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Solvent Contributions to Small-Angle X-Ray Scattering from Macromolecular Solutions

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ABSTRACT: Theoretical methods are developed which quantitatively account for solvent contributions to the inner portion of small-angle X-ray patterns obtained from macromolecular solutions. The usual assumption of a homogeneous solvent is shown to be inadequate. The presence of solvent is shown to significantly affect observed values of the radius of gyration for certain cases. The effect is strongly dependent on the macromolecular size, relative mean electron density of the macromolecule and solvent, and the degree of inner solvation. Polyelectrolyte–counterion systems are considered in detail. An interpretation of recent data on the binding of cations to phenylalanine specific *t*-RNA is included.

I. Introduction

Several attempts to include the effects of solvent into the formalism of small-angle X-ray scattering from macromolecular solutions have been made previously. Stuhrmann and Kirste[1,2] suggest a method for eliminating solvent effects which arise because of intramolecular fluctuations in electron density. Their method involves studying the macromolecule successively in several solvents which differ in mean electron density. This technique was employed to study myoglobin in glycerin–water, glucose–water, and saccharose–water mixed solvents. One difficulty with this approach lies in finding several solvents which differ suitably in mean electron density while at the same time leave the macromolecular structure unaltered. Hyman and Vaughan[3] have described the effect of solvent for sphere-like molecules. Luzzati and coworkers[4] have developed theoretical methods which apply to polyelectrolyte systems. Their theory involves the parameterization of fundamental scattering relations. Each of these methods have merit, but they suffer from a common defect: each assumes a homogeneous solvent. This assumption appears reasonable since internal solvent structure is short ranged and will not be observed at small angles. We have no quarrel with this argument. However, there are other problems which arise from the homogeneous solvent model which are not related to internal solvent structure. In a loose sense the problems arise from a consideration of where the macromolecular domain ends and the domain of the solvent begins. We illustrate the problem below.

In the small-angle region the exact electron density, $\rho(\mathbf{r})$, of the scattering system is not observed. Instead a locally averaged electron density is perceived. This local average is not well defined, and may vary over the volume occupied by one or several atoms. If, after this averaging, inhomogeneities of macromolecular scope persist, small-angle scattering is observed. To illustrate the difficulties with the assumption of a homogeneous solvent, consider a hypothetical one-component, two-phase, monatomic system in which solid-phase fragments of macromolecular dimensions are dispersed in a liquid phase. We assume that the solid phase has frozen out with a mean electron density identical with that of the liquid. Would this system exhibit small-angle scattering? If we simply assume a homogeneous solvent we are inclined to think not, because of the equal electron densities of the dispersed and liquid phases. However, this is not the case. One would observe small-angle scattering, however small, due to a discontinuity of the locally averaged electron density at the phase boundaries, represented by a depletion of electron density at the interface. The situation is illustrated in Figure 1 for a one-dimensional case. The atoms of a solid phase fragment are depicted as points. Although at small angles atoms scatter as points, they do not interact physically with each other as points and consequently their centers are restricted to minimum distances of approach. The atomic centers in the liquid phase are as-

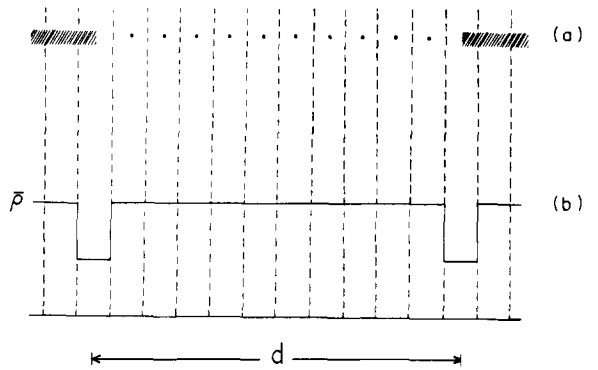

Figure 1. One-dimensional analog of a monatomic solid fragment frozen out of a liquid. (a) Atomic centers of the solid are depicted as points, which are localized because of interatomic interactions. Atomic centers of the liquid are depicted by the shaded regions. Other than being subject to a distance of minimum approach from the solid they are assumed randomly distributed. (b) Locally averaged electron density of the system. Local averaging is performed between adjacent dashed lines. The locally averaged electron density exhibits two discontinuities separated by a distance of macromolecular dimensions, d. Although the mean electron densities of the solid and liquid phases are equal the discontinuities lead to small angle scattering.

sumed randomly distributed in the space not excluded to them by the solid. They are depicted by the shaded region adjacent to the solid phase. The locally averaged electron density is shown immediately below in the figure, with local averaging being performed within the space between adjacent vertical (dashed) lines. Although the particular method of local averaging is somewhat arbitrary, all methods produce the same result, a depleted zone of electron density at either end of the one-dimensional fragment and representing an inhomogeneity of macromolecular dimensions, which consequently leads to small-angle scattering.

It is clear that the assumption of a homogeneous solvent poses problems which are not immediately apparent. We analyze the scattering system below and hopefully resolve these problems.

II. General Theoretical Considerations

We are interested in the intensity of coherently scattered X-rays in the small angle region by all components of a solution. The usual experimental procedure is to observe the scattering curve for a sequence of solutions which vary in solute concentration. Derived parameters are then extrapolated to infinite dilution. In this way the effect of inter-solute particle interference is minimized. Accordingly, we will attempt to describe the contributions to the observed intensity for solutions which are extremely dilute. For this purpose it is convenient to consider a single polymeric solute molecule in a large amount of solvent.

The intensity of coherently scattered X-rays (in electron units) is given by

$$i(\mathbf{h}) = \int\int \rho(\mathbf{r})\rho(\mathbf{r}')e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' \qquad (1)$$

where $\mathbf{h}$ is the scattering vector with magnitude equal to $4\pi(\sin\theta)/\lambda$, 2θ is the scattering angle, and λ is the wavelength of X-rays. The quantity $\rho(\mathbf{r})$ is the electron density at the point defined by the vector $\mathbf{r}$. The integrations are over the irradiated volume.

Let

$$\rho(\mathbf{u}) = \rho_m(\mathbf{u}) + \rho_s(\mathbf{u}) \qquad (2)$$

where $\rho_m(\mathbf{u}) = 0$ when $\mathbf{u}$ defines a point in the solvent and $\rho_s(\mathbf{u}) = 0$ when $\mathbf{u}$ defines a point in the solute. Substitution of (2) into (1) yields a four-term expression which resolves the intensity into identifiable contributions.

$$i(\mathbf{h}) = \int_m\int_m \rho_m(\mathbf{r})\rho_m(\mathbf{r}')e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' + \int_s\int_m \rho_s(\mathbf{r})\rho_m(\mathbf{r}')e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' + \int_m\int_s \rho_m(\mathbf{r})\rho_s(\mathbf{r}')e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' + \int_s\int_s \rho_s(\mathbf{r})\rho_s(\mathbf{r}')e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' \qquad (3)$$

in which the subscripts "s" and "m" indicate integration over those parts of the irradiated volume occupied by the solvent and macromolecule, respectively. The first term on the right side of (3) describes the scattering due to the solute molecule. Its properties are well known. The contributions due to solvent appear in the remaining three terms. To a large extent, the balance of this paper deals with an examination of these last three terms.

The second and third terms on the right side of (3) describe the scattering due to electron pairs, each of which is split between the solvent and the macromolecule. In any single observation, all the allowed positions of the solvent molecules (relative to the macromolecule) will contribute to the intensity. We assume the solvent molecules have random orientation (but not position). In aqueous solution the electron distribution within a solvent molecule is nearly spherically symmetric so that this assumption is of little consequence. Note that this assumption does not rule out internal solvent structure since the ordering of solvent molecule centers about each other is still allowed. In order to perform the desired averaging, we need to know how the solvent molecule centers are ordered with respect to the macromolecule. We choose an origin at the electronic center of mass of the macromolecule and inquire about the distribution of solvent molecule centers about this origin. In general, this may be a complicated function depending not only on the shape of the macromolecule but also on the gradient of solvent density encountered as one moves out from the macromolecule into the bulk solvent. At the least we expect a zero density of solvent molecule centers in a region somewhat larger than that occupied by the electrons of the macromolecule, e.g., say that region bounded by the "surface" of the macromolecule plus a shell of thickness about equal to a van der Waals radius of solvent. The simplest assumption that we can make is that outside this region the density of solvent centers is uniform and equal to that found in bulk solvent. This is certainly the essential feature of the distribution. Consequently we ignore any buildup or depletion of solvent centers that may exist near the macromolecular surface. This assumption is discussed further in section V.

Under these assumptions we may average over solvent-center positions and thus simplify the second and third terms of (3). Consider for example the second term which we write as a product: $\int_s\rho_s(\mathbf{r})e^{i\mathbf{h}\cdot\mathbf{r}}\mathrm{d}V\int_m\rho_m(\mathbf{r})e^{-i\mathbf{h}\cdot\mathbf{r}}\mathrm{d}V$. From the first integral we separate out the spherically averaged solvent-molecule structure factor $\bar{F}_s$. At small angles $\bar{F}_s$ may be taken as equal to the number of electrons per solvent molecule, Z_s. (In the Appendix we describe an extension to larger angles for which $\bar{F}_s \neq Z_s$.) The second integral above represents the amplitude scattered by the macromolecule. Aside from the fact that the macromolecule excludes solvent centers from a hole, the detailed structure of the macromolecule is independent of the distribution of solvent centers. Consequently, we need only

average the first integral above over solvent center positions with the result

$$\left\langle \int_s \rho_s(\mathbf{r}) e^{i\mathbf{h}\cdot\mathbf{r}} dV \right\rangle = N_s \bar{F}_s \langle e^{i\mathbf{h}\cdot\mathbf{r}} \rangle = N_s Z_s \langle e^{i\mathbf{h}\cdot\mathbf{r}} \rangle \quad (4)$$

where N_s is the number of irradiated solvent molecules. The brackets about the quantity $e^{i\mathbf{h}\cdot\mathbf{r}}$ indicate averaging over the allowed positions of $\mathbf{r}$, the vector from the origin to the center of a solvent molecule. This quantity, according to the assumption made above of a random distribution of solvent centers outside the hole, is given by

$$\langle e^{i\mathbf{h}\cdot\mathbf{r}} \rangle = \frac{1}{V_s} \int_{V_s} e^{i\mathbf{h}\cdot\mathbf{r}} dV \quad (5)$$

in which the integration is over the volume, V_s, available to the *centers* of solvent molecules. Defining the mean solvent electron density, $\bar{\rho}_s$, as $N_s Z_s / V_s$ and combining (4) and (5) we write

$$\left\langle \int_s \rho_s(\mathbf{r}) e^{i\mathbf{h}\cdot\mathbf{r}} dV \right\rangle = \bar{\rho}_s \int_{V_s} e^{i\mathbf{h}\cdot\mathbf{r}} dV \quad (6)$$

The third term on the right side of (3) yields to a similar analysis.

We label the volume of the hole excluded to solvent centers V_H. The quantity $(V_H + V_S)$ constitutes the entire irradiated volume. We may then, in view of the above result, write for the second and third terms of (3) respectively

$$\int_s \int_m \rho_s(\mathbf{r}) \rho_m(\mathbf{r}') e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')} dV dV' = \bar{\rho}_s \int_{V_s} \int_{V_H} \rho_m(\mathbf{r}') e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')} dV dV' \quad (7)$$

and

$$\int_m \int_s \rho_m(\mathbf{r}) \rho_s(\mathbf{r}') e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')} dV dV' = \bar{\rho}_s \int_{V_H} \int_{V_s} \rho_m(\mathbf{r}) e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')} dV dV' \quad (8)$$

The last term on the right side of (3) contains contributions from electrons belonging entirely to the solvent. All of the solvent-structure information which is contained in the scattering pattern appears in this term. We denote this last term which describes solvent–solvent scattering by i_{ss}. It corresponds to the intensity scattered by a system composed of solvent which contains a hole of macromolecular dimensions. There are two distinct contributions to i_{ss}. The first is due to the fact that the solvent now possesses long-range order by virtue of its exclusion from the relatively large hole. The second contribution is due to the internal structure characteristic of the solvent, i.e., the ordering of solvent molecules about each other. We examine below each of these contributions. For this purpose it is convenient to represent i_{ss} as a double sum, i.e.,

$$i_{ss}(\mathbf{h}) = Z_s^2 \sum_j \sum_k e^{i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)} \quad (9)$$

where $\mathbf{r}_j$ and $\mathbf{r}_k$ are vectors to the centers of the jth and kth solvent molecules, respectively. We break the sum (eq 9) into two parts: that for which $j = k$ and that for which $j \neq k$.

$$i_{ss}(\mathbf{h}) = Z_s^2 [N_s + N_s(N_s - 1)\langle e^{i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)} \rangle_{j \neq k}] \quad (10)$$

The brackets, $\langle\ \rangle$, in (10) indicate averaging over all allowed values of the vectors $\mathbf{r}_j$ and $\mathbf{r}_k$, since we have assumed that each pair of solvent molecules contributes identically.

Let

$$\langle e^{i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)} \rangle_{j \neq k} = \frac{1}{V_s^2} \times \int_V \int_V P_{SS}(\mathbf{r}_j, \mathbf{r}_k) P_{SH}(\mathbf{r}_j, \mathbf{r}_k) e^{i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)} dV_j dV_k \quad (11)$$

in which the integrations are over the entire irradiated volume. The quantity P_{SS} is a probability function that describes the solvent structure, and P_{SH} is a probability function that accounts for the fact that solvent molecules are excluded from the hole which defines V_H. For a sample of pure solvent, with volume V, $P_{SS} dV_j dV_k / V^2$ represents the probability that the vector $\mathbf{r}_j$ ends in the volume element dV_j while at the same time $\mathbf{r}_k$ ends in dv_k. The function P_{SH} vanishes when either $\mathbf{r}_j$ or $\mathbf{r}_k$ ends in the hole. It has unit value otherwise in accordance with previous assumptions.

The individual effects of the hole and the solvent structure can be separated as follows. We write

$$\langle e^{i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)} \rangle_{j \neq k} = J(\mathbf{h}) + K(\mathbf{h}) \quad (12)$$

where

$$J(\mathbf{h}) = \frac{1}{V_s^2} \int_V \int_V (P_{SS} - 1) P_{SH} e^{i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)} dV_j dV_k \quad (13)$$

$$K(\mathbf{h}) = \frac{1}{V_s^2} \int_V \int_V P_{SH} e^{i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)} dV_j dV_k \quad (14)$$

The function $J(\mathbf{h})$ may be simplified by asserting some general properties of the solvent structure. We assume that $P_{SS} = P_{SS}(|\mathbf{r}_j - \mathbf{r}_k|)$, and that P_{SS} approaches unity rapidly for $|\mathbf{r}_j - \mathbf{r}_k|$ greater than a few molecular dimensions. That is, P_{SS} is a spherically symmetric function of intermolecular separation and the solvent structure is relatively short range. Accordingly, the integrand in $J(\mathbf{h})$ vanishes for most values of $(\mathbf{r}_j - \mathbf{r}_k)$. As a consequence the effect of the hole probability function, P_{SH}, is negligible in $J(\mathbf{h})$. Only in the immediate neighborhood of the hole does P_{SH} influence the value of the integral in $J(\mathbf{h})$. Very few irradiated solvent molecules are involved, and these contribute negligibly. In this manner, the hole affects this integral in the same way as the constraint of a finite irradiated volume introduces negligible edge effects. Accordingly, we set $P_{SH} = 1$ in $J(\mathbf{h})$ and reduce the volume of integration to that available to solvent molecule centers, V_s, That is, $J(\mathbf{h})$ is given by

$$J(\mathbf{h}) = \frac{1}{V_s^2} \int_{V_s} \int_{V_s} (P_{SS} - 1) e^{i\mathbf{h}\cdot(\mathbf{r}_j - \mathbf{r}_k)} dV_j dV_k \quad (15)$$

Thus the term $J(\mathbf{h})$ contains contributions to i_{ss} due only to solvent structure. The term $K(\mathbf{h})$ includes only the effect of the hole.

Neither $J(\mathbf{h})$ nor $K(\mathbf{h})$ can be evaluated without a detailed knowledge of the functions P_{SS} and P_{SH}. Fortunately such evaluation is unnecessary. We will require, however, the functional dependence of J on V_s. Let $\mathbf{u} = \mathbf{r}_j - \mathbf{r}_k$. Noting that $P_{SS} = P_{SS}(u)$ rapidly approaches unity for large u we perform one of the integrations indicated in $J(\mathbf{h})$ and the other over the angular variables with the result

$$J(h) = \frac{4\pi}{h V_s} \int_0^\infty (P_{SS} - 1) u \sin(hu) du \quad (16)$$

That is $J(h)$ is inversely proportional to V_s.

At the outset we assumed, for convenience, one macro-

molecule in a large amount of solvent. Contributions to the intensity due to intermolecular interference are thus avoided, and the intensity $i(\mathbf{h})$ is normalized per solute molecule. The relative contributions of $J(h)$ and $K(\mathbf{h})$ need to be examined further since in any given sample each macromolecular hole contributes a term $K(\mathbf{h})$. The value of $J(h)$ is only minimally influenced by the number of solute molecules which cause small changes in V_s. Accordingly, we write $\langle e^{i\mathbf{h}\cdot(\mathbf{r}_j-\mathbf{r}_k)}\rangle$ more properly as $J(h) + N_H K(\mathbf{h})$, where N_H is the number of macromolecules (or holes) in the irradiated sample. The term $i_{ss}(\mathbf{h})$ is then correctly written as

$$i_{ss}(\mathbf{h}) = \frac{N_s}{N_H} Z_s^2[1 + (N_s - 1)(J + N_H K)] \quad (17)$$

The contribution of solvent structure to the intensity is contained entirely in the quantity J. This contribution, although very small, may be removed by subtracting from the sample scatter an appropriate function of the intensity scattered by a blank, i.e., a sample containing only solvent with the same irradiated volume and measured under the identical conditions as was the solution. Let I^0 be the total observed intensity scattered by such a blank containing N_s^0 solvent molecules. We define $J^0(h)$ as the quantity corresponding to $J(h)$ when the sample is the blank, i.e.,

$$J^0 = \frac{4\pi}{hV}\int_0^\infty (P_{SS} - 1)u \sin(hu)\mathrm{d}u \quad (18)$$

We define

$$I_{ss} = N_H i_{ss} \quad (19)$$

and assert the following relations.

$$I^0 = Z_s^2 N_s^0[1 + (N_s^0 - 1)J^0] \quad (20)$$

$$V = V_s + N_H V_H \quad (21)$$

$$J = (V/V_s)J^0 \quad (22)$$

$$N_s = N_s^0(1 - N_H V_H/V) \quad (23)$$

Equation 20 follows from arguments similar to those leading to (17); (21) simply accounts for the extra holes in the partition of V; (22) follows from (16) and (18); (23) follows from (21) assuming $N_s \propto V_s$ and $N_s^0 \propto V$.

Replacing $(N_s - 1)$ and $(N_s^0 - 1)$ with N_s and N_s^0, respectively, we find from eq 19, 20, 21, 22, and 23

$$(I_{ss} - (N_s/N_s^0)I^0)/N_H = Z_s^2 N_s^2 K \quad (24)$$

and

$$(I_{ss} - I^0)/N_H = Z_s^2 N_s K - \frac{V_H}{V} I^0 \simeq Z_s^2 N_s K \quad (25)$$

Consider now the quantity $K(\mathbf{h})$

$$K(\mathbf{h}) = \frac{1}{V_s^2}\int_V\int_V P_{SH} e^{i\mathbf{h}\cdot(\mathbf{r}_j-\mathbf{r}_k)}\mathrm{d}V_j\mathrm{d}V_k \quad (26)$$

Since P_{SH} vanishes inside the hole but has unit value otherwise, we write

$$K(\mathbf{h}) = \frac{1}{V_s^2}\int_{V_s}\int_{V_s} e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' \quad (27)$$

We combine (25) and (27) and note that the mean solvent electron density, $\bar{\rho}_s$, is given by $N_s Z_s/V_s$, with the result

$$(I_{ss} - I^0)/N_H = \bar{\rho}_s^2 \int_{V_s}\int_{V_s} e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' \quad (28)$$

Let $\Delta i(\mathbf{h})$ be the difference (per macromolecule) between the intensities scattered by the solution and blank, i.e.,

$$\Delta i(\mathbf{h}) = i(\mathbf{h}) - I^0(\mathbf{h})/N_H \quad (29)$$

We combine eq 3, 7, 8, 19, 28, and 29, noting the equivalence of $\int_m\int_m \rho_m(\mathbf{r})\rho_m(\mathbf{r}')e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V'$ and $\int_{V_H}\int_{V_H} \rho_m(\mathbf{r})\rho_m(\mathbf{r}')e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V'$ with the result

$$\Delta i(\mathbf{h}) = \Big[\int_{V_H}\int_{V_H} \rho_m(\mathbf{r})\rho_m(\mathbf{r}') + \bar{\rho}_s\int_{V_H}\int_{V_s} \rho_m(\mathbf{r}) + \bar{\rho}_s\int_{V_s}\int_{V_H} \rho_m(\mathbf{r}') + \bar{\rho}_s^2\int_{V_s}\int_{V_s}\Big] e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' \quad (30)$$

The expression 30 is equivalent to the intensity (per macromolecule) that would be scattered by a system of macromolecules that occupy holes somewhat larger than the macromolecules and which are imbedded in a homogeneous solvent of electron density $\bar{\rho}_s$. The dimensions of the holes may be obtained by extending the macromolecular dimensions by a quantity of the order of a van der Waals radius of solvent, the extension being made at the macromolecule–solvent interface. Thus, the usual assumption of a homogeneous solvent is almost justified. It becomes correct when the hole sizes are extended in the manner indicated. The consequences of this seemingly small difference are examined in section III.

The integrals in (30) over V_s can be transformed into integrals over V_H by noting that at all accessible angles sample shape scattering is not observed, i.e.,

$$\int_V e^{i\mathbf{h}\cdot\mathbf{r}}\mathrm{d}V = \int_{V_H} e^{i\mathbf{h}\cdot\mathbf{r}}\mathrm{d}V + \int_{V_s} e^{i\mathbf{h}\cdot\mathbf{r}}V = 0 \quad (31)$$

or

$$\int_{V_s} e^{i\mathbf{h}\cdot\mathbf{r}}\mathrm{d}V = -\int_{V_H} e^{i\mathbf{h}\cdot\mathbf{r}}\mathrm{d}V \quad (32)$$

By (30) and (32) we have

$$\Delta i(\mathbf{h}) = \int_{V_H}\int_{V_H} \{\rho_m(\mathbf{r})\rho_m(\mathbf{r}') - \bar{\rho}_s[\rho_m(\mathbf{r}) + \rho_m(\mathbf{r}')] + \bar{\rho}_s^2\} e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\mathrm{d}V\mathrm{d}V' \quad (33)$$

Let

$$F(\mathbf{h}) = \int_{V_H} \rho_m(\mathbf{r})e^{i\mathbf{h}\cdot\mathbf{r}}\mathrm{d}V \quad (34)$$

and

$$\Phi(\mathbf{h}) = \int_{V_H} e^{i\mathbf{h}\cdot\mathbf{r}}\mathrm{d}V \quad (35)$$

The quantity $F(\mathbf{h})$ is the structure factor of the macromolecule; $\Phi(\mathbf{h})$ is the amplitude scattered by a homogeneous object with uniform electron density whose dimensions are those of a hole. Combination of eq 33, 34, and 35 yields

$$\Delta i(\mathbf{h}) = FF^* - \bar{\rho}_s(F\Phi^* + F^*\Phi) + \bar{\rho}_s^2\Phi\Phi^* \quad (36)$$

Since the macromolecules assume random orientation with respect to the X-ray beam, we average over all macromolecular orientations, i.e.,

$$\Delta i(h) = \langle FF^*\rangle - \bar{\rho}_s(\langle F\Phi^*\rangle + \langle F^*\Phi\rangle) + \bar{\rho}_s^2\langle\Phi\Phi^*\rangle \quad (37)$$

We now examine the quantities $\langle F\Phi^*\rangle$ and $\langle F^*\Phi\rangle$.

$$\langle F\Phi^*\rangle = \int_{V_H}\int_{V_H} \langle\rho_m(\mathbf{r})e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\rangle\mathrm{d}V\mathrm{d}V' \quad (38)$$

and

$$\langle F^*\Phi\rangle = \int_{V_H}\int_{V_H} \langle\rho_m(\mathbf{r})e^{-i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}\rangle\mathrm{d}V\mathrm{d}V' \quad (39)$$

Averaging over macromolecular orientations is equivalent

to averaging over orientations of **h**. Since $\rho_m(\mathbf{r})$ is independent of the scattering vector orientation,

$$\langle F\Phi^* \rangle = \int_{V_H} \int_{V_H} \rho_m(\mathbf{r}) \langle e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')} \rangle dV dV' \quad (40)$$

and

$$\langle F^*\Phi \rangle = \int_{V_H} \int_{V_H} \rho_m(\mathbf{r}) \langle e^{-i\mathbf{h}\cdot\mathbf{r}-\mathbf{r}'} \rangle dV dV' \quad (41)$$

the bracketed quantities in (40) and (41) are well known to be equal and given by $\sin(h|\mathbf{r}-\mathbf{r}'|)/(h|\mathbf{r}-\mathbf{r}'|)$. Consequently $\langle F\Phi^* \rangle = \langle F^*\Phi \rangle$ and from (36) we have

$$\Delta i(h) = \langle FF^* \rangle - 2\bar{\rho}_s \langle F\Phi^* \rangle + \bar{\rho}_s^2 \langle \Phi\Phi^* \rangle \quad (42)$$

The first term of (42) represents the intensity scattered by a dilute gas of macromolecules. The second term arises from interference between X-rays scattered by pairs of electrons, one electron of each pair belonging to the macromolecule and the other to the solvent. The last term is due to interference caused by electrons belonging entirely to the solvent. It corresponds to the scattering that would be observed from a homogeneous solvent into which holes have been placed.

III. The Effect of Solvent on the Determination of the Radius of Gyration

The contribution of the first term in (42) to the inner part of the scattering curve is well known from the work of Guinier.[5] Let us examine the remaining two terms for the inner regions of the scattering curve. Consider, first, the quantity $\langle F\Phi^* \rangle$. It is convenient to replace the integral in (34) with a sum of integrals over each of the atoms in the macromolecule. Since henceforth we are concerned with extremely small angles we replace atomic scattering factors with atomic numbers, Z_j, and write accordingly

$$F(\mathbf{h}) = \sum_j Z_j e^{i\mathbf{h}\cdot\mathbf{r}_j} \quad (43)$$

where $\mathbf{r}_j$ is a vector from the origin to the center of the jth atom. Thus, we may write

$$\langle F\Phi^* \rangle = \sum_j Z_j \int_{V_H} \langle e^{i\mathbf{h}\cdot(\mathbf{r}_j-\mathbf{r})} \rangle dV \quad (44)$$

or

$$\langle F\Phi^* \rangle = \sum_j Z_j \int_{V_H} \frac{\sin(h|\mathbf{r}_j-\mathbf{r}|)}{h|\mathbf{r}_j-\mathbf{r}|} dV \quad (45)$$

We expand the integrand in (45) in powers of h, and since only the inner part of the scattering curve is being considered here, we drop terms higher than quadratic. Accoringly, we write

$$\langle F\Phi^* \rangle = \sum_j Z_j \int_{V_H} \left[1 - \frac{h^2}{6}(\mathbf{r}^2 + \mathbf{r}_j{}^2 - 2\mathbf{r}\cdot\mathbf{r}_j)\right] dV \quad (46)$$

There are four terms in $\langle F\Phi^* \rangle$ as given by (46) which we examine in turn. The first term is equal to $V_H \Sigma_j Z_j$. We define a mean electron density inside a hole, $\bar{\rho}_m$, as $\Sigma_j Z_j / V_H$. The first term may then be written as $\bar{\rho}_m V_H{}^2$. In order to evaluate the remaining terms of $\langle F\Phi^* \rangle$ we note that although each of these terms is origin dependent, $\langle F\Phi^* \rangle$ is not. The quantity $\langle F\Phi^* \rangle$ depends only on differences between vectors within a hole. Consequently, the origin may be chosen for convenience. We have already located it at the electronic center of mass of the macromolecule. Let us examine the second term of $\langle F\Phi^* \rangle$ which is given by $-(\bar{\rho}_m V_H h^2/6) \int_{V_H} \mathbf{r}^2 dV$. The integral $\int_{V_H} \mathbf{r}^2 dV$ may be written as $V_H R_H{}^2$, where R_H is the radius of gyration of a homogeneous object with the dimensions of a hole. Thus, the second term of $\langle F\Phi^* \rangle$ is given by $-\bar{\rho}_m V_H{}^2 h^2 R_H{}^2/6$. The third term of $\langle F\Phi^* \rangle$ may be written in terms of the radius of gyration of the macromolecule, R. It is given by $-\bar{\rho}_m V_H{}^2 h^2 R^2/6$. The fourth term of $\langle F\Phi^* \rangle$ vanishes as a result of origin choice, since $\Sigma_j Z_j \mathbf{r}_j = 0$. Combining these results yields for $\langle F\Phi^* \rangle$

$$\langle F\Phi^* \rangle = \bar{\rho}_m V_H{}^2 \left[1 - \frac{h^2}{6}(R_H{}^2 + R^2)\right] \quad (47)$$

The quantity $\langle \Phi\Phi^* \rangle$ may be evaluated in a similar manner to yield

$$\langle \Phi\Phi^* \rangle = \int_{V_H} \int_{V_H} \langle e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')} \rangle dV dV' = \int_{V_H} \int_{V_H} \frac{\sin(h|\mathbf{r}-\mathbf{r}'|)}{h|\mathbf{r}-\mathbf{r}'|} dV dV' \quad (48)$$

Expansion of the integrand in (48) in powers of h yields for $\langle \Phi\Phi^* \rangle$ to the quadratic term

$$\langle \Phi\Phi^* \rangle = \int_{V_H} \int_{V_H} \left[1 - \frac{h^2}{6}(\mathbf{r}^2 + \mathbf{r}'^2 - 2\mathbf{r}\cdot\mathbf{r}')\right] dV dV' \quad (49)$$

The first term of $\langle \Phi\Phi^* \rangle$ as given by (49) is simply $V_H{}^2$. The second and third terms are each equal to $-V_H{}^2 h^2 R_H{}^2/6$. The fourth term is vanishingly small unless there is a concentration of relatively heavy (or light) atoms at one end of the macromolecule, for suppose a homogeneous object with the dimensions of a hole. If this object has a center of mass coincident with the origin, i.e., the electronic center of mass of the macromolecule, then $\int_{V_H} \mathbf{r} dV = 0$ and the fourth term vanishes. The essential coincidence of the origin and the center of mass of the homogeneous object is assured, barring an unusual concentration of electrons at one end of the macromolecule. Combining these results for each term of $\langle \Phi\Phi^* \rangle$ we find

$$\langle \Phi\Phi^* \rangle = V_H{}^2 \left(1 - \frac{h^2}{3} R_H{}^2\right) \quad (50)$$

The quantity $\langle FF^* \rangle$ is well known from the work of Guinier.[5] It may be written as

$$\langle FF^* \rangle = \bar{\rho}_m{}^2 V_H{}^2 \left(1 - \frac{h^2}{3} R^2\right) \quad (51)$$

Combination of eq 37, 47, 50, and 51 yields the following equivalent expressions valid for the inner part of the scattering curve.

$$\Delta i(h) = V_H{}^2 (\bar{\rho}_m - \bar{\rho}_s)^2 \left[1 - \frac{h^2}{3} \frac{(\bar{\rho}_m R^2 - \bar{\rho}_s R_H{}^2)}{(\bar{\rho}_m - \bar{\rho}_s)}\right] \quad (52a)$$

and

$$\Delta i(h) = V_H{}^2 (\bar{\rho}_m - \bar{\rho}_s)^2 \exp\left[-\frac{h^2}{3} \frac{(\bar{\rho}_m R^2 - \bar{\rho}_s R_H{}^2)}{(\bar{\rho}_m - \bar{\rho}_s)}\right] \quad (52b)$$

Examination of (52b) reveals that the customary plot of $\log \Delta i(h)$ vs. h^2 yields a limiting slope of $-R_0{}^2/3$, where

$$R_0{}^2 = \frac{\bar{\rho}_m R^2 - \bar{\rho}_s R_H{}^2}{\bar{\rho}_m - \bar{\rho}_s} \quad (53)$$

In general, the quantity R_0 obtained from the limiting slope does not equal the macromolecular radius of gyration, R. In some cases these two quantities may differ markedly. It is apparent from (53) that R_0 and R represent the same quantity when $\bar{\rho}_s = 0$, i.e., for a gas of macromolecules. This case, of course, is entirely academic. There are a few systems that have been studied for which $\bar{\rho}_m \gg \bar{\rho}_s$, such as the gold sol.[6] For such systems R_0 and R are virtually identical.

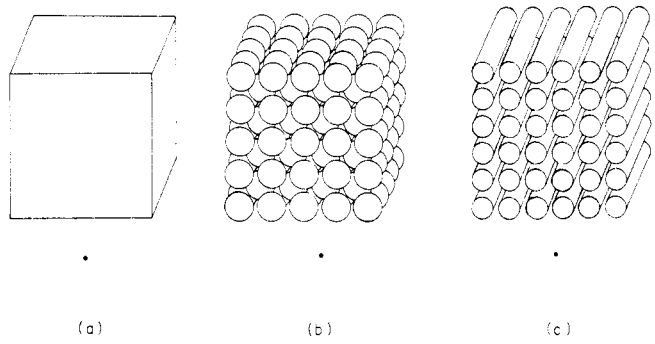

Figure 2. The effect of inner solvation on the determination of radii of gyration. Three objects are shown which are similar in size and overall shape. Each would lead to a different error in the observed radius of gyration if observed in the presence of solvent. The solvent molecule size relative to each object is indicated by a black dot under each object. In objects (b) and (c) solvent molecules fill the interstitial spaces. Errors in observed R are computed assuming a mean solvent electron density of 0.3 electron/Å^3 and an electron density of 0.4 electron/Å^3 within the homogeneous regions of each object. (a) Homogeneous cube: error = 11%. (b) Homogeneous spheres arranged on a body-centered cubic lattice: error = 1%. (c) Homogeneous parallel rods with centers on a simple square lattice: error = 3%.

However, for the common case encountered experimentally, $\bar{\rho}_m$ and $\bar{\rho}_s$ differ only by about 25%. Accordingly, a detailed scrutiny of factors involved in (53) is indicated.

The relationship between R and R_0 may be more easily understood by examining a rearrangement of (53), i.e.

$$(R_0/R)^2 = \left[\bar{\rho}_m - \left(\frac{R_H}{R}\right)^2 \bar{\rho}_s\right]/(\bar{\rho}_m - \bar{\rho}_s) \qquad (54)$$

from which it is seen that the deviation of R_H/R from unity determines the difference between R and R_0. Note that even if the macromolecule were homogeneous, $R_H/R \neq 1$. In this event both R and R_H would represent radii of gyration of homogeneous objects but of different sizes. It is of interest to quantitatively assess the effect of solvent on the limiting slope of the Guinier plot. For this purpose we will examine several models for the solute. These models are not intended to accurately represent a macromolecule, but instead serve to identify those features of a macromolecule which determine the extent to which R_0/R differs from unity. We suppose, first, that the macromolecule may be represented by a regular solid globular object with uniform electron density. For this case, the hole in the solvent is defined by the outer surface of a shell of thickness δ which surrounds the macromolecule. This model is depicted schematically in Figure 2a. The quantity, δ is of the order of a van der Waals radius of solvent and will be small compared to some representative linear dimension of the macromolecule, say l. Consequently, we may conveniently expand the quantity $(R_H/R)^2$ in powers of δ/l dropping quadratic and higher terms. We find $(R_H/R)^2 \simeq 1 + 2(\delta/l)$. If the object representative of the solute molecule has symmetry, the quantity, l, may be precisely defined; if not it is simply of the order of a linear dimension of the macromolecule. For example, if the object is an ellipsoid with semi-axes a, b, and c, l is given by $(a^2 + b^2 + c^2)/(a + b + c)$, or for an elliptic cylinder with semi-axes a and b, and height h, l is given by $(a^2 + b^2 + h^2/3)/(a + b + 2h/3)$. Typical values of l, encountered in small-angle experiments, are in the range of 20 to 100 Å. The van der Waals radius of water is about 1.4 Å giving the corresponding range of $(R_H/R)^2$ as 1.03 to 1.14. The effect on the limiting slope may be computed from (54). For this purpose we assume somewhat typical values for $\bar{\rho}_m$ and $\bar{\rho}_s$ of 0.4 and 0.3, respectively, with the result that R exceeds R_0 by 5 to 31%, the smaller objects yielding the larger differences. The magnitude of the discrepancy is unacceptable in view of the better agreement generally observed between radii of gyration determined experimentally and those calculated from known molecular structures, e.g., myoglobin,[7-9] haemoglobin,[10-12] and lysozyme.[13] (Krigbaum and Godwin[14] observed rather poor agreement for the case of chymotrypsinogen, but they convincingly demonstrated, from the small-angle scattering pattern, a significant conformational change in solution which accounts for the discrepancy.) The apparent difficulty presented by the usually good agreement between crystal and solution radii of gyration is resolved if one recognizes the inadequacy of representing a globular macromolecule by a homogeneous regular solid object as a model for scattering. As evidenced by space-filling models of known structures, the macromolecular surface is highly irregular and deeply pitted, being characterized by grooves and channels which lead to inner solvation. The effect of inner solvation on the ratio R_H/R is marked. To illustrate this effect we examine two models which are computationally convenient and for which the solvent is intimately associated with the macromolecule. Consider first an object of macromolecular dimensions which consists of a set of homogeneous spheres of radius a centered on a body-centered cubic lattice with lattice parameter d. The lattice points form a cube with N points on an edge. The model is illustrated in Figure 2b. The hole from which solvent centers are excluded is now disconnected. It is defined by the outer surfaces of shells of thickness δ about each sphere. The solvent occupies the interstitial spaces in the array of spheres. For this model it is easily shown that

$$\left(\frac{R_H}{R}\right)^2 = 1 + \frac{2(\delta/a) + (\delta/a)^2}{\frac{5}{12}\left[\frac{N(N-1)^3(N-2) + N^3(N^2-1)}{N^3 + (N-1)^3}\right](d/a)^2 + 1} \qquad (55)$$

The ratio (R_H/R) depends on several parameters: the lattice spacing d, the sphere radius a, the number of lattice points on an edge N, and the van der Waals radius of the solvent δ. All but the last are adjustable. However, regardless of the particular choice of d, a, and N, the resulting ratio (R_H/R) is closer to unity than that for a comparable regular solid object of similar dimensions. The reason may be understood by imagining a sphere constructed about the center of mass of an object (Figure 3). Let this sphere have a radius equal to the radius of gyration (R) of the object. Suppose now we define a new, larger object by adding a shell of thickness δ to all the surfaces of the original object. The new object (with radius of gyration R_H) consists of the old object plus its shell. If the original object contains pores or groves, much of the shell, which now may be disconnected, falls within the sphere of radius R. Those portions of the shell falling within the sphere of radius R contribute to R_H in such a way as to make R_H smaller than R. Those portions of the shell outside the sphere of radius R contribute in such a way as to make R_H larger than R. The net effect is one in which the contributions of the shell to R_H largely cancel one another tending to make R_H and R similar in magnitude. Contrast this with the case of a nonporous object say a sphere of radius r, for which $R = (3/5)^{1/2}r$. A new object made by addition of a shell of thickness δ to the surface of this sphere is another sphere of radius $(r + \delta)$. All of the shell falls outside R, with the result that R_H is considerably greater than R.

In most cases the effect of inner solvation on the ratio

R_H/R is dramatic. Consider for example a particular choice of parameters for the model described above. We must exercise some care in this choice because the validity of (55) depends on the fact that neighboring spheres plus their shells do not overlap. This requires that $d \geq (4/3^{1/2})(a + \delta)$. Bearing this restriction in mind we choose as an example: $N = 5$, $(\delta/a) = 0.2$, and $(d/a) = 2.8$ (the smallest allowed value of d being $2.77a$). By (55) we find $(R_H/R)^2 = 1.006$. We wish now to compute this quantity for a solid object of comparable size and overall shape and examine the consequences of pores on the limiting slope. We choose for comparison a solid cube with side $(N - 1)d$ or in this case $4d$. The total volume of the spheres is 56% that of the solid cube. For the solid cube we compute $(R_H/R)^2 = 1.07$. The effect on the limiting slope may be estimated from (54) again by choosing $\bar{\rho}_m$ and $\bar{\rho}_s$ to be 0.4 and 0.3, respectively. By (54) the porous model yields $(R_0/R) = 0.99$, whereas the solid model yields $(R_0/R) = 0.89$. Thus if the scattering object were a solid cube the radius of gyration computed from the observed limiting slope would be in error by about 11% because of the effect of solvent. The introduction of inner solvation via the model described reduces the discrepancy to about 1%, well within the experimental error associated with the technique.

The degree of intimacy with which the solvent and macromolecule are associated plays an important role in determining the extent of the effect of solvent on the limiting slope. For this purpose we introduce another computationally convenient model (Figure 2c) which is inner solvated by means of channels. We consider a model made of parallel, identical, right circular cylinders, of radius a and height h. The centers of the cylinders are placed on a simple square lattice with lattice parameter d. The lattice points form a square with N points on a side. For this model it is easily shown that

$$\left(\frac{R_H}{R}\right)^2 = 1 + \frac{[1 + \frac{1}{3}(h/a)](\delta/a) + \frac{5}{6}(\delta/a)^2}{\frac{(N^2-1)}{6}(d/a)^2 + \frac{1}{12}\left(\frac{h}{a}\right)^2 + \frac{1}{2}} \quad (56)$$

The validity of (56) depends on the fact that neighboring rods plus their shells do not overlap. This requires that $d \geq 2(a + \delta)$. As an example we choose the parameters to yield a somewhat cubic object with a radius of gyration similar in value to that of the previous example: $N = 6$, $(\delta/a) = 0.2$, $(d/a) = 2.4$ (the smallest allowed value), and $(h/a) = 12$. By (56) we find $(R_H/R)^2 = 1.02$. We choose for comparison a solid cube with side $5d$. The total volume of the cylinders is 78.5% that of the cube. For the cube we compute $(R_H/R)^2 = 1.07$. The effect of these values on the limiting slope is again estimated by choosing $\bar{\rho}_m$ and $\bar{\rho}_s$ to be 0.4 and 0.3 respectively in (54). The parallel rod model yields $(R_0/R) = 0.97$, whereas the solid cube yields $(R_0/R) = 0.89$. The discrepancy between the "observed" and actual radius of gyration is reduced from 11 to 3% by the introduction of solvent filled channels and computed for the model described above.

IV. Scattering from Solutions of Polyelectrolytes

We have seen that to a large extent the ability to essentially ignore the presence of solvent in radius of gyration determinations is fortuitous, resulting from the fact that macromolecules in solution are not similar to solid homogeneous objects. The formalism developed above can be used to do more than illustrate the good fortune that results from inner solvation. In this section we will show how to interpret the limiting slopes of the Gunier plot which are obtained from solutions of polyelectrolytes in such a way as to gain a measure of the extent of counterion binding.

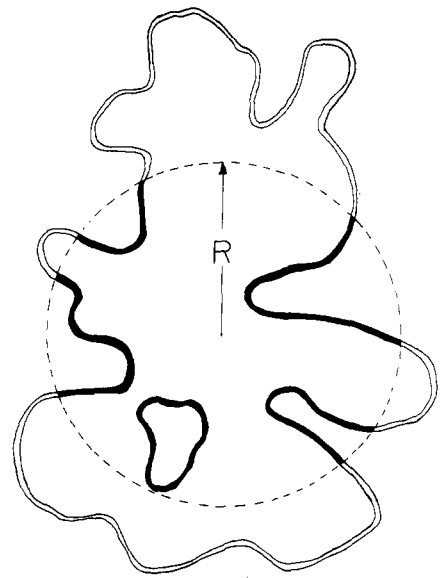

Figure 3. Contributions to the radius of gyration (R_H) of a homogeneous object whose boundaries are formed by extending the boundaries of a macromolecule by a shell of thickness equal to a van der Waals radius of solvent. A sphere of radius R equal to the radius of gyration of the macromolecule and centered on the center of mass of the macromolecule is shown by the dashed line. The darkened portions of the shell fall within the sphere. They contribute so as to make $R_H < R$. Those portions of the shell outside the sphere contribute so as to make $R_H > R$. For macromolecules which are inner-solvated by means of pores or grooves, the two kinds of contributions tend to cancel making R_H approximately equal to R.

Consider a polyelectrolyte solution which contains but one species of counterion. Since the counterion atmosphere follows the solute molecule in its travels through the solution, we may in a formal sense regard the macromolecule plus its counterion atmosphere as a single scattering object. In order to apply the methods developed above to the polyelectrolyte system we partition the volume excluded to solvent–molecule centers, V_H, into two parts: that due to the macromolecule and to the associated counterions. We label these volumes $V_{H,m}$ and $V_{H,c}$, respectively. We denote the electron content of the macromolecule and counterions by Z_m and Z_c, respectively. The essential validity of this partition rests on the fact that (53) does not depend on V_H being the volume of a connected region. We expect difficulty only when the counterions are bound to the macromolecule without intervening solvent, i.e., "inner-sphere" complexes. In this event it becomes difficult to make a nonarbitrary partition of the excluded volume between the two species. When the counterions are bound with intervening solvent, i.e., "outer-sphere" complexes, the indicated partition of V_H is readily conceptualized.

Let us examine (53) with the thought of applying it to the polyelectrolyte system. The quantity R represents the radius of gyration of the scattering object which in this case consists of the macromolecule plus its counterions. As before, R_H is the radius of gyration of the hole from which solvent–molecule centers are excluded. Both macromolecule and counterions contribute to this hole. It is convenient to introduce two addition radii of gyration. Accordingly, let R_m be the radius of gyration of the macromolecule, computed without contributions from the counterions. Let R_c be the radius of gyration of the counterion atmosphere, computed without contributions from the macromolecule, i.e., as if the counterion atmosphere existed in the absence of the macromolecule. (Unless otherwise stipu-

lated all radii of gyration defined here and below are computed from an origin located at the electronic center of mass of the macromolecule plus its counterions.)

The radius of gyration of the scattering object is related to contributions from the macromolecule and counterions as follows.

$$R^2 = \frac{Z_m R_m^2 + Z_c R_c^2}{Z_m + Z_c} \tag{57}$$

In a similar manner, R_H is related to macromolecular and counterion contributions. To establish this relationship, we define $R_{H,m}$ and $R_{H,c}$ as the radii of gyration of homogeneous objects occupying the volumes $V_{H,m}$ and $V_{H,c}$, respectively. Accordingly, we have

$$R_H^2 = \frac{V_{H,m} R_{H,m}^2 + V_{H,c} R_{H,c}^2}{V_{H,m} + V_{H,c}} \tag{58}$$

The quantity $\bar{\rho}_m$ likewise has two contributions:

$$\bar{\rho}_m = \frac{Z_m + Z_c}{V_{H,m} + V_{H,c}} \tag{59}$$

By eq 53, 57, 58, and 59 we find

$$R_0^2 = \frac{(Z_m R_m^2 - \bar{\rho}_s V_{H,m} R_{H,m}^2) + (Z_c R_c^2 - \bar{\rho}_s V_{H,c} R_{H,c}^2)}{(Z_m - \bar{\rho}_s V_{H,m}) + (Z_c - \bar{\rho}_s V_{H,c})} \tag{60}$$

Let us now examine the quantities R_c and $R_{H,c}$. For simple counterions, R_c^2 is simply the mean square distance, $\langle r_c^2 \rangle$, from the origin to the counterion centers. It is easily shown that

$$R_{H,c}^2 = \langle r_c^2 \rangle + R_{ion}^2 = R_c^2 + R_{ion}^2 \tag{61}$$

where R_{ion} is the radius of gyration of a homogeneous object occupying the hole about one counterion, and is computed from the counterion center. The magnitudes of R_c^2 and R_{ion}^2 differ greatly. The quantity R_c is the radius of gyration of the counterions computed from the center of mass of the scattering object. It is larger than (but of the order of) the radius of gyration of the macromolecule. The quantity R_{ion} is the radius of gyration of a sphere with radius equal to the sum of an ionic radius plus a van der Waals radius of solvent. Typically, simple ions in water will yield values for R_{ion}^2 of about 5 Å^2, whereas the smallest macromolecules likely to be examined by small-angle X-rays will yield values of R_c^2 greater than say 225. Thus, in extreme cases, R_{ion}^2 will be approximately 2% of R_c^2 and more typically about 0.5%. Consequently, we neglect R_{ion}^2 with respect to R_c^2 in (61), i.e.

$$R_{H,c}^2 \simeq R_c^2 \tag{62}$$

and modify (60) accordingly:

$$R_0^2 = \frac{(Z_m R_m^2 - \bar{\rho}_s V_{H,m} R_{H,m}^2) + R_c^2 (Z_c - \bar{\rho}_s V_{H,c})}{(Z_m - \bar{\rho}_s V_{H,m}) + (Z_c - \bar{\rho}_s V_{H,c})} \tag{63}$$

The quantity R_c, which appears in (63), does not depend so much on the particular species of counterion present or the extent to which the counterions are bound. Rather, it depends essentially on the distribution of these ions about the macromolecule. The counterions more or less outline the surface of the macromolecule upon which charged sites reside. The distribution of these sites on the polyelectrolyte molecule therefore determines the value of R_c. This quantity should be close to but somewhat larger than the root mean square distance of the charged sites from the origin. Thus, R_c is a parameter determined by the nature of the macromolecule rather than the particular choice of counterion. Let us keep this in mind while we examine (63). All of the quantities which bear a subscript "m" are determined only by the macromolecule. All of the quantities (except R_c) which bear a subscript "c" are determined by the type of counterions and/or the extent to which they are bound. The quantity R_c really belongs to the first class and in this sense should bear the subscript "m" rather than "c". The quantity $\bar{\rho}_s$ depends essentially on the nature of the solvent. In dilute solutions, $\bar{\rho}_s$ is virtually independent of the nonsolvent components. It is instructive to write (63) so as to emphasize the dependence of the parameters on the various components:

$$R_0^2 = \frac{A_{m,s} + B_m (Z_c - \bar{\rho}_s V_{H,c})}{C_{m,s} + (Z_c - \bar{\rho}_s V_{H,c})} \tag{64}$$

for which the quantities subscripted "m,s" or "m" do not depend on the nature of the counterions or the extent to which they are bound.

From (64) one sees that as the counterion type or concentration is changed the only effect on the limiting slope of the Gunier plot occurs by virtue of a change in the quantity $(Z_c - \bar{\rho}_s V_{H,c})$. Correlation of this quantity with observed values of R_0 for various counterions may therefore shed light on the extent of counterion binding.

An alternative and useful form of (64) is obtained by writing Z_c and $V_{H,c}$ in terms of the number (n_c) of counterions bound to one macromolecule, the number (z_c) of electrons per counterion, and the volume $(\upsilon_{H,c})$ excluded to solvent–molecule centers by one counterion, i.e., $Z_c = n_c z_c$ and $V_{H,c} = n_c \upsilon_{H,c}$. Then by (64) we have

$$R_0^2 = \frac{A_{m,s} + B_m n_c (z_c - \bar{\rho}_s \upsilon_{H,c})}{C_{m,s} + n_c (z_c - \bar{\rho}_s \upsilon_{H,c})} \tag{65}$$

The quantities z_c and $\bar{\rho}_s$ are generally known; $\upsilon_{H,c}$ may be computed from the ionic radius of the counterion and the van der Waals radius of solvent. The extent of binding is reflected through the quantity n_c.

We provide an illustration of the application of these methods to recent data. Pilz et al.[15] report values of R_0 observed for phenylalanine specific t-RNA for a variety of counterions present in excess. A seemingly puzzling result was obtained. Although R_0 was found to vary with the type of counterion employed, the value observed in excess aqueous Cs^+ was found within experimental error to be equal to that found for excess aqueous Ba^{2+}. Since Cs^+ and Ba^{2+} are isoelectronic, the obvious inference is that these ions are bound to the same extent. This interpretation presents some difficulty since the two ions are very different in both size and charge. The authors point out this difficulty and correctly infer that the difficulty stems from the absence of a satisfactory theoretical treatment of the effect of solvent. The apparently anomalous result is easily explained. The important ionic parameter as seen from (65) is not z_c but rather the quantity $(z_c - \bar{\rho}_s \upsilon_{H,c})$. Inspection of (65) reveals that in order for Cs^+ and Ba^{2+} to yield the same R_0, the quantity $n_c(z_c - \bar{\rho}\upsilon_{H,c})$ must be identical in each system. Thus, for this case

$$\frac{n_c(Ba^{2+})}{n_c(Cs^+)} = \frac{z_c(Cs^+) - \bar{\rho}_s \upsilon_{H,c}(Cs^+)}{z_c(Ba^{2+}) - \bar{\rho}_s \upsilon_{H,c}(Ba^{2+})} \tag{66}$$

The quantities $z_c(Cs^+)$ and $z_c(Ba^{2+})$ are each equal to 54. Since the solutions examined were dilute we take $\bar{\rho}_s$ to be 0.33, the bulk electron density of water. The quantity $\upsilon_{H,c}$ is computed as the volume of a sphere of radius equal to the sum of the ionic radius and the van der Waals radius of water. We used Pauling radii of 1.69 and 1.35 Å for Cs^+ and Ba^{2+}, respectively, and 1.40 Å for the van der Waals radius of water. The insertion of these values into (66) gives a

ratio of bound Ba^{2+} to Cs^+ of 0.52. That is, within experimental error the binding of Ba^{2+} relative to Cs^+ is stoichiometric.

V. Discussion

The effect of solvent on the small-angle X-ray scattering from macromolecular solutions can be described without the a priori assumption of a homogeneous solvent. The internal solvent structure is of little importance at these angles, but can be subtracted out if desired by observing the scattering from a blank. However, the distribution of solvent molecules with respect to the macromolecular solute plays an important role in determining the observed scattering. In order to account for this effect it is usual to assume a homogeneous solvent. As has been pointed out, this raises questions as to a definition of the macromolecular surface. Indeed such an assumption obscures some of the information available from the scattering pattern. It is, however, necessary to assume something about this distribution. We have assumed that solvent–molecule centers are randomly distributed within the region not excluded to them by the macromolecules. Additionally we have assumed that the atoms of both the macromolecules and solvent are hard, so that this distribution breaks sharply at the macromolecule–solvent interface.

This assumption requires some scrutiny. More precisely, let us examine the distribution of solvent–molecule centers about the macromolecular center of mass. Surely, this distribution is not a step function as has been assumed. In the wide-angle region such an assumption would prove disastrous. However, at small angles local averaging wipes out features which persist over relatively short distances. That is, at small angles the distinction between a distribution of solvent centers which breaks sharply and then has additional features over several ångströms and one which is uniform after the break is negligible. The essential feature is the break itself. Of course, this argument applies equally well to the assumption of homogeneous solvent. In fact we have shown that this latter assumption becomes essentially correct if the homogeneous solvent is assumed to begin some distance (roughly a van der Waals radius of solvent) away from the macromolecule's surface. Although this distance is much smaller than some linear dimension of a macromolecule, its implications appear to be far from negligible in certain cases.

Appendix

We have shown that at suitably small angles, where the solvent molecules may be safely regarded as point charges, the difference between the intensities scattered by a dilute solution of macromolecules and a blank is equivalent to the intensity that would be observed from a model system in which the macromolecules occupy holes; the hole size is obtained by extending the macromolecular dimensions by a van der Waals radius of solvent at the macromolecule–solvent interface; the holes are imbedded in a homogeneous solvent of electron density equal to that of bulk solvent. This theorem may be easily extended to wider angles.

The restriction to small angles is introduced in (4) by equating $\bar{F}_s$ with Z_s and in (9) by replacing $\overline{F_s^2}$ with Z_s^2. We define two effective solvent electron densities which are functions of the scattering angle. Let

$$\rho_s'(h) = \bar{F}_s/V_s = \bar{\rho}_s\bar{F}_s/Z_s \tag{67}$$

and

$$\rho_s^{\ddagger}(h) = (\overline{F_s^2})^{1/2}/V_s = \bar{\rho}_s(\overline{F_s^2})^{1/2}/Z_s \tag{68}$$

Development similar to that which leads to (30) results in an analogous relation.

$$\Delta i(\mathbf{h}) = \Big[\int_{V_H}\int_{V_H}\rho_m(\mathbf{r}) + \rho_s'\int_{V_H}\int_{V_s}\rho_m(\mathbf{r}) + \rho_s'\int_{V_s}\int_{V_H}\rho_m(\mathbf{r}') + \rho_s^{\ddagger 2}\int_{V_s}\int_{V_s}\Big]e^{i\mathbf{h}\cdot(\mathbf{r}-\mathbf{r}')}dV\,dV' \tag{69}$$

For solvents with molecules about the size of water molecules, the quantities ρ_s' and $\rho_s^{\ddagger}$ differ negligibly over all normally observed scattering angles including wide angles. For example, in the case of water using the oxygen nucleus as an origin for the computation of $\bar{F}_s$, we find that $\rho_s^{\ddagger}(0.2)/\rho'(0.2) = 1.002$ and $\rho_s^{\ddagger}(3.8)/\rho'(3.8) = 1.003$. Thus we replace ρ_s' with $\rho_s^{\ddagger}$ in (69), and restate the theorem: the difference between the intensities scattered by a dilute solution of macromolecules and a blank is equivalent to the intensity that would be observed from a model system in which the macromolecules occupy holes; the hole size is obtained by extending the macromolecular dimensions by a van der Waals radius of solvent at the macromolecule–solvent interface; the holes are imbedded in a solvent of electron density $(\overline{F_s^2})^{1/2}/Z_s$; the solvent may otherwise be regarded as homogeneous.

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