$\mathrm{d}\mu_3 = \mathrm{d}\mu_X + \mathrm{d}\mu_Y ,$$

and evaluate using eqs. (12) and (15)

$$D_2 = -v_P(\mathrm{d}\ln C_2/\mathrm{d}r)^{-1}.$$

The result is ($C_P \equiv C_2$, $C_Y \equiv C_3$)

$$D_2 = \frac{1}{f_P(\mathrm{d}\ln C_2/\mathrm{d}r)} \times \frac{\dfrac{\mathrm{d}\mu_2}{\mathrm{d}r} - \dfrac{\mathrm{d}\mu_3}{\mathrm{d}r}\dfrac{iZC_3}{f_Y}\left((f_X^{-1} + f_Y^{-1})C_3 + \dfrac{iZ}{f_X}\right)^{-1}}{1 + \dfrac{i^2Z^2}{f_P}C_2\left((f_X^{-1} + f_Y^{-1})C_3 + \dfrac{iZ}{f_X}C_2\right)^{-1}}. \tag{16}$$

For the purpose of practical evaluation we may now consider the special condition, already discussed, that in the mixed polyelectrolyte simple salt system μ_3 is constant and $\mathrm{d}\mu_3/\mathrm{d}r$ vanishes.* If we introduce this condition into eq. (16) we obtain

$$\frac{1}{D_2}\frac{\mathrm{d}\mu_2}{\mathrm{d}\ln C_2} = f_P + \frac{i^2Z^2C_2}{(f_X^{-1} + f_Y^{-1})C_3 + iZf_X^{-1}C_2}, \tag{17}$$

where

$$\frac{1}{RT}\frac{\mathrm{d}\mu_2}{\mathrm{d}\ln C_2} = 1 + \frac{\mathrm{d}\ln y_2}{\mathrm{d}\ln C_2} = 1 + 2A_2M_2c_2 + \dots .$$

---

* It is advisable to perform the classical diffusion experiment across a phase boundary consisting of a polyelectrolyte solution in dialysis equilibrium with the solution of the simple salt.

Deviations from ideal behavior are expressed in terms of either the activity coefficient y_2 or the second virial coefficient A_2. At vanishing concentration C_2

$$D_2^0 = RT/f_P. \tag{18}$$

If the frictional coefficient f_P is identical for diffusion and sedimentation it may be eliminated between eqs (18) and (10) to yield an equation analogous to the Svedberg equation for two component systems.

In the salt-free case $C_3 = 0$ and eq. (16) reduces to

$$\frac{1}{D_2}\frac{d\mu_2}{d\ln C_2} = f_P + iZf_X, \tag{19}$$

which is a well known result for salt-free polyelectrolyte solutions and may be considered in conjunction with eq. (11) for the sedimentation coefficient; at vanishing concentration C_2 the Svedberg equation is recovered.

3. Sedimentation and diffusion coefficients of polyelectrolytes by the methods of irreversible thermodynamics

We first consider the system PX_Z, XY in the terms of the three flows J_P^1, J_Y^1 and J_X^1, taken with respect to the solvent, component 1, in terms of the gradients of the total chemical potentials (including gravitational and electrical terms) of the ionic species, $\bar\mu_P$, $\bar\mu_Y$ and $\bar\mu_X$; it is convenient to use flows, forces, concentrations and electrical charges *per mole*

$$\begin{aligned} -J_P^1 &= L_{PP}\,\mathrm{grad}\,\bar\mu_P + L_{PY}\,\mathrm{grad}\,\bar\mu_Y + L_{PX}\,\mathrm{grad}\,\bar\mu_X,\\ -J_Y^1 &= L_{YP}\,\mathrm{grad}\,\bar\mu_P + L_{YY}\,\mathrm{grad}\,\bar\mu_Y + L_{YX}\,\mathrm{grad}\,\bar\mu_X,\\ -J_X^1 &= L_{XP}\,\mathrm{grad}\,\bar\mu_P + L_{XY}\,\mathrm{grad}\,\bar\mu_Y + L_{XX}\,\mathrm{grad}\,\bar\mu_X. \end{aligned} \tag{20}$$

The dissipation function Φ which is equal to the entropy production times T is [17,18]

$$\Phi = -J_P^1\,\mathrm{grad}\,\bar\mu_P - J_Y^1\,\mathrm{grad}\,\bar\mu_Y - J_X^1\,\mathrm{grad}\,\bar\mu_X. \tag{21}$$

With these definitions the system is well determined, the phenomenological coefficients satisfy the Onsager reciprocal relations ($L_{ik} = L_{ki}$) and we therefore have six independent coefficients. Fluxes with respect to the solvent are not usually measurable and it is therefore preferable to transform these fluxes to a more convenient reference frame. We choose the frame fixed with respect to the local center of volume which [19] moves with a velocity v^V

$$v^V = \sum_k v_k \bar V_k C_k, \tag{22}$$

where v_k is the velocity of species k with respect to the frame fixed to the cell, the $\bar V_k$ are partial molal volumes, and the summation is over all species. The flows J_k^V with respect to the center of volume frame are related to the flows J_k (relative to the cell) by

$$J_k^V = C_k(v_k - v_k^V) = J_k - C_k v_k^V, \tag{23}$$

and, with use of eq. (22)

$$J_k^V = J_k - C_k \sum_i \bar V_i J_i. \tag{24}$$

Substitution of $J_k^1 = C_k(v_k - v_1)$ into eq. (24) demonstrates that we also have

$$J_k^V = J_k^1 - C_k \sum_i \bar V_i J_i^1, \tag{25}$$

where the summation now does not include the solvent ($J_i^1 = 0$); the last equation will be used in our transformation of flows in this section.

We now transform eqs. (20) to three alternate flows: J_Y^V, J_P^V and total electrical current J_{el} (which we will eventually set equal to zero); subsequently we will also assume the mean volume velocity to be zero for low concentrations of solutes or if the partial molar volumes do not depend on concentrations [20,21] and the total volume flow is zero. Under these conditions J_k^V will be approximated by J_k.

The matrix M transforming the old flows of eq. (20) to the new flows is (cf. eq. (25) for the flows of matter)

$$M = \begin{pmatrix} 1 - C_P\bar V_P & -C_P\bar V_Y & -C_P\bar V_X \\ -C_Y\bar V_P & 1 - C_Y\bar V_Y & -C_Y\bar V_X \\ -\nu\mathcal{F} & -\mathcal{F} & \mathcal{F} \end{pmatrix}, \tag{26}$$

where ν is the number of charges per macromolecule, $\mathcal{F}$ the Faraday electrochemical equivalents, and the new flows are

$$\begin{aligned} J_P^V &= (1 - C_P\bar V_P)J_P^1 - C_P\bar V_Y J_Y^1 - C_P\bar V_X J_X^1,\\ J_Y^V &= -C_Y\bar V_P J_P^1 + (1 - C_Y\bar V_Y)J_Y^1 - C_Y\bar V_X J_X^1,\\ J_{el} &= \mathcal{F}(-\nu J_P - J_Y + J_X). \end{aligned} \tag{27}$$

To obtain the new forces X_k associated with these fluxes we take into account the invariance of the dissipation function when expressed in either the new forces-fluxes system or in the original system. The general relation between forces satisfying this requirement is given by [18]

$$X_{\text{new}} = -(\tilde{M}^{-1})\, X_{\text{old}}, \tag{28}$$

where $\tilde{M}^{-1}$ is the transpose of the inverse of the matrix M defined in eq. (26). The new forces X_{P}, X_{Y} and X_{el} associated to J_{P}^V, J_{X}^V and J_{el} are written explicitly as functions of the grad $\bar{\mu}_k$'s

$$X_2 \equiv X_{\text{P}} = -\frac{1}{C_1\bar{V}_1}\,[(1-C_3\bar{V}_3)\,\text{grad}\,\bar{\mu}_2 + C_3\bar{V}_2\,\text{grad}\,\bar{\mu}_3],$$

$$X_3 \equiv X_{\text{Y}} = -\frac{1}{C_1\bar{V}_1}\,[(C_2\bar{V}_3\,\text{grad}\,\bar{\mu}_2 + (1-C_2\bar{V}_2)\,\text{grad}\,\bar{\mu}_3],$$

$$X_{\text{el}} = -\frac{\text{grad}\,\bar{\mu}_{\text{X}}}{\mathcal{F}} - \frac{\bar{V}_{\text{X}}}{\mathcal{F}C_1\bar{V}_1}\,(C_2\,\text{grad}\,\bar{\mu}_2 + C_3\,\text{grad}\,\bar{\mu}_3). \tag{29}$$

Since grad $\bar{\mu}_2$ and grad $\bar{\mu}_3$ correspond to the total chemical potential of the electroneutral salt (no electrical forces are involved in grad $\bar{\mu}_3$ and grad $\bar{\mu}_2$) and only quantities corresponding to electroneutral components appear in X_{P} and X_{Y}, we may identify

$$J_2^V \equiv J_{\text{P}}^V, \qquad X_2 \equiv X_{\text{P}},$$

and

$$J_3^V \equiv J_{\text{Y}}^V, \qquad X_3 \equiv X_{\text{Y}}.$$

Eqs. (29) for the X_k are identical with the equations for the set of forces χ, ψ and ϕ of Mijnlieff and Overbeek [6] and Mijnlieff [7]. In their procedure, cf. Mijnlieff [8] eq. (3) for instance, the total potential $\bar{\mu}$ is split into terms depending on concentration, pressure and ultracentrifugal and electrostatic fields, respectively.

For our further deliberations we approximate the flows J_k^V by the experimental flows J_k with respect to a frame of reference fixed to the cell and also note that, at low concentrations of solutes $(1-C_2\bar{V}_2)$, $(1-C_3\bar{V}_3)$ and $C_1\bar{V}_1$ are close to unity and the terms in C_2 and C_3 vanish. Under these conditions the forces of eqs. (29) assume the simple forms

$$X_2 = -\text{grad}\,\bar{\mu}_2, \quad X_3 = -\text{grad}\,\bar{\mu}_3,$$

$$X_{\text{el}} = -\text{grad}\,\bar{\mu}_{\text{X}}/\mathcal{F}, \tag{30}$$

which is the form used by Varoqui and Schmitt [9]. Caution has to be exercised with respect to this simplification because, whereas the case of vanishing concentration C_2 is often realized, the concentration C_3 of the added simple salt may be quite high in a given experimental situation.

With the newly defined flows and forces we may write the phenomenological equations

$$J_2 = L_{22}X_2 + L_{23}X_3 + L_{2\text{e}}X_{\text{el}},$$

$$J_3 = L_{32}X_2 + L_{33}X_3 + L_{3\text{e}}X_{\text{el}}.$$

$$J_{\text{el}} = L_{\text{e}2}X_2 + L_{\text{e}3}X_3 + L_{\text{ee}}X_{\text{el}}. \tag{31}$$

We next apply the condition of zero current $(J_{\text{el}}=0)$ and substitute the electrical force X_{el} in J_2 and J_3 to obtain the final system of phenomenological euqations for the transport of electroneutral components

$$J_2 = \mathcal{L}_{22}X_2 + \mathcal{L}_{23}X_3, \qquad J_3 = \mathcal{L}_{32}X_2 + \mathcal{L}_{33}X_3, \tag{32}$$

where the coefficients

$$\mathcal{L}_{22} = L_{22} - L_{\text{e}2}^2/L_{\text{ee}}, \qquad \mathcal{L}_{23} = L_{23} - L_{\text{e}2}L_{\text{e}3}/L_{33}. \tag{33}$$

Both the L_{ij} and the $\mathcal{L}_{ij}$ satisfy the Onsager relations and the system at zero current is therefore defined by three phenomenological coefficients only. A fundamental discussion of flow processes of ionic species and neutral components in electrolyte solutions in the ultracentrifuge has been given by Fujita [19].

Varoqui and Schmitt relate the phenomenological coefficients from eqs. (32) and (33) to frictional coefficients by the formulation of Spiegler [22]. In a quasi-stationary state the applied forces acting on a particle at constant speed are opposed by all frictional forces exerted on the particle by the surrounding medium, the total force acting on the particle being equal to zero. For particle P, for instance

$$\text{grad}\,\bar{\mu}_{\text{P}} = F_{\text{PX}} + F_{\text{PY}} + F_{\text{P1}}. \tag{34}$$

The frictional force F_{ij} refers to the interaction of one mole of particle i with all particles j in its vicinity. From this definition one derives the relationship

$$C_iF_{ij} = C_jF_{ji}. \tag{35}$$

The frictional force is proportional to the relative velocities of particles i and j

$$F_{ij} = -f_{ij}(v_i - v_j). \tag{36}$$

f_{ij} is the interparticle friction coefficient and v_i and v_j are the mean time averaged velocities of i and j; if the flows are referred relative to the solvent ($v_1 = 0$), the following equations are obtained

$$\begin{aligned} -\operatorname{grad}\bar{\mu}_P &= f_{PX}(v_P - v_X) + f_{PY}(v_P - v_Y) + f_{P1}v_P, \\ -\operatorname{grad}\bar{\mu}_Y &= f_{YX}(v_Y - v_X) + f_{YP}(v_Y - v_P) + f_{Y1}v_Y, \\ -\operatorname{grad}\bar{\mu}_X &= f_{XY}(v_X - v_Y) + f_{XP}(v_X - v_P) + f_{X1}v_X. \end{aligned} \tag{37}$$

Eqs. (37) are solved for the velocities v_i, these are multiplied by the concentrations C_i to give the macroscopic fluxes J_i and a set of equations similar to eqs. (20) is obtained. By identification, Varoqui and Schmitt obtain the relationship between the phenomenological and the friction coefficients. They derive two limiting cases. For excess salt and vanishing polymer concentration

$$C_3 \gg C_2,$$

$$\mathcal{L}_{22} = \frac{C_2}{f_{P1} + (C_3/C_2)(f_{YP} + f_{XP})} = \frac{C_2}{f_{P1} + f_{PY} + f_{PX}},$$

$$\mathcal{L}_{23} = \frac{C_3(f_{YP} + f_{XP}) - \nu C_2 f_{X1}}{(f_{Y1} + f_{X1})[f_{P1} + (C_3/C_2)(f_{YP} + f_{XP})]}$$

$$= \frac{C_2(f_{PY} + f_{PX} - \nu f_{X1})}{(f_{Y1} + f_{X1})(f_{P1} + f_{PY} + f_{PX})}. \tag{38}$$

The second form of the phenomenological coefficients is obtained by the use of eqs. (35) and (36).

For the case of zero salt

$$C_3 = 0, \quad \mathcal{L}_2 = \frac{C_2}{f_{P1} + \nu f_{X1}}, \qquad \mathcal{L}_{23} = 0. \tag{39}$$

For sedimentation in a centrifugal field we write [23] s_2 in terms of the Onsager coefficients of eq. (38) and obtain

$$s_2^0 = \frac{\bar{M}_2 - \bar{M}_3\left(\nu - \dfrac{f_{PX} + f_{PY}}{f_{X1}}\right)\left(1 + \dfrac{f_{Y1}}{f_{X1}}\right)^{-1}}{f_{P1} + f_{PY} + f_{PX}} \tag{40}$$

which is identical with the limiting form of eq. (8) if the interparticle frictions f_{PX} and f_{PY} (not considered in the previous derivation) are set equal to zero and only the particle-solvent frictions f_{P1}, f_{Y1} and f_{X1} are considered ($\nu = iZ$).

For the case of zero salt we find

$$C_3 = 0, \qquad s_2 = \bar{M}_2/(f_{P1} + \nu f_{X1}), \tag{41}$$

which is identical with eq. (11). A similar correspondence with the previously derived equations applies for the expressions for the diffusion coefficients.

Is it possible to evaluate the interparticle frictions f_{PX} and f_{PY} and to evaluate their relative importance with respect to f_{P1}? In principle they are measurable by way of the measurement of self-diffusion coefficients of small ions with the help of radioactive tracer ions. Varoqui and Schmitt [9], for instance, obtain

$$D_X' = RT/(f_{XY} + f_{X1} + f_{XX}),$$

$$D_X = RT/(f_{XP} + f_{XY} + f_{X1} + f_{XX}), \tag{42}$$

where D_X' and D_X are the self-diffusion coefficients at a given salt concentration in the absence and in the presence of the polyelectrolyte component, respectively. It is assumed that the values of f_{XY}, f_{X1} and f_{XX} are unaffected by the addition of the polyelectrolyte. A similar expression is obtained for D_Y' and D_Y. On the basis of these relations we have

$$f_{XP} = (RT/D_X')(D_X'/D_X - 1),$$

$$f_{YP} = (RT/D_Y')(D_Y'/D_Y - 1). \tag{43}$$

In the absence of salt the counterion mobility may be determined with accuracy even at low polyelectrolyte concentrations. In the presence of excess salt D_Y'/D_Y and D_X'/D_X become close to unity as C_2 decreases and therefore the limiting coefficients f_{XP} and f_{YP} cannot be determined with any accuracy and no such results are available.

Acknowledgement

Supported in part by a grant from the Stiftung Volkswagenwerk.

References

[1] K.O. Pedersen, J. Phys. Chem. 62 (1958) 1282.
[2] R.A. Robinson and R.H. Stokes, Electrolyte Solutions (Butterworths, London, 1959).

[3] E.F. Casassa and H. Eisenberg, Adv.Protein Chem. 19 (1964) 287.

[4] Z. Alexandrowicz and E. Daniel, Biopolymers 1 (1963) 447.

[5] Z. Alexandrowicz and E. Daniel, Biopolymers 6 (1968) 1500.

[6] P.F. Mijnlieff and J.Th.G. Overbeek, Koninkl. Neder. Akad. Wetenschap. Proc. Ser. B65 (1962) 221.

[7] P.F. Mijnlieff, Koninkl. Neder. Akad. Wetenschap. Proc. Ser. B65 (1962) 334, 343, 355, 364.

[8] P.F. Mijnlieff, in: Ultracentrifugal Analysis, ed. J.W. Williams (Academic Press, New York, 1963) p. 81.

[9] R. Varoqui and A. Schmitt, Biopolymers 11 (1972) 1119.

[10] M. Nagasawa and Y. Eguchi, J. Phys. Chem. 71 (1967) 880.

[11] T. Svedberg and K.O. Pedersen, The Ultracentrifuge (Oxford University Press, London, 1940).

[12] R. Ziccardi and V. Schumaker, Biopolymers 10 (1971) 1701.

[13] H. Eisenberg, J. Chem. Phys. 36 (1962) 1837.

[14] G. Cohen and H. Eisenberg, Biopolymers 6 (1968) 1077.

[15] H.E. Auer and Z. Alexandrowicz, Biopolymers 8 (1969) 1.

[16] M. Nagasawa and H. Fujita, J. Amer. Chem. Soc. 86 (1964) 3005.

[17] A. Katchalsky and P.F. Curran, Nonequilibrium Thermodynamics in Biophysics (Harvard University Press, Cambridge, Massachusetts, 1965).

[18] D.D. Fitts, Nonequilibrium Thermodynamics (McGraw–Hill, New York, 1962).

[19] H. Fujita, Foundations of Ultracentrifugal Analysis (John Wiley and Sons, 1975).

[20] S.R. de Groot, P. Mazur and J.Th.G. Overbeek, J. Chem. Phys. 20 (1952) 1825.

[21] J.G. Kirkwood, R.L. Baldwin, P.J. Dunlop, L.J. Gosting and G. Kegeles, J. Chem. Phys. 33 (1960) 1505.

[22] K.S. Spiegler, Trans. Far. Soc. 54 (1958) 1408.

[23] H. Eisenberg, Biological Macromolecules and Polyelectrolytes in Solution (Oxford University Press, London, 1976).