A general ellipsoid cannot always serve as a model for the rotational diffusion properties of arbitrarily shaped rigid molecules

(molecular shape/hydrodynamic properties/rotational relaxation)

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ABSTRACT An ellipsoidally shaped body, or more commonly, an ellipsoid of revolution, is generally assumed to serve as a convenient model for evaluating the rotational diffusion properties of macromolecules. If Perrin’s equations for the rotational diffusion coefficients of general ellipsoids can be shown to generate all possible rotational diffusion coefficients, then there would exist at least one equivalent ellipsoidal shape for every arbitrarily shaped rigid body. We investigated the problem by first generating a space, r-space, representing all possible ellipsoidal shapes. We then generated another space, D-space, representing all possible combinations of rotational diffusion coefficients. We then mapped r-space into D-space by using Perrin’s equations. Ellipsoidal shapes map into diffusion space in a well-defined manner. The mapping is either 1:1, 2:1, or 3:1; several distinctly different regions of r-space map onto the same regions of D-space. Thus, for some combinations of rotational diffusion coefficients, more than one ellipsoid can be used as a model. Not all of D-space is covered by the mapping of r-space. Therefore, there are combinations of rotational diffusion coefficients that cannot be generated from ellipsoidally shaped bodies. Several examples of rigid body shapes with nonellipsoidal diffusion properties are described.

A general ellipsoid† or, more commonly, an ellipsoid of revolution, serves as a convenient model for analyzing the rotational diffusion properties of macromolecules by various experimental techniques (1–4). It has not been established that a rigid body of arbitrary shape can always be represented by such a model. If Perrin’s equations (1) for general ellipsoids generate all possible rotational diffusion coefficients, then at least one equivalent general ellipsoidal shape should exist for every arbitrarily shaped rigid body. We evaluated the problem by first generating a two-dimensional space, r-space, representing all possible ellipsoidal shapes. Then we generated another two-dimensional space, D-space, representing all possible equivalent rotational diffusion coefficients. Upon mapping r-space into D-space, not all of D-space was covered, indicating that there are combinations of rotational diffusion coefficients that cannot be generated from ellipsoidally shaped bodies.

r-Space

Without loss of generality, we can designate the semi-axes of a general ellipsoid such that \( a_1 \geq a_2 \geq a_3 \). For the purposes of this analysis, ellipsoid shape is important, not size. Because multiplying a combination of semi-axes by any real number does not alter ellipsoid shape, the range of ellipsoidal shapes can be represented by two independent asymmetric parameters:

\[
r_1 = \frac{a_2 - a_3}{a_1 - a_3}; \quad r_2 = a_1/a_3.
\]

(1)

\( r_1 \) measures the degree to which an ellipsoid is prolate or oblate; for prolate ellipsoids \( r_1 = 0 \), and for oblate ellipsoids \( r_1 = 1 \). \( r_2 \) measures the elongation between major and minor axes, indicating departure from a sphere, where \( r_2 = 1 \). Whereas \( r_1 \) is bounded between 0 and 1, \( r_2 \geq 1 \) but unbounded. For every distinctly shaped ellipsoid there is a unique point in r-space, except for spheres, which correspond to a line with \( r_2 = 1 \). Conversely, except for spherical shapes on the line \( r_2 = 1 \), every point in r-space represents a distinctly shaped ellipsoid.

D-Space

In similar fashion, let us label the principal values of the rotational diffusion tensor such that \( D_1 \geq D_2 \geq D_3 \). The rotational diffusion tensor can be regarded as belonging to a prolate-like body for \( D_1 > D_2 = D_3 \), to an oblate-like body for \( D_1 = D_2 > D_3 \), and to a spherical body for \( D_1 = D_2 = D_3 \). For the purposes of this analysis, the absolute magnitudes of the rotational diffusion coefficients are not important, only their relative magnitudes. Multiplying the rotational diffusion coefficients by any real number does not change their relative magnitudes and, therefore, is trivial. Thus, the relation between the three rotational diffusion coefficients can be represented in diffusion space by two asymmetry parameters \( X \) and \( Y \) in which

\[
X = \frac{(D_2 - D_3)}{(D_1 - D_3)}; \quad Y = \frac{D_1}{D_3}.
\]

(2)

The collective space of all such \( X,Y \) points is referred to as D-space. Because \( X = 0 \) when \( D_2 = D_3 \), and \( X = 1 \) when \( D_1 = D_2 \), the boundary line \( X = 0 \) represents prolate-like degeneracy and the boundary line \( X = 1 \) represents oblate-like degeneracy. Similarly, the boundary line \( Y = 1 \) represents spherical degeneracy. D-Space covers all possible combinations of principal values of the rotational diffusion tensor. A rigid body of any shape has three rotational diffusion coefficients and the rotational diffusion tensor of every possible rigid body has an associated point in D-space.

Perrin’s ellipsoid expressions

According to Perrin (1), who corrected the original 1893 derivation of Edwardes, the frictional coefficient \( C_i \) for slow, steady rotations of an asymmetric ellipsoid in a viscous fluid about the principal axis \( i \) is

\[
C_i = \frac{16 \pi \eta}{3} \frac{a_i^2 + a_j^2}{a_i^2 P_j + a_j^2 P_i},
\]

(3)

in which \( \eta \) is the viscosity, \( a_i \) the general ellipsoid semiaxis \( i \), and \( P_i \) the elliptic integral

\[
P_i = \int_0^a \frac{ds}{(a_i^2 + s^2)^{1/2}}.
\]

(4)

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In this paper, the terms general ellipsoid, asymmetric ellipsoid, and ellipsoid are used interchangeably. Prolate ellipsoids, oblate ellipsoids, and spheres are special cases of ellipsoids collectively referred to as ellipsoids of revolution.
in which
\[ \gamma(s) = [(a_1^2 + s)(a_2^2 + s)(a_3^2 + s)]^{1/2}. \]

The integrals \( P_i \) for \( i = 1, 2, 3 \) are related by the identities:
\[ \sum_{i=1}^{3} P_i = 2/(a_1a_2a_3); \quad \sum_{i=1}^{3} a_i^2 P_i = S, \]
in which \( S = \int_0^1 (1/\gamma(s))ds \). The rotational diffusion coefficient corresponding to axis \( i \) is
\[ D(a_i) = kT/C_i, \]
in which \( k \) is Boltzmann's constant and \( T \) the absolute temperature. As shown below, the order of the principal values of the rotational diffusion tensor represented by Perrin's equations may be different from the order of the semi-axes of the ellipsoid; i.e., \( D_i \neq D(a_i) \).

**Mapping \( r \)-space into \( D \)-space**

We mapped \( r \)-space into \( D \)-space by using Perrin's expressions. Prolate ellipsoids, represented along the line \( r_1 = 0 \), mapped on the line \( X = 0 \); oblate ellipsoids along the line \( r_1 = 1 \) mapped on the line \( X = 1 \); and spheres represented by the line \( r_2 = 1 \) mapped on the line \( Y = 1 \).

Other lines generated in \( r \)-space by a computer calculation were mapped into \( D \)-space. Lines were defined for various constant values of \( r_2 \); for each \( r_2 \) value, 101 values of \( r_1 \) equally spaced between 0 and 1 were used. For each point on such a line, the semi-axes were expressed in terms of Eq. 1 as:
\[ a_1 = r_2a_3; \quad a_2 = [r_1r_2 + (1 - r_1)]a_3. \]

In Eq. 8, the semi-axes are all proportional to \( a_3 \), which determines the ellipsoidal size for a given \( r_1, r_2 \) pair. Setting \( a_3 = 1 \), the elliptic integrals were evaluated by the DCADRE subroutine program in the International Mathematics and Statistics Library. The resulting rotational diffusion coefficients were used to generate an \( X, Y \) pair according to Eq. 2.

In Fig. 1A, 10 curves are plotted in \( D \)-space for \( r_2 \) values between 1 (sphere) and 2.46. The curves form a family, distinct, without crossovers, nested in order of the \( r_2 \) values. As \( r_1 \) moves from 0 to 1, the corresponding value of \( X \) moves monotonically from 0 to 1. The value \( r_2 = 2.46 \) generates the maximum \( Y \) (\( Y = 1.26, X = 1 \)) corresponding to an oblate ellipsoid. As the degree of oblateness increases further as \( r_2 \to \infty \), the value of \( Y \) decreases monotonically to 1; i.e., both an infinitely thin flat disk and a sphere are characterized by \( D_1 = D_2 = D_3 \). On the other hand, there is no such reversal in \( D \)-space for prolate ellipsoids (\( X = 0 \); \( Y \) increases continuously from 1 to \( \infty \) *parri passu* with \( r_2 \). In Fig. 1B, seven curves are plotted from \( r_2 = 2.46 \) to the limit \( r_2 \to \infty \). The first point of each curve (which is not depicted) generated by \( r_1 = 0 \), occurs at \( X = 0 \). For each curve, as \( r_1 \) increases, the corresponding \( Y \) value decreases. At first, as \( r_1 \) increases from 0, the \( X \) value also increases from 0. However, for further increases of \( r_1 \), the trend reverses and \( X \) decreases, each curve returning to \( X = 0 \). As \( r_1 \) increases still further to approach 1, \( X \) increases to 1. The curves continue to be ordered, but approach a boundary such that a well-defined region of \( D \)-space is not filled by ellipsoids of any shape. The limit curve when \( r_2 \to \infty \) is derived in the *Appendix* and is shown to depend only on \( a_1 \) and \( a_2 \) by the relations:
\[ X = a_2^2/(a_1^2 + a_2^2); \quad Y = P_2/P_1, \]
where \( P_1 \) and \( P_2 \) are given in Eq. 4 with \( a_3 = 0 \).

**Structure and degeneracies of the mapping**

The shaded areas of Fig. 2, representing the area of \( D \)-space covered by \( r \)-space, were obtained by combining the areas covered by the curves for \( r_2 \leq 2.46 \) and \( r_2 \geq 2.46 \) (Fig. 1). There are regions of \( D \)-space that cannot be associated by this mapping with any region of \( r \)-space. Thus, there are certain combinations of rotational diffusion coefficients that cannot be attributed to general ellipsoids. Furthermore, the mapping is not one to one. The boundary of the mapped region is delineated by two intersecting curves; one is associated with the beginning of turnaround behavior for oblate ellipsoids where \( r_2 = 2.46^6 \) and the other is the limit curve generated as \( r_2 \to \infty \). These two curves divide \( D \)-space into four regions. In region I, bounded by segments \( O \) and \( QQ \), each \( X, Y \) pair is uniquely generated by one ellipsoidal shape. Region II, bounded by segments \( OO \) and \( Q\), is double degenerate; two distinct shapes generate the same \( X, Y \) pair. Region III, bounded by segments \( O \) and \( Q\), is triply degenerate; three distinct shapes generate the same \( X, Y \) pair. Last and most important, no point in region IV corresponds to a general ellipsoid.

When examined, \( r \)-space consists of definite regions as does

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4 Turnaround behavior does not begin at precisely \( r_2 = 2.46 \) but is slightly dependent on the value of \( X \), although the discrepancy is small on the scale of \( D \)-space in Fig. 2. The exact boundary curve was obtained by examining many curves with nearby values of \( r_2 \) and varied smoothly from \( r_2 = 2.46^6 (Y = 1.26) \) for oblate ellipsoids to \( r_2 = 2.3 (Y = 2.2) \) for prolate ellipsoids. The \( Y \) values close to the beginning of turnaround were plotted in the figures rather than those precisely on the curve \( r_2 = 2.46 \).
Ellipsoidal nonuniqueness

The relationship between some ellipsoidal shapes and the rotational diffusion coefficients generated from them is not one to one and, therefore, cannot be unique. Points in region I of D-space are uniquely associated with ellipsoids where \( r_2 \geq 2.46 \). Nonuniqueness appears in regions II and III of D-space where ellipsoids with \( r_2 \leq 2.46 \) are necessarily constrained. For such ellipsoids there will always be one or two ellipsoids with \( r_2 \geq 2.46 \) that have the same \( X,Y \) value. Thus, several attributions of the rotational diffusion tensor are always possible if \( Y \) is less than about 1.4 (point c, Fig. 2) in regions II or III.

Because prolate ellipsoids are usually used as convenient models, it is of interest to note that any ellipsoid corresponding to a point on curve \( p_2 \) of Fig. 3 with \( r_2 \geq 2.46 \) behaves like another prolate ellipsoid corresponding to a point on line \( p_1 \) of Fig. 3 with \( r_2 \leq 2.46 \) such that \( D_2 = D_3 \) and \( D_1 < D_2 \). Table 1 lists some ellipsoids that are not prolate, but that have two degenerate rotational diffusion coefficients such that they behave in a prolate-like manner. They correspond to points along curve \( p_2 \) from the first ellipsoid that is prolate (\( Y = 2.2 \)) to the infinitely thin disk (\( Y = 1 \)). In order to give insight into the actual shapes, the three semi-axes are given in addition to \( r_1 \) and \( r_2 \).

Ellipsoidal insufficiency: Other models

Combinations of rotational diffusion coefficients that map in region IV cannot be attributed to general ellipsoids. Although methodologies exist for evaluating the frictional resistance tensor and determining the rotational diffusion coefficients of more general shapes, their use has thus far been restricted to cases of cylindrical symmetry (5, 6). Distinctive cases with differences from ellipsoids have not been reported. Therefore, we present several such definitive cases which establish the fact that model shapes with nonellipsoidal diffusion properties, indeed, do exist. In addition, these cases suggest some behavioral trends.

Table 1. Ellipsoids that are not prolate (corresponding to points on curve \( p_2 \), but have two degenerate rotational diffusion coefficients

<table>
<thead>
<tr>
<th>( a_1 )</th>
<th>( a_2 )</th>
<th>( a_3 )</th>
<th>( r_1 )</th>
<th>( r_2 )</th>
<th>( Y )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0</td>
<td>2.3</td>
<td>2.2</td>
</tr>
<tr>
<td>3.0</td>
<td>1.6</td>
<td>1.0</td>
<td>0.3</td>
<td>3.0</td>
<td>2.1</td>
</tr>
<tr>
<td>4.0</td>
<td>2.6</td>
<td>1.0</td>
<td>0.53</td>
<td>4.0</td>
<td>1.8</td>
</tr>
<tr>
<td>5.0</td>
<td>3.5</td>
<td>1.0</td>
<td>0.63</td>
<td>5.0</td>
<td>1.7</td>
</tr>
<tr>
<td>7.5</td>
<td>5.9</td>
<td>1.0</td>
<td>0.75</td>
<td>7.5</td>
<td>1.4</td>
</tr>
<tr>
<td>10.0</td>
<td>8.4</td>
<td>1.0</td>
<td>0.82</td>
<td>10.0</td>
<td>1.3</td>
</tr>
<tr>
<td>25.0</td>
<td>23.3</td>
<td>1.0</td>
<td>0.93</td>
<td>25.0</td>
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</tr>
<tr>
<td>( \infty )</td>
<td>( \infty )</td>
<td>1.0</td>
<td>1.0</td>
<td>( \infty )</td>
<td>1.0</td>
</tr>
</tbody>
</table>
The dashed curve in Fig. 2 is generated by a long rigid rod bent at its center; it is an example of a rigid body whose rotational diffusion coefficients cannot always be represented by an equivalent ellipsoid. The body was evaluated by considering two equal prolate ellipsoids of axial ratio 10:1 joined at their ends (unpublished data). No correction was made for hydrodynamic interactions between the two ellipsoids. Positions along the curve correspond to various bending angles $\beta$. At $\beta = 0$, the rod is not bent and may be represented by a prolate ellipsoid as $X = 0$. As $\beta$ increases, the degree of bending increases. Initially, the rotational diffusion coefficients of the bent rod lie in region I and may be represented by a general ellipsoid. At $\beta = 110^\circ$, the rod enters region IV, where it cannot be so represented. At $\beta = 125^\circ$, the asymmetry parameter reaches $X = 1$ and then decreases as the bending angle is increased further. At $\beta = 140^\circ$, region I is reentered and the body can be represented by a general ellipsoid. At $\beta = 180^\circ$, the rod is completely bent back upon itself and can again be represented as a prolate ellipsoid. The F(ab)2 fragment of immunoglobulin is a biologically important macromolecule of this type.

Two related cases also illustrate behavior that cannot be attributed to general ellipsoids. These cases have relevance for macromolecular assemblies. First, consider $n$ equal spheres each with translational frictional resistance coefficient $f$ placed at the center and the vertices of a planar regular polygon of $n - 1$ sides. Connections between the spheres are assumed to be rigid and without frictional resistance. The spheres are far enough apart that hydrodynamic interaction effects can be neglected. The spheres are small enough compared to the distance $R = |R|$ from the center of the polygon so that frictional resistance to rotations about the center of the polygon involves only lever arm terms, such as components of the dyadic $fRR$, and neglects the rotational contribution for rotations of a sphere about its own center. The frictional resistance of the polygonal figure to rotations about the normal to the plane is one-half that for rotations about any axis in the plane, as determined by construction from methods of Happel and Brenner (7). This is true for any $n \geq 3$ (triangles, squares, etc.). Denoting $D_0$ as the diffusion coefficient for rotation about the normal, $D_1 = D_2 = 2D_3$ which leads to $X = 1$, $Y = 2$ and plots in region IV of $D$-space.

In the last example, all $n$ spheres are simply replaced by identical prolate ellipsoids with the long symmetry axis normal to the plane. The semiaxes are small compared to $R$. In the limit of infinite axial ratio, the translational frictional resistance for motion in the direction of the semimajor axis of a prolate ellipsoid is one-half that for sideways motion. In this limit $D_1 = D_2 = 4D_3$, which leads to $X = 1$, $Y = 4$. Although it might be difficult for macromolecular assemblies of subunits to fulfill these structural conditions, it seems reasonable that macromolecular assemblies with perpendicular symmetry axes may exist where $X = 1$ and $Y > 1.26$. If the prolate symmetry axes are tilted, or the subunits are not identical, etc., results other than those with $X = 1$ would be generated.

There may be other physical reasons why bodies cannot be represented by ellipsoids in diffusion space. Rigid bodies with intrinsic coupling between rotational and translational motion studied by Brenner (8, 9) may show significant deviations from ellipsoidal modeling behavior. For small bodies, the hydrodynamic equation with boundary conditions used to derive Perrin’s equations may not be appropriate. Hu and Zwanzig (10) attempted to deal with this problem, deriving rotational frictional resistance coefficients for ellipsoids of revolution with the low Reynold’s number Stoke’s equation of hydrodynamics by using slip boundary conditions instead of the usual stick boundary conditions; even this derivation may not be valid for bodies comparable in size to solvent molecules. Last, proteins, nucleic acid, and many man-made polymers are polyelectrolytes, in which the coupling to charge fluctuations in the solvent may result in additional torques.

The implications of these results on characterization of rigid body rotational relaxation expressions for various experimental techniques such as time-dependent fluorescence depolarization and transient birefringence will be considered in subsequent communications.

Appendix: Ellipsoidal limit curve in D-space

The limit curve in $D$-space for ellipsoids when $r_2 \rightarrow \infty$ is derived as follows. Referring to Osborn (10), we obtain from Eqs. 3 and 7

$$D'_1 = (a_3^2M + a_3^2N)/(a_3^2 + a_3^2)$$

$[A-1a]$

$$D'_2 = (a_3^2L + a_3^2N)/(a_3^2 + a_3^2)$$

$[A-1b]$

$$D'_3 = (a_3^2L + a_3^2M)/(a_3^2 + a_3^2)$$

$[A-1c]$

in which $D'_i = \eta D(a_i/kT, L = nP_1, M = nP_2, N = nP_3$, and $n = 2\pi a_3a_2a_3$.

In order to treat the limit, we expand the denominators of Eqs. A-1 to order $r_2^2$

$$D'_1 = M + r_2^2(a_1/a_2)^2N$$

$[A-2a]$

$$D'_2 = L + r_2^2N$$

$[A-2b]$

$$D'_3 = L + r_2^2(a_2/a_3)^2M$$

$[A-2c]$

Case a. Consider a very slender ellipsoid such that $r_2 \gg (a_2/a_3)$ $\geq 1$. In order to approach the limit as $r_2 \rightarrow \infty$, the expressions for $L$, $M$, and $N$ are written to order $r_2^2$ as (11)

$$L/4\pi = O(r_2^2)$$

$$M/4\pi = a_3/(a_2 + a_3) + O(r_2^2)$$

$$N/4\pi = a_2/(a_2 + a_3) + O(r_2^2)$$

$[A-3]$

Substituting Eqs. A-3 into Eqs. A-2b, A-2c, and A-1a yields

$$D'_1 = a_2a_3/(a_2^2 + a_3^2) + O(r_2^2)$$

$$D'_2 = O(r_2^2)$$

$$D'_3 = O(r_2^2)$$

$[A-4]$

Using Eqs. A-4 in the definitions of Eq. 2, as $r_2 \rightarrow \infty$

$$X \rightarrow 0 \quad \text{and} \quad Y \rightarrow \infty$$

$[A-5]$

If $r_2 \gg (a_2/a_3)$, Eq. A-5 also holds for intermediate shapes where $r_2 \gg (a_2/a_3) \gg 1$.

Case b. Consider a very flat ellipsoid where $r_2 \geq (a_2/a_3) \gg 1$. The approximate expressions for $L$, $M$, and $N$ to order $(r_2^2)$ are (11)

$$L/4\pi = (r_2^2)/(1 - e^2)^{1/2}(K - E)/e^2$$

$$M/4\pi = (r_2^2)(E - (1 - e^2)K)/e^2(1 - e^2)^{1/2}$$

$$N/4\pi = 1 - [(r_2^2)/E/(1 - e^2)^{1/2}]$$

$[A-6]$

$K$ and $E$ are complete elliptic integrals with argument $e$, given as

$$e = [1 - (a_2/a_1)^2]^{1/2}$$

$[A-7]$

with $K \geq E$, $L$, $M$, and $N$ are bounded for all ellipsoids. $E$ is bounded for all ratios $a_1/a_2$, whereas $K$ is finite for all finite $a_1/a_2$ values.

Substituting Eqs. A-6 into Eqs. A-2a, A-2b, and A-1c, we obtain to first order in $r_2^{-1}$
\[ D_1 = M \]
\[ D_2 = L \]
\[ D_3 = \frac{a_2^3 L + a_3^3 M}{(a_1^3 + a_2^3)} \]  \[ \text{[A-8]} \]

From Osborn's tables (11) or by examining Eqs. A-6, \( M \geq L \)
with equality holding when \( a_1 = a_2 \) for the very flat oblate ellipsoid. Thus, the ordering of \( D_1 \) is \( D_1 \geq D_3 \geq D_2 \). Using Eq. 2, we have
\[ X = \frac{(D_3 - D_2)/(D_3 - D_2)}{a_2^2/a_3^2} = \frac{a_2^2}{(a_1^2 + a_2^2)} \]
\[ Y = \frac{D_1/D_2}{M/L}. \]  \[ \text{[A-9]} \]

Observing the Eqs. A-6 are obtained by substituting \( a_3 = 0 \)
into Osborn's exact expressions (11), Eqs. A-9 are written equivalently as
\[ X = \frac{a_2^2}{(a_1^2 + a_2^2)} \]
\[ Y = \frac{P_2}{P_1}, \]  \[ \text{[A-10]} \]
in which \( P_1 \) and \( P_2 \) are given by Eq. 4 with \( a_3 = 0 \). Eqs. A-10
are exact when \( r_2 \to \infty \), with \( a_1 \) finite. For an infinitely flat disk
where \( r_2 = (a_2/a_3) \to 1 \), \( X = 1/2 \) and \( Y = 1 \).

As the ratio \( a_1/a_2 \) increases, leading to intermediate shapes
where \( r_2 \gg (a_2/a_3) \to 1, e \to 1 \) and \( E \to 1 \), while \( (1 - e^2)K \to 0 \) (12, 13). From Eqs. A-6, \( M/L \) diverges; therefore, Eqs. A-10
lead to
\[ X \to 0 \] and \[ Y \to \infty. \]  \[ \text{[A-11]} \]

This is the same result derived in case a for intermediate shapes.
Therefore, the expressions in Eq. 9 (A-10) for the \( X,Y \) limit curve
apply to all shapes as \( r_2 \to \infty \).

Last, we note that the qualitative behavior of the \( X,Y \) limit curve
can be obtained from the approximation \( P_1/P_2 \approx a_2/a_1 \)
given by van de Hulst (13). Thus, from Eqs. A-10 we obtain
\[ Y^2 \approx \left( \frac{1}{X} \right) - 1. \]  \[ \text{[A-12]} \]

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