

Victor A. Bloomfield
Department of Biochemistry,
Molecular Biology, and
Biophysics,
University of Minnesota,
1479 Gortner Avenue,
Saint Paul, MN 55108

Received 6 December 1999;
accepted 9 March 2000

Static and Dynamic Light Scattering from Aggregating Particles

Abstract: We use standard hydrodynamic and light scattering theories to calculate the total intensity and dynamic light scattering properties of random aggregates of spherical particles containing up to ten spheres. When the aggregates have dimensions comparable to the wavelength of light, intraaggregate interference effects can dramatically reduce the apparent size of the aggregates. These results could be significant in interpreting DNA condensation, protein polymerization, and other biomolecular aggregation reactions. © 2000 John Wiley & Sons, Inc. *Biopoly* 54: 168–172, 2000

Keywords: diffusion; aggregation; polymerization; molecular weight determination

INTRODUCTION

Light scattering is one of the most important ways to characterize macromolecules and colloids. Using modern instrumentation, one can readily obtain the weight average molecular weight and radius of gyration from total intensity (static) light scattering, and the hydrodynamic (Stokes) radius from dynamic light scattering.^{1–3} Theory shows that scattering and hydrodynamic behavior are strongly nonlinear functions of size, due to hydrodynamic interaction if the particles are composed of multiple subunits, and intraparticle interference if they are comparable in size to the wavelength of scattered light. However, it is often not appreciated how misleading these nonlinear effects can be in some practical cases.

Such issues arise in the data we have recently published⁴ on the kinetics of DNA condensation by multivalent cations. The primary condensed particles are toroids, with typical inner radii of 15 nm and outer radii of 50 nm.⁵ These dimensions are large enough to produce a particle scattering form factor significantly

less than unity.⁴ Electron micrographs show that these toroids aggregate into small clusters, stabilized either by DNA strand sharing between the toroids or by the same ion-mediated intermolecular attractions that produce DNA–DNA attraction within a toroid.⁶ The center-to-center distance between adjacent toroids within these aggregates is about 100 nm, the same order of magnitude as the wavelength of the laser light used in scattering experiments (488 nm in vacuo or 367 nm in aqueous solution with refractive index 1.33). Therefore, there is considerable reduction in both light scattering intensity due to intraaggregate interference, and in frictional resistance due to hydrodynamic interaction. Both of these effects tend to make the aggregates seem smaller, when characterized by light scattering techniques, than they really are.

The purpose of this article is to estimate quantitatively the magnitude of these effects. We model the aggregating particles as spheres, rather than attempting to take into account the geometrical complexities of toroids or other less symmetrical shapes.

Correspondence to: Victor A. Bloomfield; email: victor@tc.umn.edu

Biopolymers, Vol. 54, 168–172 (2000)

© 2000 John Wiley & Sons, Inc.

METHODS

Aggregates containing up to ten spheres were generated on a cubic lattice. Starting with a dimer of two spheres in contact, aggregates containing n spheres were produced by randomly choosing one of the $n - 1$ spheres (with coordinates of the next smaller aggregate), adding the n th sphere at one of the six adjacent sites on the lattice, checking for double occupancy, and repeating until an empty site was found. In the results reported in this paper, 100 random aggregates of each size were used, but the results are very insensitive to the number of aggregates sampled.

The mean-square radius R_G^2 was calculated from the equation⁷

$$R_G^2 = \frac{1}{2n^2} \sum_{i=1}^n \sum_{j=1}^n R_{ij}^2 \quad (1)$$

where R_{ij} is the distance between the centers of spheres i and j .

Translational frictional coefficients of n -mers were calculated from the Kirkwood equation⁸⁻¹⁰ for spheres of radius R :

$$\begin{aligned} f_n &= \frac{nf_1}{1 + (R/n) \sum_{i=1}^n \sum_{j \neq i}^n (1/R_{ij})} \\ &= \frac{nf_1}{1 + (2R/n) \sum_{i=2}^n \sum_{j=1}^{i-1} (1/R_{ij})} \end{aligned} \quad (2)$$

If we set the lattice spacing (distance L between touching spheres) = 1, then $R = \frac{1}{2}$. While the Kirkwood theory is known to be accurate only to about 10%, this is well within the range of variation for these random arrays, and the qualitative results that are the aim of this study will be entirely adequate.

The intensity of light scattered from an array of n particles, relative to scattering at $q = 0$ from a single point particle, is¹¹

$$I_n(q) = n^2 P_n(q) \quad (3)$$

where

$$P_n(q) = P_1(q) S_n(q) \quad (4)$$

$$S_n(q) = \frac{1}{n^2} \sum_{i=1}^n \sum_{j=1}^n \frac{\sin(qR_{ij})}{qR_{ij}} \quad (5)$$

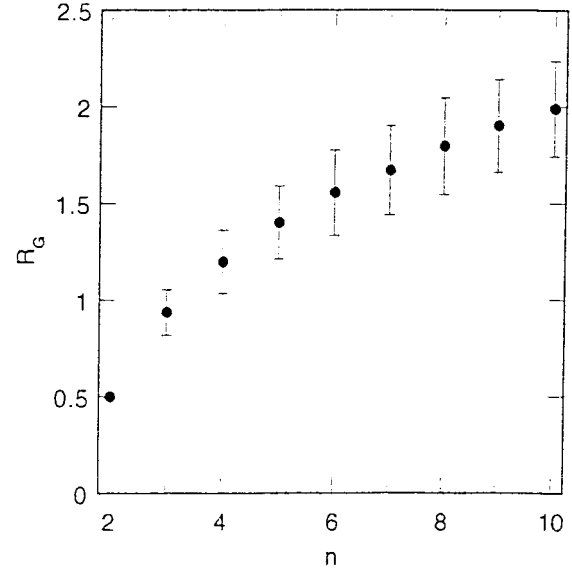


FIGURE 1 Rms radius of gyration $R_G = \langle R_G^2 \rangle^{1/2}$ as a function of number of particles n in the array, calculated according to Eq. (1) for 100 arrays of randomly placed but contiguous point particles on a cubic lattice.

and for a sphere of radius R ,

$$P_1(q) = \left\{ \frac{3}{(qR)^3} [\sin(qR) - qR \cos(qR)] \right\}^2 \quad (6)$$

The scattering vector q is defined as

$$q = \frac{4\pi n_0}{\lambda_0} \sin\left(\frac{\theta}{2}\right) \quad (7)$$

where n_0 is the solution refractive index, λ_0 is the wavelength of the scattered light in vacuo, and θ is the scattering angle. For scattering with the 488 nm line of an argon ion laser in aqueous solution ($n_0 = 1.334$) from aggregates composed of spherical particles with $R = 50$ nm, $qR = 0.445, 0.859, 1.21, \text{ and } 1.49$ at $\theta = 30^\circ, 60^\circ, 90^\circ, \text{ and } 120^\circ$, respectively. The smallest values of qL are double these. The values of $P_1(q)$ corresponding to these scattering angles are 0.96, 0.86, 0.74, and 0.63, respectively. We have assumed the validity of Rayleigh-Debye scattering in these equations, which should be adequate even though qR is not small, because $\Delta n q R \ll 1$, where Δn is the difference in refractive index between solute particles and solvent.

RESULTS AND DISCUSSION

Figure 1 shows the rms radius of gyration $R_G = \langle R_G^2 \rangle^{1/2}$ as a function of n , calculated according to Eq. (1) for arrays of randomly placed but contiguous point particles on a cubic lattice.

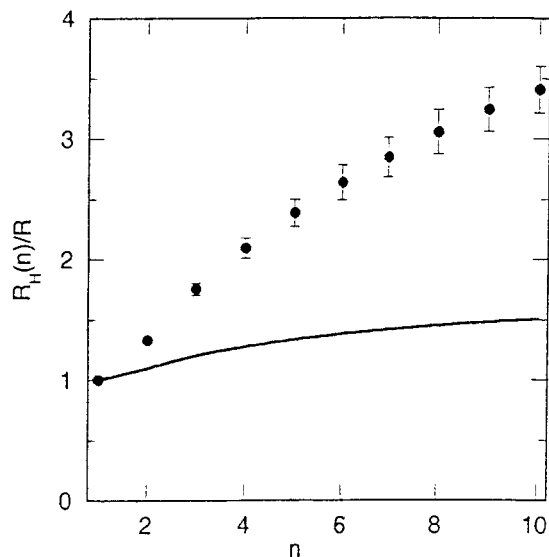


FIGURE 2 Ratio of hydrodynamic radii of n -mer and monomer for the arrays in Figure 1. The solid line is $n^{1/3}$, which would be obtained if the spheres coalesced into a single solid sphere.

Figure 2 shows the ratio of hydrodynamic radii [equal to the ratio of frictional coefficients f_n/f_1 calculated according to Eq. (2)] for the same arrays. The solid line in Figure 2 is $n^{1/3}$, which would be obtained if the spheres coalesced into a single solid sphere. The array is clearly more expanded than that.

Figure 3 shows $\rho = R_G/R_H$ as a function of n . Burchard^{2,3} has shown that ρ can be used to distin-

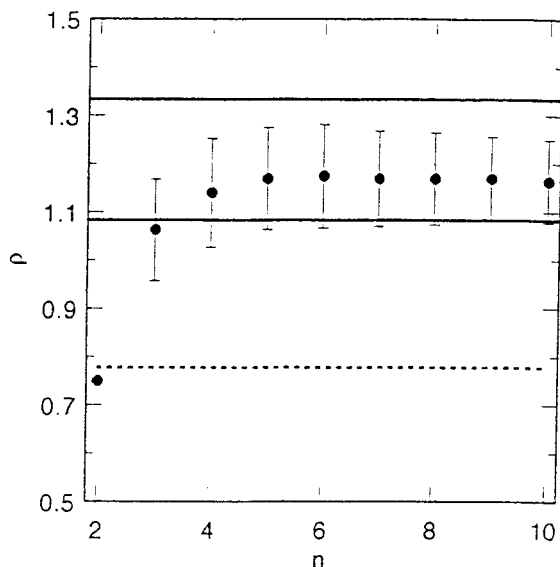


FIGURE 3 Ratio of radius of gyration to hydrodynamic radius. Dotted line is at $\rho = 0.778$ for solid spheres. Solid lines are for regular star molecules in a Θ solvent with four arms (upper, $\rho = 1.33$) and $\gg 1$ arms (lower, $\rho = 1.079$).

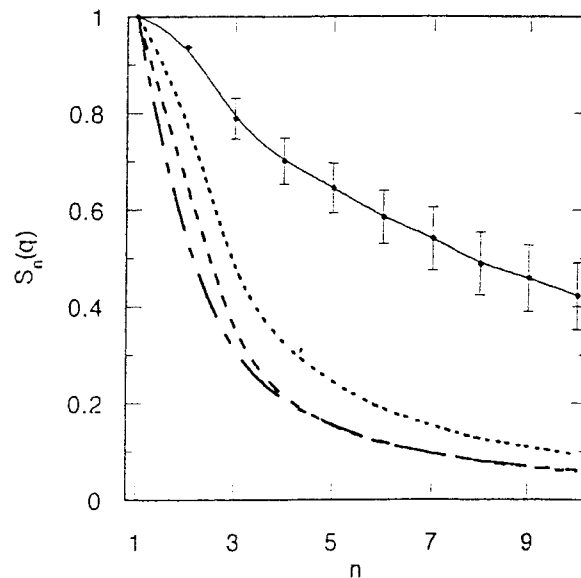


FIGURE 4 Aggregate scattering factor $S_n(q)$ as a function of n for $\theta = 30$ (—), 60 (---), 90 (— · —), and 120 (— · —) deg assuming $R = 50$ nm and minimum interparticle distance $L = 2R$, corresponding to $qL = 0.89, 1.72, 2.41,$ and 2.98 . For clarity, error bars are given only for the 30 deg curve.

guish among various models for polymer structure. The dotted line in Figure 3 is at the value $\rho = 0.778$ for solid spheres. The upper and lower solid lines are for regular star molecules in a Θ solvent with four arms (upper) or $\gg 1$ arms (lower), with $\rho = 1.33$ and 1.079 , respectively. Our random aggregates are intermediate between these two cases, with $\rho \approx 1.17$, a result that seems physically reasonable.

Figure 4 shows $S_n(q)$ as a function of n for $\theta = 30^\circ, 60^\circ, 90^\circ,$ and 120° assuming $R = 50$ nm. For clarity, typical error bars are attached only to the 30 deg curve. The profound depression of scattering intensity due to intermolecular interference is evident from this figure. Of course, $S_n(q) \approx 1$ for all n at or near $\theta = 0^\circ$, as can be achieved with modern low-angle light scattering instruments.

These results are combined in Figure 5 to show the scattering of random aggregates of n -mers relative to monomers. If there were no intra-aggregate interference, these curves should vary as n^2 . In fact, even at the lowest scattering angle of 30° that is normally achievable without specialized low-angle instrumentation, the curve only reaches 42 for $n = 10$. At more common experimental scattering angles, the intensity is reduced by a factor of 10 or more for the larger aggregates. This indicates that light scattering will strongly underestimate the number of monomers in such aggregates except at scattering angles quite near 0° .

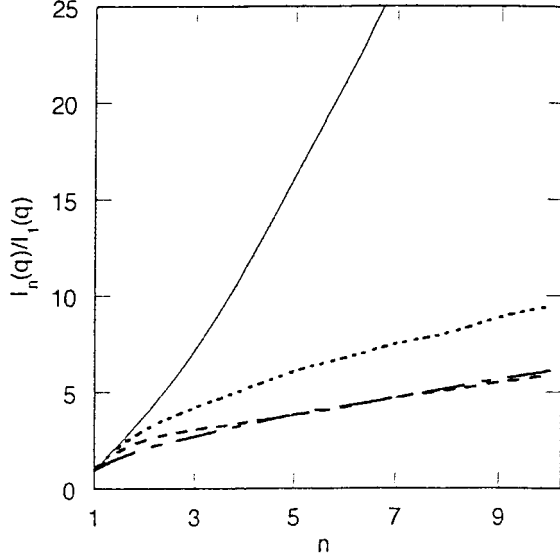


FIGURE 5 Scattering of random aggregates of n -mers relative to monomers, at scattering angles of 30° (—), 60° (---), 90° (— · —), and 120° (----). The 30° curve extends to 42 at $n = 10$.

This underweighting of large aggregates will be reflected in the apparent weight-average degree of polymerization $\langle dp \rangle_w$ and z -average diffusion coefficient $\langle D \rangle_z$. The apparent $\langle dp \rangle_w$ as measured by light scattering is defined as

$$\langle dp \rangle_w = \frac{\sum_{i=1}^n i^2 N_i P_1(q) S_i(q)}{\sum_{i=1}^n i N_i} = \frac{\sum_{i=1}^n i C_i P_1(q) S_i(q)}{\sum_{i=1}^n C_i} \quad (8)$$

where N_i and C_i are the molar and weight concentrations of species i , respectively. We can evaluate these averages if we make an assumption about the distribution of species. In Table I we show calculations for two idealized cases: (1) equal numbers of each species ($N_i = \text{constant}$) and (2) equal masses of each species ($C_i = \text{constant}$).

The entries $\langle M \rangle_w / M_1$ (M is molecular weight) are calculated by neglecting the monomer form factor $P_1(q)$. These results are remarkable, because they show that depression of scattering intensity through the intra-aggregate form factor, when $S(q)_i < i^{-1}$, can lead to apparent molecular weights even less than the monomer. Of course, the details depend on the distribution of aggregate sizes; the two distributions we have used here were chosen solely for simplicity.

Using the same notation, $\langle D \rangle_z$ can be written¹²

Table I Apparent Weight-Average Degree of Polymerization as a Function of Scattering Angle Under the Assumption of Equal Numbers or Equal Masses of Each Aggregate from Monomer to 10-mer

	θ (deg)				
	0	30	60	90	120
$P_1(q)$	1.0	0.96	0.86	0.74	0.63
Equal numbers					
$\langle dp \rangle_w$	7.0	3.44	0.94	0.53	0.45
$\langle M \rangle_w / M_1$	7.0	3.59	1.10	0.71	0.71
Equal masses					
$\langle dp \rangle_w$	5.5	2.96	1.00	0.60	0.49
$\langle M \rangle_w / M_1$	5.5	3.08	1.16	0.82	0.78

$$\langle D \rangle_z = \frac{\sum_{i=1}^n i^2 N_i D_i(q) S_i(q)}{\sum_{i=1}^n i^2 N_i S_i(q)} = \frac{\sum_{i=1}^n i C_i D_i(q) S_i(q)}{\sum_{i=1}^n i C_i S_i(q)} \quad (9)$$

Combining this with the Einstein equation $D = kT/f$, we find

$$\frac{\langle D \rangle_z}{D_1} = \frac{\sum_{i=1}^n i^2 N_i S_i(q) (f_i/f_1)^{-1}}{\sum_{i=1}^n i^2 N_i S_i(q)} = \frac{\sum_{i=1}^n i C_i S_i(q) (f_i/f_1)^{-1}}{\sum_{i=1}^n i C_i S_i(q)} \quad (10)$$

These results have been obtained using standard theories, from which it is well known that intraparticle interference will cause a diminution in the intensity of scattered light if the particles have dimensions comparable to the wavelength (more precisely, if $qR_G \approx 1$). The main purpose of this paper has been to demonstrate the strikingly large effects that interfer-

Table II Ratio $\langle D \rangle_z / D_1$ as a Function of Scattering Angle Under the Assumption of Equal Numbers or Equal Masses of Each Aggregate from Monomer to 10-mer

	θ (deg)				
	0	30	60	90	120
Equal numbers	0.35	0.36	0.40	0.41	0.41
Equal masses	0.39	0.42	0.5	0.54	0.53

ence can have on aggregate size determination in a case of practical interest. It is clear that light scattering measurements of DNA condensation and of other biomolecular association reactions should be carried out under conditions that minimize the formation of large aggregates, and at scattering angles as low as possible to minimize intraparticle interference effects. Since many biomolecular structures are in the 100 nm size range, our findings should have general qualitative validity.

REFERENCES

1. Berne, B. J.; Pecora, R. *Dynamic Light Scattering with Applications to Chemistry, Biology, and Physics*; Wiley Interscience: New York, 1976; 376 pp.
2. Burchard, W.; Schmidt, M.; Stockmayer, W. H. *Macromolecules* 1980, 13, 1265–1272.
3. Burchard, W. In *Laser Light Scattering in Biochemistry*; Harding, S. E.; Sattelle, D. B.; Bloomfield, V. A., Eds.; Royal Society of Chemistry: Cambridge, 1992; pp 3–22.
4. He, S.; Arscott, P. G.; Bloomfield, V. A. *Biopolymers* 2000, 53, 329–341.
5. Arscott, P. G.; Li, A.-Z.; Bloomfield, V. A. *Biopolymers* 1990, 30, 619–630.
6. Rouzina, I.; Bloomfield, V. A. *J Phys Chem* 1996, 100, 9977–9989.
7. Zimm, B. H.; Stockmayer, W. H. *J Chem Phys* 1949, 1301.
8. Kirkwood, J. G. *Rec Trav Chim Pays Bas* 1949, 68, 649.
9. Kirkwood, J. G. *J Polym Sci* 1954, 12, 1.
10. Bloomfield, V. A.; Dalton, W. O.; van Holde, K. E. *Biopolymers* 1967, 5, 135–148.
11. Tanford. *Physical Chemistry of Macromolecules*; Wiley: New York, 1961; Chap 5.
12. Koppel, D. E. *J Chem Phys* 1972, 57, 4814–4820.