Excluded Volume for Pairs of Triaxial Ellipsoids at Dominant Brownian Motion

J. M. RALLISON* AND S. E. HARDING†

*University of Cambridge, Department of Applied Mathematics and Theoretical Physics, Silver Street, Cambridge CB3 9EW, and †University of Cambridge, Department of Biochemistry, Tennis Court Road, Cambridge CB2 1QW, England

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An expression is given for the volume excluded by one rigid convex particle to another when Brownian motion dominates the orientation statistics. Explicit numerical results are presented for pairs of triaxial ellipsoids. Implications for the determination of macromolecular size and shape by measurement of the second virial coefficient are discussed. © 1985 Academic Press, Inc.

1. INTRODUCTION

One of the ways in which the shape of a biological macromolecule in solution may be inferred from macroscopically observable solution properties is through the second virial coefficient, B, which arises in the analysis of osmosis and sedimentation equilibrium. B can be interpreted, after due allowance for other factors, in terms of the excluded volume or "molecular covolume," u, between pairs of molecules and can thus be calculated for various simple model shapes. Such shapes as have hitherto been available are the sphere (1), the rod (2–4), and spheroids (5–7). All these shapes are axisymmetric, and so involve only one aspect ratio. For many macromolecules, restriction to such simple shapes is a poor approximation to the true conformation in solution: an extension of the formulation to cover the triaxial ellipsoid with *two* aspect ratios may, where applicable, represent a considerable improvement.

In this study, the earlier contributions of Isihara (5) and Winzor *et al.* (6, 7) describing the covolume for pairs of axisymmetric ellipsoids are extended to the general case of pairs of triaxial ellipsoids (centrally symmetric). As before, the theory only caters for *pairs* of excluding macromolecules (and not triplets, etc.) and hence its applicability is restricted to dilute dispersions. The extension to cover more concentrated dispersions is formidable and is only briefly discussed here. Nevertheless, our excluded volume formulation may provide the basis, if combined with other macroscopic solution properties of ellipsoids such as viscosity and electric birefringence, for providing reasonable estimates for the 3 axial dimensions of macromolecules in solution.

Conversely, in the case when estimates of the three axial dimensions of a biological macromolecule are known from, say, X-ray crystallography, our formulation may provide the basis for improved methods of making allowance for thermodynamic nonideality in the interpretation of physical measurements.

2. THE PROBLEM FOR GENERAL PARTICLE SHAPES

At first sight, the calculation of the volume u_{AB} excluded to a point P of a rigid particle B, by the presence of a second, A, involves fixing the relative orientations of B and A, calculating the volume of the region swept out by P as B moves around A while remaining in contact with it, and finally averaging this volume over all possible choices of the

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relative orientation (see Fig. 1). In cases where the orientation statistics are nonuniform, this is probably the easiest way to compute the excluded volume.

In the case where Brownian motion dominates, however, a simpler method exists, which in essence involves performing the orientational average first (all relative orientations of A and B now being equally probable). This technique was demonstrated by Isihara (5), but the mathematics in his paper is somewhat abstract and complicated and we sketch below a simpler method which arrives at the same conclusion, and illustrates clearly the cases in which the shortcut will fail, and in which the more laborious technique sketched above must be used.

Let the surface of A be specified parametrically by coordinates (u, v). Then at a fixed relative orientation of B for the configuration in which B touches A at $\mathbf{x}(u, v)$ the position of P is given as $\mathbf{X}(u, v) = \mathbf{x}(u, v) + \mathbf{y}(u, v)$ as shown in Fig. 1. Now extend the particles to families of geometrically similar surfaces in which the "axes" of A and B are stretched by amounts λ_A and λ_B. In that case,

$$\mathbf{X}(u, v) = \lambda_A \mathbf{x}(u, v) + \lambda_B \mathbf{y}(u, v)$$

and, for these stretched particles, the volume V swept out by P is

$$V = \tfrac{1}{3}\left|\int\int \mathbf{X}\cdot\frac{\partial \mathbf{X}}{\partial u}\wedge\frac{\partial \mathbf{X}}{\partial v}\,dudv\right|$$

as may be shown by the divergence theorem. Provided A and B are convex, the limits of integration are independent of the sizes of A and B and hence of λ_A, λ_B. Hence on averaging over all relative orientations we must have a cubic expression for U_{AB}, namely

$$u_{AB} = \bar{V} = c_1\lambda_A^3 + c_2\lambda_A^2\lambda_B + c_3\lambda_A\lambda_B^2 + c_4\lambda_B^3.$$

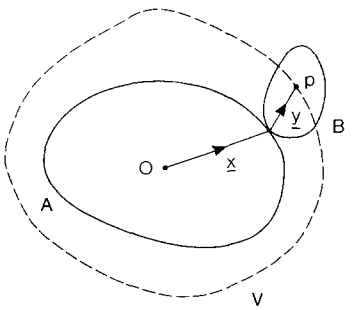

FIG. 1. Volume excluded to point P for a fixed relative orientation of particle B to particle A.

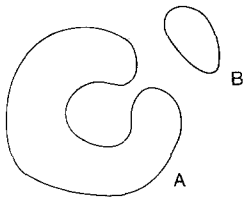

FIG. 2. Case where u_{AB} is not a polynomial.

For nonconvex shapes (e.g., that shown in Fig. 2) the limits of integration for u, v do depend on λ_A, λ_B and thus u_{AB} is no longer of the form above. It is worth noting in passing that because two touching spheres do not form a convex shape, the analogous expression for u_{AB} for *three* spheres is not a polynomial, and hence that there is no simple analog of the Isihara treatment for more than two particles.

Next suppose that $\lambda_B \to 0$. Then at leading order u_{AB} becomes just the volume of A, V_A, so that

$$c_1 = V_A,$$

and similarly,

$$c_4 = V_B,$$

and further, c_2 may be identified as the volume of the thin shell swept out by (the mean position of) P as B moves round A (see Fig. 3). Let R be the distance of P from the tangent plane at $\mathbf{x}$, and denote by $\bar{R}(\mathbf{x})$ the mean value of R as the orientation of B is varied (the thickness of the shell at $\mathbf{x}$), all orientations being equally probable. Then

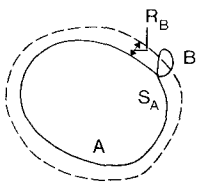

FIG. 3. Case when $\lambda_B/\lambda_A \to 0$, "shell"-excluded volume.

$$c_2 = S_A\langle \bar{R}\rangle_A,$$

where S_A is the surface area of A, and $\langle\ \rangle_A$ denotes a surface mean over contact points $\mathbf{x}$ of A.

Now because λ_B is small, A may be regarded as being locally planar (with slight smoothing of corners and edges if necessary). Hence $\bar{R}$ depends on $\mathbf{x}$ only through the normal $\mathbf{n}$ (to A) at $\mathbf{x}$. But since Brownian motion is dominant, and in calculating $\bar{R}$ all relative orientations are averaged with equal probability, $\bar{R}$ is independent of $\mathbf{n}$ and hence also of $\mathbf{x}$. This is not, of course, true if the orientation statistics are nonuniform. Thus $\langle \bar{R}\rangle_A = \bar{R}$ independently of A, and hence $\bar{R}$ depends only on P and the shape of B, and may be computed as the mean distance of P from a tangent plane to B, all directions of the normal (to B) being equally probable (see Fig. 4). Thus if θ, ϕ are spherical polar coordinates at P, and $\mathbf{X}(\theta, \phi)$ is the position of a point $\mathbf{X}$ of B, then $\mathbf{X}$ is single-valued by convexity and

$$\bar{R} = \frac{1}{4\pi}\int_{\text{unit sphere}} \mathbf{X}(\theta, \phi)\cdot\hat{\mathbf{r}}\sin\theta d\theta d\phi.$$

By the divergence theorem, if $\mathbf{X}$ is replaced by $\mathbf{X} + \mathbf{d}$ where $\mathbf{d}$ is a constant vector, $\bar{R}$ is unchanged, and hence $\bar{R}$ is independent of the choice of reference point P in B; thus it is proper to speak of $\bar{R}$ (and hence u_{AB}) without reference to P, and $\bar{R}$ as R_B. For a smooth surface (twice differentiable) Isihara has identified R_B as the surface mean radius of curvature $R_B = \langle 1/2(R_1 + R_2)\rangle_B$ where R_1, R_2 are the principal radii of curvature of the surface of B.

On combining these results we have finally Isihara's general formula

$$u_{AB} = V_A + V_B + S_AR_B + S_BR_A.$$

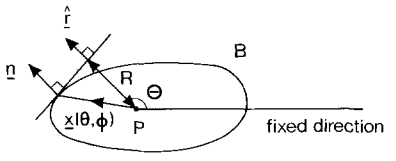

FIG. 4. Definition of R_B.

In the particular case where A, B are identical this gives

$$u_{red} = u_{AA}/V_A = 2 + 2R_AS_A/V_A, \quad [1]$$

where the dimensionless quantity u_{red} is the "reduced" molecular covolume.

Alternatively, if B is a sphere of radius r,

$$u_{AB} = V_A + rS_A + 4\pi r^2R_A + \tfrac{4}{3}\pi r^3, \quad [2]$$

and thus if u_{AB} can be computed, S_A, R_A (and hence u_{red}) can be identified as appropriate coefficients of powers of r. This method is used by Winzor *et al.* (6, 7) and below.

3. APPLICATION TO TRIAXIAL ELLIPSOIDS

We now turn to the problem of computing R, S, and u_{red} for a general triaxial ellipsoid, of semi-axes a, b, c. We first compute the volume excluded by the ellipsoid to the center of a sphere of radius r.

Let the ellipsoid be specified parametrically by $x = (a\cos u\cos v, b\cos u\sin v, c\sin u)$, $-\pi/2 \leqslant u \leqslant \pi/2$, $0 \leqslant v \leqslant 2\pi$. Then the normal $\mathbf{n}$ at (u, v) is

$$\mathbf{n} = (\cos u\cos v/a, \cos u\sin v/b, \sin u/c)\Delta,$$

where

$$\Delta^{-2} = \frac{\cos^2 u\cos^2 v}{a^2} + \frac{\cos^2 u\sin^2 v}{b^2} + \frac{\sin^2 u}{c^2}.$$

Hence the center of a sphere of radius r which touches the ellipse at (u, v) is at

$$\mathbf{X} = [(a + r\Delta/a)\cos u\cos v,$$
$$(b + r\Delta/b)\cos u\sin v, (c + r\Delta/c)\sin u],$$

and as u, v vary, $\mathbf{X}$ sweeps out a surface S_r which encloses a volume V_r given by

$$V_r = \tfrac{1}{3}\int_{S_r}\mathbf{X}\cdot d\mathbf{S}_r$$
$$= \tfrac{8}{3}\left|\int_0^{\pi/2}\int_0^{\pi/2}\mathbf{X}\cdot\frac{\partial\mathbf{X}}{\partial v}\wedge\frac{\partial\mathbf{X}}{\partial u}dudv\right|.$$

It is then a matter of straightforward but tedious algebra to evaluate the integrand and collect powers of r to obtain by means of Eq. [2] the results

$$S = \tfrac{8}{3}\int_0^{\pi/2}\int_0^{\pi/2} \cos u\,du\,dv \left\{\left(\frac{bc}{a}+\frac{ca}{b}+\frac{ab}{c}\right)\Delta - \sin^2 v\cos^2 v\cos^2 u\,\Delta^3 c\left(\frac{b}{a}-\frac{a}{b}\right)\left(\frac{1}{a^2}-\frac{1}{b^2}\right)\right.$$
$$\left. - \sin^2 u\cos^2 u\,\Delta^3\left(\frac{\cos^2 v}{a^2}+\frac{\sin^2 v}{b^2}-\frac{1}{c^2}\right)\left[c\left(\frac{b\cos^2 v}{a}+\frac{a\sin^2 v}{b}\right)-\frac{ab}{c}\right]\right\} \quad [3]$$

and

$$R = \frac{2}{3\pi}\int_0^{\pi/2}\int_0^{\pi/2} \cos u\,du\,dv \left\{\left(\frac{a}{bc}+\frac{b}{ac}+\frac{c}{ab}\right)\Delta^2\right.$$
$$- \sin^2 v\cos^2 v\cos^2 u\,\Delta^4\left(\frac{1}{a^2}-\frac{1}{b^2}\right)\frac{1}{c}\left(\frac{b}{a}-\frac{a}{b}\right)$$
$$\left. - \sin^2 u\cos^2 u\,\Delta^4\left(\frac{\cos^2 v}{a^2}+\frac{\sin^2 v}{b^2}-\frac{1}{c^2}\right)\left[\frac{1}{c}\left(\frac{b\cos^2 v}{a}+\frac{a\sin^2 v}{b}\right)+\frac{c}{ab}-\frac{b}{ac}-\frac{a}{bc}\right]\right\}. \quad [4]$$

In each case it is possible to perform one of the double integrations analytically, but the results are so complicated as to be opaque, and for numerical purposes it is as easy to start from the double integrals.

4. RESULTS

Without loss of generality we may take $a \geqslant b \geqslant c$. Rescaling lengths by $(abc)^{1/3}$ (so that the volume is $4\pi/3$) then implies that only the ratios of lengths are relevant, so let $\mu = a/b \geqslant 1$ and $\nu = b/c \geqslant 1$. The cases of axisymmetric ellipsoids $\mu = 1$ (oblate) $\nu = 1$ (prolate) are then given by Isihara (5) and by Winzor *et al.* (6, 7) and displayed in Table I. The asymptotic limits for rods and disks are easily derived. For the general case we have two new asymptotic limits. First, the general ellipsoidal near-sphere for which $\mu \approx 1$, $\nu \approx 1$. After some algebra the results in Table I are obtained: it is noteworthy that the first correction from sphericity is quadratic, reflecting the fact that the sphere minimizes R and S over all particles of volume $4\pi/3$. Second, for a tape-shape particle with $a \gg b \gg c$ we find that the longest axis a is crucial as regards R (so that the tape is asymptotically like a rod), but the intermediate axis b is also important as regards S (and thus the coefficient is the same as that for a disk).

TABLE I

Asymptotic Results

Particle	Region	R	$S/4\pi$	u_{rel}
Near-sphere $\epsilon^2 = (\mu-1)^2 + (\nu-1)^2 + (\mu-1)(\nu-1)$ $\lvert\mu-1\rvert \ll 1$, $\lvert\nu-1\rvert \ll 1$	I	$1+\frac{8}{45}\epsilon^2$	$1+\frac{8}{45}\epsilon^2$	$8(1+\frac{4}{15}\epsilon^2)$
Rod: $\nu = 1$, $\mu \gg 1$		$\frac{1}{2}\mu^{2/3}$	$\frac{\pi}{4}\mu^{1/3}$	$3\pi\mu/4 + 2$
Disk: $\nu \gg 1$, $\mu = 1$		$\frac{\pi}{4}\nu^{1/3}$	$\frac{1}{2}\nu^{2/3}$	$3\pi\nu/4 + 2$
Tape: $\nu \gg 1$, $\mu \gg 1$	II	$\frac{1}{2}\mu^{2/3}\nu^{1/3}$	$\frac{1}{2}\mu^{1/3}\nu^{2/3}$	$\frac{3}{2}\mu\nu + 2$
Axisymmetric particles:				
Prolate: $\nu = 1$		$\frac{1}{2}\mu^{2/3}\left\{1+\frac{1}{\mu(\mu^2-1)^{1/2}}\cosh^{-1}\mu\right\}_1$	$\frac{1}{2}\mu^{1/3}\left\{\frac{1}{\mu}+\frac{\mu}{(\mu^2-1)^{1/2}}\cos^{-1}\frac{1}{\mu}\right\}_2$	$\frac{3}{4}\mu\{\ \}_1\{\ \}_2 + 2$
Oblate: $\mu = 1$		$\frac{1}{2}\nu^{1/3}\left\{\frac{1}{\nu}+\frac{\nu\cos^{-1}\frac{1}{\nu}}{(\nu^2-1)^{1/2}}\right\}_1$	$\frac{1}{2}\nu^{2/3}\left\{1+\frac{\cosh^{-1}\nu}{\nu(\nu^2-1)^{1/2}}\right\}_2$	$\frac{3}{4}\nu\{\ \}_1\{\ \}_2 + 2$

For intermediate values of μ and ν a numerical evaluation is needed. When μ, ν are moderate in size (less than 10, say) computation is straightforward, but when μ, ν become larger the integrand is highly peaked near $u = 0$, or $\pi/2$, and hence new variables

$$s' = \frac{a \sin u}{(a - c) \sin u + c} \quad 0 \leqslant s' \leqslant 1,$$

$$v' = \frac{av}{(a - b)v + b\pi/2} \quad 0 \leqslant v' \leqslant 1$$

are appropriate. The integrals in [3] and [4] may then be evaluated for specified (μ, ν) by Gaussian numerical routines (for example, the NAG package DO1DAF (8)). In Table II we give values of u_{red} correct to 3 decimal places for axial ratios from (1, 1) to (10, 10).

In Figs. 5–7 we show contours of constant R, S, and u_{red} in the (μ, ν) plane. The dotted lines delineate the regions within which the asymptotic results for near-spheres (I) and tapes (II) given in Table I differ by less than 5% from the numerical results. It is remarkable that whereas R and S depend very differently on μ and ν, u_{red} is symmetric in μ, ν (which extends Isihara's observation that prolate and oblate spheroids have the same excluded volume). Thus for instance, the normalized self-excluded volume for an ellipsoid of axes 1:2:8 is the same as that for one of axes 1:4:8.

5. DISCUSSION

The excluded volume u, has been related by, for example, Ogston and Winzor (6), to the second virial coefficient B from osmometry in terms of an operationally defined "effective" molar covolume (6, 1):

$$U = 2BM^2, \qquad [5]$$

where M is the molecular weight and U, the *molar* covolume (ml/mole), is related to u by (1) $U = uN_A$, N_A being Avogadro's number. U as defined by Eq. [5] was termed an "effective" covolume in the sense that there may be other contributions to the observed nonideality parameter B, notably the effect of charge on the macromolecule. If the experiment is not conducted at the isoionic point (i.e., the net charge, Z, is nonzero), Eq. [5] has been modified to (6, 9)

$$U = 2BM^2 - \frac{Z^2}{2m_0}, \qquad [6]$$

m_0 being the concentration of the uni-univalent supporting electrolyte. An improvement to the second term on the right-hand side of Eq. [6] has recently been given by Wills *et al.* [(10), especially Eq. [A.9]]. Other terms involving macroion–electrolyte and electrolyte–electrolyte interactions, as originally given by Scatchard (11), are generally

TABLE II

Values of u_{red} as a Function of (a:b:c) for a General Triaxial Ellipsoid ($a \geqslant b \geqslant c$)

	b/c									
a/b	1†	2	3	4	5	6	7	8	9	10
*1	8.000	9.077	10.908	12.956	15.104	17.308	19.547	21.810	24.090	26.383
2	9.077	11.568	14.701	18.034	21.463	24.947	28.465	32.006	35.564	39.134
3	10.908	14.701	19.149	23.817	28.593	33.431	38.308	43.212	48.136	53.074
4	12.956	18.034	23.817	29.843	36.005	42.232	48.506	54.812	61.141	67.487
5	15.104	21.463	28.573	36.005	43.559	51.196	58.888	66.616	74.371	82.146
6	17.308	24.947	33.431	42.434	51.196	60.256	67.377	78.541	87.735	96.953
7	19.547	28.465	38.308	48.506	58.888	69.377	77.936	90.544	101.187	111.855
8	21.810	32.006	43.212	54.812	66.616	78.541	90.544	102.601	114.698	126.823
9	24.090	35.564	48.136	61.141	74.371	87.735	101.187	114.698	128.252	141.839
10	26.393	39.134	53.074	67.487	82.146	96.953	111.855	126.823	141.839	156.891

* This row corresponds to an oblate ellipsoid.

† This column corresponds to a prolate ellipsoid.

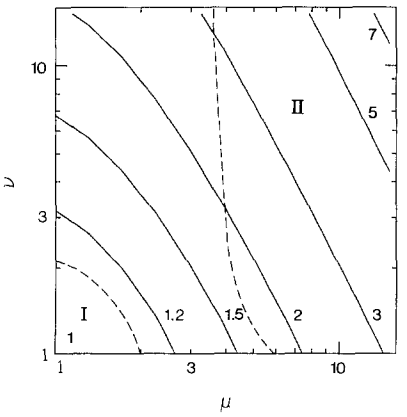

FIG. 5. Contours of constant R in the (μ, ν) plane. The dotted lines delineate regions where the asymptotic values in Table I are accurate to within 5%.

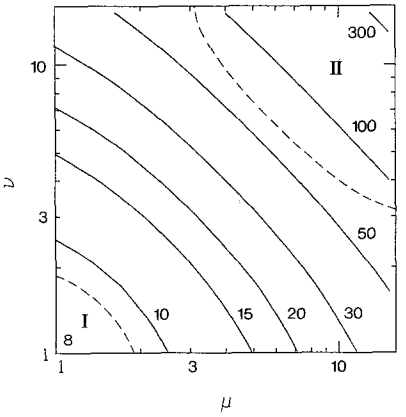

FIG. 7. Contours of constant u_{red} in the (μ, ν) plane.

negligible in comparison to those of Eq. [6] (6, 9).

It will be apparent from Fig. 7 that although a given value of (μ, ν) uniquely specifies a value for u_{red}, the converse is not true: a given value of u_{red} specifies a *line solution* of possible values for (μ, ν). Thus in order to determine (μ, ν) uniquely the solution must be combined graphically with the line solution of another measurable triaxial ellipsoidal property. An additional problem is that in order to determine empirically the shape function u_{red}, the volume V, of a macromolecule *in solution* is required (cf. Eq. [1]). This problem can be circumvented by use of another suspension property which also depends on V, for example, the viscosity increment for triaxial ellipsoids (12, 13) to give a volume-independent function II (14), which, of course, also has line solution properties. Graphical inversion procedures employing the combination of II with other volume-independent triaxial properties to produce (μ, ν) uniquely for a given macromolecule will be described elsewhere.

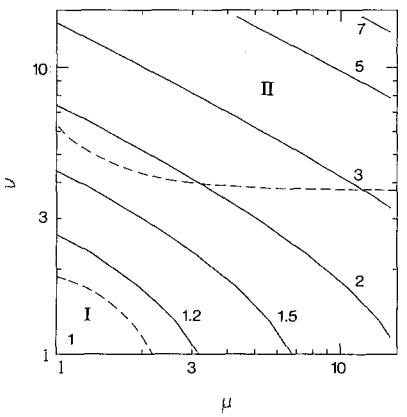

FIG. 6. Contours of constant $S/4\pi$ in the (μ, ν) plane.

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