

Hard-Sphere Hydrodynamics as Reference State in Velocity Cross-Correlation Analysis of Real Systems

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The ability of hard-sphere hydrodynamics to model translational motions in real systems is discussed. The possibility to split the excluded volume effect on mutual diffusion into thermodynamic and hydrodynamic contributions is established by using accurate experimental activity and mutual diffusion data. The combination of hard-sphere theory at the second order of volume fraction for mutual diffusion, intradiffusion, and thermodynamic properties allows calculation of velocity cross-correlation coefficients (VCC), herein proposed as new standard coefficients. The comparison of these new standards with real VCCs, corresponding to several aqueous solutions of conventional solutes and macromolecules, tests the applicability of this procedure. The new VCC analysis scales the excluded volume effect and allows more meaningful discussion about correlation of translational motion in solution, especially in the case of the cross-term solute–solvent VCCs in macromolecule–solvent mixtures. Possible extension to analytically quantify correlation between internal motions of biopolymers is briefly discussed.

I. Introduction

The interaction between solute and solute, solvent and solvent, and solute and solvent in aqueous solutions is still a formidable task. In the past decade, the inversion of the Kirkwood–Buff theory¹ was successfully used to analyze binary² and ternary systems.³ This theory allows derivation of preferential solvation analysis from thermodynamic data, such as partial molar volume or excess enthalpy or Gibbs free energy. The preferential solvation (PS) defines what each molecule “sees” around it, using the Kirkwood–Buff integrals, $G_{\alpha\beta}$ ⁴

$$G_{\alpha\beta} = \int_0^\infty [g_{\alpha\beta}(R) - 1] 4\pi R^2 dR \quad (1)$$

where $g_{\alpha\beta}(R)$ is the angle-averaged pair correlation function and R is the interparticle distance. The quantity in eq 1 is related to many thermodynamic equilibrium properties.⁵

A few years ago, some authors have suggested the use of a correct reference state as standard for a meaningful PS analysis.^{6,7} Sometimes, hard-core potential masks specific attractive interactions; therefore, it was suggested to subtract from Kirkwood–Buff integrals the contributions associated with excluded volume or combinatorial entropy effects. This correction is especially necessary when size mismatch of components is present. Therefore, this issue is particularly relevant in the case of macromolecule–solvent systems. Recently, we extracted this size effect, testing it on the homologous chemical series of poly(ethylene glycol) (PEG).⁸

The present work is intended to transfer this same concern to another kind of analysis, the velocity cross-correlation analysis (VCA), conveying complementary information to the PS.⁹ Analogously to the PS analysis, the VCA provides information about the spatial correlation between two distinct molecules.

The difference is important from a dynamic standpoint. In fact, for velocity cross-correlation analysis, the main quantities are the integrals⁹

$$f_{\alpha\beta} = \left\{ N \frac{x_\beta}{3} \int_0^\infty \langle u_\alpha^{(i)}(0) u_\beta^{(j)}(0) \rangle_v dt \right\}_{v \rightarrow \infty} \quad (2)$$

that are dynamic properties, mixture of equilibrium and non-equilibrium physical quantities. In eq 2, x_j is the molar fraction of j , N is the total number of particles in the system, t is the time, and the velocities v refer to different particles α and β , even if $i = j$. The f_{ij} in eq 2 is given in the baricentric reference frame, and the pointed brackets represent a canonical ensemble average. Correlations in eq 2 are named “cross-” because we follow the velocity correlation between two distinct particles i and j (even though belonging to the same species) not the same particle (as in the case of velocity *self*-correlation in intradiffusion).

The distinct diffusion coefficients (DDCs) of Friedman and Mills⁹ offer an alternative way to discuss correlation of motion, if referred to the same reference frame, and they are simply related to the f_{ij} by a composition factor.¹⁰ Distinct diffusion coefficients are a measure of the displacement of a particle i due to the displacement of a particle j , where the particles are distinct even if species are the same. For obviating to distortion in the DDCs from eventual large mass and volume differences between solvent and solute, it was conveniently suggested to use the number fixed frame of reference.¹⁰ Equations to switch from a frame of reference to another and from VCCs to DDCs are reported in ref 10.

Weingärtner¹¹ highlighted the possibility to couple the preferential solvation and the velocity correlation analyses as a powerful tool to understand the molecular mechanism of the transport in solution. In the present paper, the risk in using the usual velocity correlation analysis for macromolecule–solvent mixtures, such as polymer–solvent or protein–solvent, is discussed.

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The velocity cross-correlation analysis has proved to be useful in investigating the complex behavior of the transport mechanism for many systems. It has been studied for many binary systems of electrolytes¹² and nonelectrolytes¹³ with different degrees of nonideality. Positive¹¹ and negative deviation from the Raoult's law¹⁴ and near-ideality systems¹⁵ were the subject of several studies. It is desirable that the velocity correlation analysis be reliable even for macromolecule–solvent systems. Until now, the standard procedure was based on the comparison with an ideal system for which the balance of the moment equation is applied.¹⁶ Recently, based on the usual procedure, a preliminary velocity cross-correlation analysis along the homologous series of PEG (poly(ethylene glycol)) has been presented,¹⁷ emphasizing the superposition of different effects, specific and nonspecific ones. Specific effects are those related to the chemistry of the binary mixture. Nonspecific effects are due to an excluded volume effect (related to the size, shape, and aggregation state of solutes in solution) and to a combinatorial effect (related to the distribution of the conformational space of the solute). In the present paper, we propose a novel approach subtracting nonspecific effects from the velocity cross-correlation integrals. This new reference state is shown to be useful for analyzing macromolecule–solvent mixtures.

The hard-sphere hydrodynamics has been widely investigated in the literature both for binary and ternary systems.^{18–20} As the size of the real solute becomes larger, the repulsive excluded volume effect that takes part in the overall diffusion mechanism becomes larger. The study of a homologous chemical series (such as the PEG one) showed the balance between specific and nonspecific interaction changing with the molecular size.^{17,18,21–24}

Furthermore, recent mutual diffusion studies in multicomponent systems containing macromolecules have shown strong cross-correlation of motion between molecules with large size mismatch.^{19,25,26} These correlations of motion are well described by equations based on excluded volume concepts.^{18,20}

The purpose of the present study is 2-fold: (1) we analyze the excluded volume contribution on the thermodynamic and dynamic properties combined into mutual diffusion for a variety of solutes; (2) we develop a new VCC reference state, based on hard-sphere hydrodynamics, useful to the velocity cross-correlation analysis of real systems.

At the end of the paper, applications of these new standards are presented for various systems, aqueous solution of small solutes and macromolecules, highlighting the differences between the standard and new procedure. A brief review of the protein diffusion data is critically presented.

Ultimately, this paper contributes a new tool to analyze the correlation of motions between constituents of a solution, especially helpful to systems containing macromolecules. A novel general criterion to distinguish between attractive and repulsive interaction is presented.

II. Theoretical Background

Let us identify mutual diffusion coefficients of a binary system (solute(*i*)–solvent) as D_i . The intradiffusion coefficients of solute *i* and of the solvent (0) are indicated with D_i^* and D_0^* , respectively. Recently, macroscopic and microscopic differences between the mutual (or inter-, cooperative) and intra- (or self-) diffusion have been reviewed;¹⁷ therefore, the reader is directed therein for more details.

Thermodynamics of irreversible processes describes diffusive flux in *n*-component systems through Onsager coefficients, L_{ij} , and chemical potential gradients, $\nabla\mu_i$.²³

$$J_i = -\sum_{j=1}^{n-1} L_{ij} \nabla \mu_j \quad D_{ij} = \sum_{k=1}^n L_{ik} \frac{\partial \mu_k}{\partial C_j} \quad (3)$$

that for binary systems turns into

$$D_i = B_i^{(c)} M_i \quad (4)$$

Therefore, the mobility, M_i , is the hydrodynamic contribution, independent of thermodynamic terms that are included in $B_i^{(c)}$

$$B_i^{(c)} = \left(1 + \frac{\partial \ln f_i^{(c)}}{\partial \ln c_i} \right) \quad (5)$$

where $f_i^{(c)}$ is the activity coefficients in a generic concentration scale *c*. In this paper, we used both the molar fraction *x* and the molar concentration *C*. We also note here the useful thermodynamic relations $B_i^x = B_0^x$ and $B_i^C = B_i^x x_0 / \phi_0$, where ϕ_0 is the solvent volume fraction.

An equivalent approach is based on the thermodynamic expression for $(\partial \mu_i / \partial C_i)_{T,p}$ in terms of the virial expansion for the osmotic pressure. In the present paper, we have used each of the two approaches, according to the available thermodynamic data. The interplay between the concentration dependence of the thermodynamic (B_i^C) and the hydrodynamic factor (M_i) determines the concentration dependence of D_i , even though the two terms are intimately interrelated, as shown in the present paper. In the case of binary systems, differently from D_i , M_i values are dependent on the concentration scale. Using literature activity data, one can derive thermodynamic factors and evaluate the mobilities for several systems.

The increasing trend of mutual diffusion coefficients (cooperative diffusion) in polymer–solvent systems as a function of the concentration for samples with a molecular weight higher than a critical value, M_n^* , is a characteristic common to many macromolecules.²¹ It can be justified if theoretical flux equations are assumed to describe the motion. As model polymer, we consider PEG, the mutual diffusion,²¹ intradiffusion,²⁷ and activity data^{28,29} of which are extensively available for several molecular weights and along a wide range of concentration. The conventional trend of the mobilities, decreasing as the polymer concentration increases, indicates that the increasing nonideality with molecular weight sensitively affects the mutual diffusion coefficient values for PEG samples with molecular weight larger than 1000 Da.²¹ It is worth pointing out that mobilities have a similar trend, although not equal numerical values of intradiffusion data.²⁷ Indeed, the nonideality of polymer solutions is strictly related to the conformation of the segment distribution. The special case of PEG 1000 (for which no concentration dependence is observed) is due to a balance of two opposite effects, hydrodynamic and thermodynamic ones. In the case of PEG, this limiting size is small compared to diffusive data of other polymers. This limiting molecular weight for dextran in water is about 20 000,³⁰ and that for poly(acrylic acid) in water is higher than 100 000.³¹ The limiting molecular weight of PEG is due to the high value of the second virial coefficient of PEG with respect to common polymers.²⁸

A different behavior is shown by surfactants and electrolytes. For surfactants, both the thermodynamic and diffusion coefficients decrease with the solute concentration, whereas mobilities increase.³² The behavior of simple electrolytes is out of the present discussion and was treated by Miller.³³

Several attempts were made to relate directly mutual and intradiffusion coefficients, but a simple and general approach

does not yet exist. For nonelectrolytes, the Darken equation correlates mutual diffusion to intradiffusion coefficients through the thermodynamic factor

$$D_i = (x_0 D_i^* + x_i D_{(o)i}^*) B_i^x \quad (6)$$

The well-studied homologous chemical series of PEG allows testing the correlation for systems with increasing nonideality. As anticipated in a work on PEG oligomers, these polymer samples present large deviation from ideality; hence, the Darken equation is not applicable to PEGs with molecular weight larger than 400 Da.²¹ The deviation increases as the chain length increases. Actually, the Darken equation holds only when the sum of correlation integrals is zero.³⁴

The most rigorous approach to link equilibrium and non-equilibrium transport properties is based on the linear response theory.³⁴ According to this theory, a parallel use of experimental mutual diffusion, intradiffusion, and activity coefficients yields a description of correlation of motion between molecules in solution through the evaluation of main- ($i = j$) and cross- ($i \neq j$) terms velocity cross-correlation coefficients (VCCs), defined in eq 2.

The following relations hold for the VCCs¹¹

$$f_{ii} = D_i \frac{M_0^2 x_0}{M^2 B_i^x} - D_i^* \quad (7)$$

$$f_{i0} = -D_i \frac{M_i M_0}{M^2 B_i^x} x_0 = f_{0i} \frac{x_0}{x_i} \quad (8)$$

$$f_{00} = D_i \frac{M_i^2 x_i}{M^2 B_i^x} - D_0^* \quad (9)$$

where $M = M_i x_i + M_0 x_0$. In a stable mixture, B_i^x is positive, so because D_i is positive, for $x_i \neq 0$ f_{i0} must always be a negative quantity. There are restrictions among the intradiffusion and cross-correlation coefficients, accounting for the microscopic reversibility.¹⁷

The coefficients in eqs 7–9 do not play the role of an indicator for molecular association phenomena if not compared with some appropriate standard. Usually, VCCs are compared to some standards of reference proposed by Hertz¹⁶ and based on the law of effective moment conservation

$$f_{ii}^\circ = -\frac{x_i M_i D_i^* (1 - x_0 \zeta_{i0})}{M} \quad (10)$$

$$f_{i0}^\circ = -\frac{x_0 M_i D_i^* (1 + x_i \zeta_{i0})}{M} \quad (11)$$

$$f_{00}^\circ = -\frac{x_0 M_0 D_0^* (1 - x_i \zeta_{0i})}{M} \quad (12)$$

where

$$\zeta_{i0} = \frac{M_i D_i^*}{M_0 D_0^*} - 1 \quad (13)$$

$$\zeta_{0i} = \frac{M_0 D_0^*}{M_i D_i^*} - 1 \quad (14)$$

The classic standard VCC^os (eqs 10–12) are expressed only in terms of dynamic noncollective properties of the systems, that is, they are functions of the two intradiffusion coefficients. To give an interpretation of the differences between VCC and VCC^o, it is worth reviewing how the formulas for the standard VCC^os are derived. They are obtained considering that the mean value of all molecular velocities in the system is zero. Therefore there must be a movement of the remaining molecules in the opposite direction with a drift velocity. Recalling the law of linear conservation of momentum and assuming that the velocity cross-correlation function decays essentially in the same manner as the velocity self-correlation function of species i ; after normalization, eqs 10–12 are obtained. Because no specific or nonspecific interaction is assumed in VCC^o derivation, the difference between VCC and VCC^o provides information on the motion correlation between solute–solute, solute–solvent, and solvent–solvent. Typically the criterion of molecular association is that $f_{ij} > f_{ij}^\circ$.¹¹ In the presence of a large size mismatch of components, there is the risk of masking attractive self- or cross-interaction due to an excluded volume effect.³

This paper aims to propose more meaningful references, alternatives to eq 10–12, as derived in the next section.

Although the paper deals with a wide interest application field, it does not include other important issues, below reported. Recently novel experimental (through scanning tunneling microscopy)³⁶ and theoretical^{37,38} approaches have been presented for analyzing two-dimensional VCC (in surface phenomena). Similarly, the important field of electrolyte aqueous solutions, extensively discussed in the literature from a numerical simulation point of view,³⁹ is not treated in the present manuscript.

III. VCC of Hard Spheres

The theory of hard spheres (*hs*)–solvent (0) is well developed for mutual diffusion,⁴⁰ intradiffusion,⁴¹ and thermodynamic factor¹

$$D_{hs} = D_{hs}^\infty (1 - 0.90\phi_{hs} - 19.01\phi_{hs}^2 + 70\phi_{hs}^3) \quad (15)$$

$$D_{hs}^* = D_{hs}^\infty (1 - 1.73\phi_{hs} + 0.87\phi_{hs}^2) \quad (16)$$

$$B_{hs} = 1 + 8\phi_{hs} + 30\phi_{hs}^2 + 72\phi_{hs}^3 \quad (17)$$

Equation 15 was derived by Phillies. We use preferentially this equation rather than the Batchelor's one⁴² because Phillies included the sometimes-omitted tensor divergence correction to D , terms appearing intrinsically in the n -particle Smoluchowski equation. The Phillies' equation was derived from the initial slope of the dynamic structure factor, corresponding to the diffusion coefficient measured by dynamic light scattering (DLS). A parts of the experimental mutual diffusion coefficients herein used are based on classical boundary spreading instruments. Preston verified that experimentally, unless intermolecular interactions have a range comparable with a light wavelength, these two diffusion coefficients are equal.⁴³ Several experimental data on colloidal particles tested positively the decreasing trend predicted by eq 15. Anyway some data showed a positive slope 1.3–1.45 (as the Batchelor's result), but the silica spheres were stabilized by coating their surface with short (C₁₈) hydrocarbon,⁴⁴ so that they were not truly hard spheres. Anyway we present the Batchelor's equation (superscript B), and the corresponding VCCs, as well⁴²

$$D_{hs}^B = D_{hs}^\infty (1 + 1.56\phi_{hs} + 0.91\phi_{hs}^2) \quad (18)$$

Equations 16 and 17 are more widely accepted, but we would just mention alternative more recent equations for mutual and intradiffusion of hard sphere from Muramatsu–Minton⁴⁵ and Han–Herzfeld,⁴⁶ respectively, based on the definition of a target volume. In the present analysis, the solvent diffusivity is concentration-independent (equal to the pure solvent value) because of the hard-sphere model chosen. Alternative treatments for the solvent intradiffusion are based on alternative obstruction theories.⁴⁷

The substitution of eqs 15–18 in the definition of VCCs provides a novel VCC standard that will offer a new way to compare Brownian dynamic simulation to the hydrodynamic theory of hard spheres.⁴⁸ The use of standard VCC for different, more complicated model systems is limited by the absence of any analytic expression like eqs 15–17, except that for hard spheres. The idea proposed in this paper can extend to other model systems, once Brownian dynamics simulations were available to evaluate velocity cross-correlation coefficients for soft spheres.⁴⁹

This exclusion contribution to the VCCs permits recognition of the contribution of the velocity correlation due to a specific interaction as below discussed.

In Figure 1a–c, we report the VCC corresponding to a sphere of three different sizes and up to a volume fraction of 0.15, using a specific volume typical of a protein ($0.74 \text{ cm}^3 \text{ g}^{-1}$).⁵⁰ These volumes correspond to solutes that will be investigated in the following VCC analysis. We present three references: one for the standard Hertz reference (H), and the new references derived by using the Phillies' (P) and Batchelor's (B) equations (eqs 15 and 18, respectively). The Phillies' and Batchelor's results are slightly different, but both are very different from the Hertz references. The results can be summarize as follows: (a) $f_{ii}^P < f_{ii}^B < f_{ii}^H < 0$, (b) $0 > f_{i0}^H > f_{i0}^P > f_{i0}^B$, and (c) $f_{00}^B > f_{00}^P > 0 > f_{00}^H$.

The difference between Phillies' and Batchelor's results is consistent with the fact that Batchelor equation omits the divergence of the diffusion tensor. That makes the Phillies' diffusive behavior much more anticorrelated than the Batchelor's one, clearly apparent from relation (a).

The sequence in relation (b) is a consequence of the anticorrelation between hard spheres, which implies an increase of correlation between solvent–solvent and solute–solvent.

The result (c) indicates that an apparent solvent–solvent attraction can be artificially deduced from a comparison with the Hertz's reference, which should be accounted to the hard-core interaction. The Hertz's reference never accounts for a positive f_{00} , as Phillies's and Batchelor's do. This is a first evidence that the standard (Hertz) procedure can lead to misleading conclusions.

Finally, differences reported here can sensitively affect the interpretation of the velocity correlation analysis. A more meaningful analysis can be derived by comparing the experimental VCCs with the hard-sphere ones, to scale the exclusion effect from the overall analysis.

It is worth mentioning that a singularity was recognized in a theoretical study on the short-time velocity self-correlation of hard spheres⁵¹ not reported in the present analysis, which applies long times. Furthermore, some numerical simulations showed, consistently with the present analytic results, how the VCC of hard-sphere systems can be negative,⁵² underlining the important application of these phenomena to interpret quasi periodic velocity relaxation in many spectroscopic manifestations.⁵³

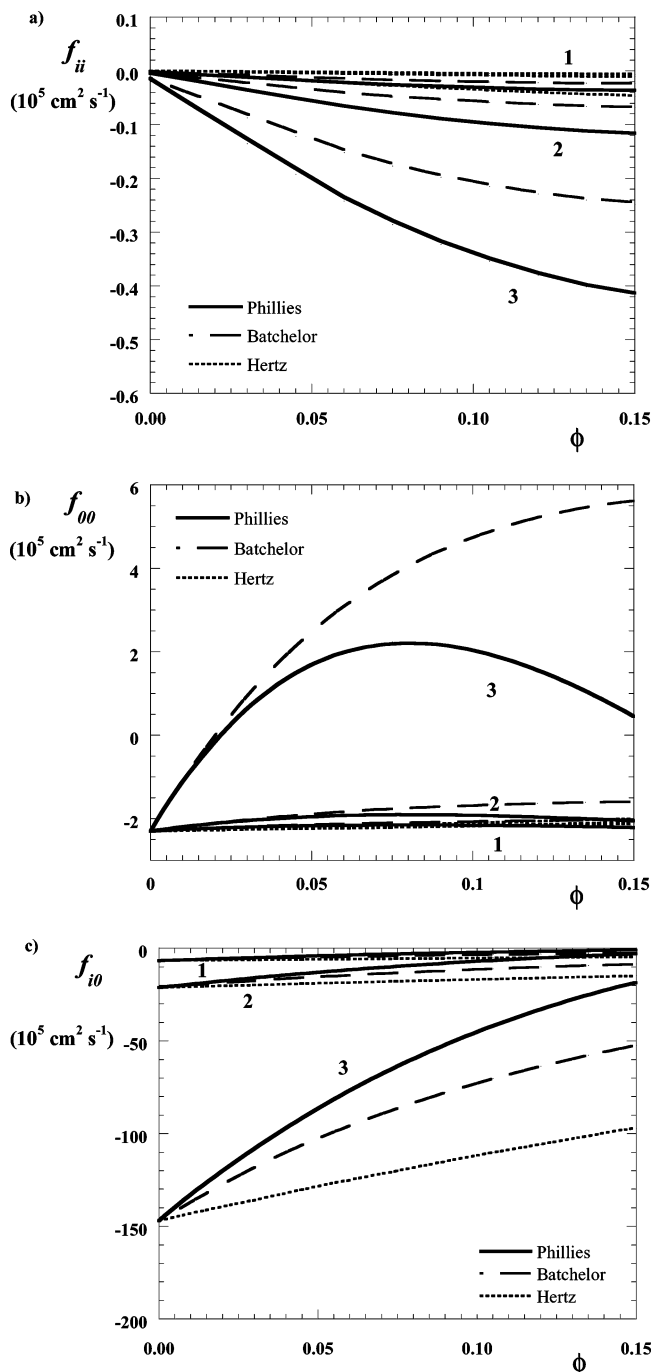


Figure 1. Velocity cross-correlation coefficients (a, f_{ii} ; b, f_{00} ; c, f_{i0}) for hard spheres (i) as a function of their volume fraction (ϕ) with three different effective volumes: (1) 0.29, (2) 3.8, and (3) $50 \text{ cm}^3 \text{ mol}^{-1}$.

IV. Source of the Experimental Data

A summary of the literature data used here (conventional solutes, polymers, proteins) is reported in Table 1. Below, we comment briefly on the protein data.

Although there are many data in the literature for virial coefficients, self- and mutual diffusion of proteins, there is much less about solvent intradiffusion. Therefore, the limited number of protein systems analyzed in this work is associated with the availability of solvent intradiffusion data. Hopefully, this lack will be filled, because often the same apparatus can measure both protein and solvent intradiffusion coefficients (see NMR–PGSE technique⁵⁴). Another limitation is the accuracy of both diffusion and virial coefficients data. In ref 55 a hierarchy of

TABLE 1: Source of Sets of Experimental Data Used in the Paper, Corresponding to Aqueous Solutions of Small Solutes, Polymers, and Proteins^a

solute	D_i	D_i^*	D_0^*	B_i^x
methyl(pyrrrolidone)	68	68	68	68
glucose	69	69	69	69
poly(ethylene glycol)s	21	27	27	28, 29
dextrans	30			28
lysozyme	60	71	70	60
β -lactoglobulin	64	64		64
ovalbumin	72	73	73	72

^a In some cases for brevity, we refer to papers wherein one can find all of the necessary references.

accuracy for diffusion technique and in refs 26 and 28 the accuracy of activity data is discussed for protein and polymer, respectively.

Systems containing proteins are not actual binary systems because of the presence of buffer and eventually salt; thus, multicomponent effects (see eq 3) may be expected. Therefore herein only data at high (larger than 0.2 M) and constant salt component concentration are taken into account for proteins; thus, they behave as bare globular particles²⁵ without any drag due to counterions. Some efforts have been devoted to the experimental determination of multicomponent diffusion coefficients in protein solutions during past few years,¹⁹ but the diffusion data are still limited just to two proteins (lysozyme and serum albumin).

V. Thermodynamic and Hydrodynamic Contributions on Mutual Diffusion

In recent papers, two important results have been obtained: the slope of intradiffusion coefficients as a function of volume fraction is equal for many globular proteins;⁵⁶ the cross-term diffusion coefficient linking the flux of “small” solute to a macromolecule concentration gradient in multicomponent systems can be well described by exclusion theory.¹⁹ It would be desirable to find such a kind of universal contribution for the mutual diffusion coefficients in “binary” systems, as well.

In eqs 15–18, the “effective” molecular volume appears to be the only parameter per solute. Before we apply the new references to real systems, we make a brief comment about the choice of the molecular volume. Elsewhere, several possibilities to evaluate it (partial molar, hydrodynamic from diffusion or viscosity, van der Waals volume) were discussed.^{20,22} The differences between these “volumes” may be attributed to the approximation of spherical shape and to the presence of solvent, which moves with the solute molecule, increasing its effective hydrodynamic radius.⁵⁷

We tried to obtain the molecular PEG volume from the experimental concentration dependence of the diffusion coefficients according to eq 15 for binary systems of a hard sphere i in a continuum. We use

$$\phi_i = C_i V_{\text{eff},i} \quad (19)$$

where $V_{\text{eff},i}$ is the effective molar volume of solute i expressed in $\text{dm}^3 \text{mol}^{-1}$. This route to obtain the molecular volume is modified after Phillies.⁵⁸

This procedure can be applied when the system does not deviate too much from a hard-sphere model, namely, the nonideality of the system must be essentially due to the molecular exclusion. This could be a reasonably good approximation for compact uncharged spherical particles but not for random coil or densely charged solutes. In the case of

polymeric random coil, it does not hold because of the combinatorial entropy effect. The last effect, as well as attractive solute–solute and solute–solvent interactions, produce an experimental trend of diffusion coefficient as a function of the solute concentration not qualitatively interpretable by eq 15. In fact, for PEG samples with molecular weight higher than 1000 Da, the diffusion coefficient increases as the concentration increases.²¹ To satisfy this condition, eq 15 would imply a negative “effective” molecular volume. Although the scaled particle theory⁵⁹ makes a sense of negative excluded volume (as in inclusion process), this is not the case. To use eq 15, we should have to “reduce” the experimental D_{ex} to a hard-sphere–solvent system. Therefore, we tried to transform the experimental D_{ex} , separating every contribution to the chemical potential different from the exclusion effect of hard spheres. We used the expression derived from the thermodynamics of the irreversible processes (eq 4). The mobility defined in eq 4, M_i , represents the diffusion coefficient of a hypothetical solution having the same hydrodynamic properties but with unitary activity coefficient at any concentration. In fact, we use B_{ex} to correct it for the concentration dependence of chemical potential, μ_{ex} . If we replace the experimental thermodynamic factor B_{ex} , introducing one relative to hard sphere, B_{hs} , we obtain a corrected diffusion coefficient, D_{cor} , consistent with the thermodynamic hypothesis on which eq 15 has been obtained

$$D_{\text{cor}} = D_{\text{ex}} \frac{B_{\text{hs}}}{B_{\text{ex}}} \quad (20)$$

The B_{ex} can be experimentally determined and the B_{hs} is evaluated by using a statistical thermodynamic theory of hard spheres (eq 17). We point out that the following procedure to evaluate the effective volume is also a test of the hard-sphere-like behavior split into thermodynamic and hydrodynamic contributions.

To evaluate the volume, an iterative procedure consisting of the following five steps has been attempted: (1) evaluation of B_{ex} from precise experimental activity coefficients of binary system (solute–water); (2) evaluation of the mobility from eq 4; (3) evaluation of B_{hs} from eq 17; (4) evaluation of D_{cor} from eq 20; (5) evaluation of V_{eff} from the concentration dependence of D_{cor} , by using eq 15.

The steps 3, 4, and 5 are iterated to self-consistency between the thermodynamic (input in step 3) and hydrodynamic volume (output of step 4). Unfortunately, this procedure does not give the expected correction for the PEG–water systems investigated ($D_{\text{cor}} \neq D_{\text{hs}}$). This is apparent from the unlikely volumes evaluated with this procedure. Nevertheless, this analysis provided useful information about the systems under study. A possible reason for this failure is the factorization of the mutual diffusion coefficient in thermodynamic factor, and mobility does not discharge all of the non-hard-sphere effects on the first term, but also on the mobility. Some wormlike mobility theory could correct this inconsistency. Much more difficult is the inclusion of specific interaction between solvent and polymer (such as the hydrogen bonding in the case of poly(ethylene glycol)–water systems). Therefore, the hydrodynamic part of the mutual diffusion coefficient reflects sensitively strong deviation from the hard-sphere behavior. The subtraction of the new standards in the VCC analysis will reveal the residual part due to specific interactions. Because of the inability of evaluating the effective volume of PEG, we have used the viscosimetric ones.⁵⁷

An analogous iterative procedure to evaluate the hydrodynamic volume was tried also for proteins listed in Table 1. This

procedure especially permits testing whether a universal behavior can be observed for the thermodynamic or hydrodynamic factors or both in mutual diffusion of proteins. In this case, the agreement between the evaluated and hydrodynamic volumes is much better, even though the better choice seems still to be the Stokes–Einstein under slip conditions. Below some details about the thermodynamic and mobility factor of protein solutions are reviewed.

Fine derived both the thermodynamic and mobility factor for γ -crystallin aqueous solution,⁶⁰ emphasizing how much the system deviates from the hard-sphere behavior. They quantified this deviation by using the Baxter adhesive model of hard spheres.

Lysozyme solutions are well studied, and unpublished data of lysozyme multicomponent diffusion as a function of protein concentration (see eq 3) are about to be provided by Albright from the Texas Christian University. When these data will be available, an extension of the current VCC theory to multicomponent systems will be required⁶¹ to give the right importance to these data. Until then accurate DLS data are available for our analysis.⁶² Muschol and Rosenberger used a DLVO pair potential to describe the mobility behavior of lysozyme solutions at different ionic strength.

For the ovalbumine, a reasonable prediction of the mutual diffusion coefficients can be made from the experimental measurement of the intradiffusion and activity coefficients by equating mobility and intradiffusion coefficients of the protein. This same correlation, although not thermodynamically rigorous has worked for other proteins.⁶³

From the analysis of the β -lactoglobulin, it has been observed that the protein–protein friction is small compared to the protein–solvent friction.⁶⁴ These results, if confirmed for all of the proteins, can yield a useful approximation in the velocity correlation analysis.

For the hemoglobin under conditions of moderate ionic strength and pH near to the isoelectric point, Minton observed good agreement between hydrodynamic volume and thermodynamic volume when eq 18 is used.⁶⁵

Finally protein hydrodynamics can be approximated by a hard-sphere behavior much better than polymers such as PEG. On the other hand, from a more detailed analysis, it seems that the main discrepancy between protein and hard spheres is about the thermodynamic features of protein solutions rather than the protein's mobility.

The observed important contribution of the excluded volume to the hydrodynamic factor in mutual diffusion of proteins agrees with the universal behavior of protein intradiffusion⁵⁶ and multicomponent cross-term diffusion coefficients¹⁷ of systems containing proteins.

VI. Application of the New Standard VCC

Once the effective volume is estimated, we can compare real VCCs with the corresponding hard-sphere reference.

In Figures 2 and 3, the comparison of two different systems is reported with the corresponding Hertz's reference. The molecular volumes correspond to ones used in Figure 1; therefore, Figure 1 contains the hard-sphere reference that we want to compare. In fact, the choice of the systems is one conventional solute (glucose, $V_{\text{eff}} = 200 \text{ cm}^3 \text{ mol}^{-1}$) and one polymer (PEG 2000, $V_{\text{eff}} = 3400 \text{ cm}^3 \text{ mol}^{-1}$).

The qualitative interpretation of velocity correlation coefficients between particles of the same species is not very different. On the contrary, the hard-sphere references allows a much more meaningful analysis for the cross-terms hard-

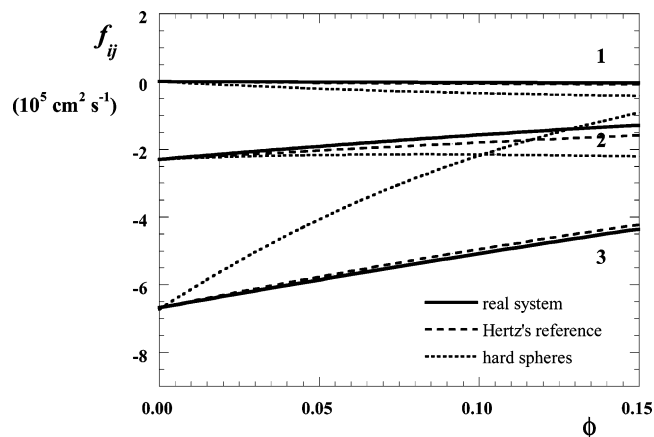


Figure 2. Comparison of real, Hertz reference, and hard-sphere VCCs, f_{ij} , in the case of glucose aqueous solutions: (1) f_{ii} ; (2) f_{00} ; (3) f_{i0} .

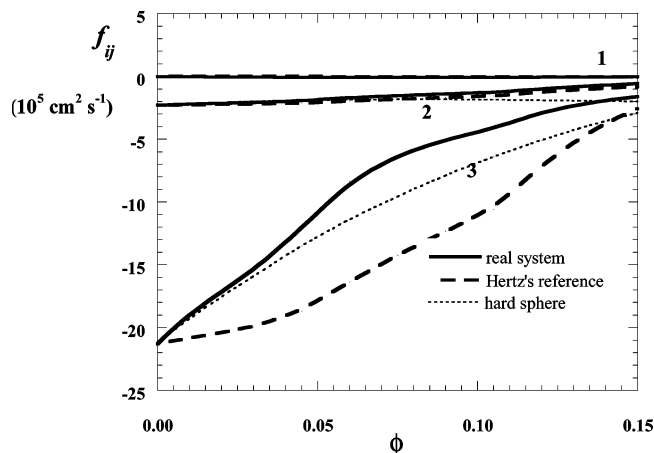


Figure 3. Comparison of real, Hertz reference, and hard-sphere VCCs, f_{ij} , in the case of PEG 2000 aqueous solutions: (1) f_{ii} ; (2) f_{00} ; (3) f_{i0} .

sphere–solvent (f_{i0}). Therefore, we suggest a combined use of both the references for the self-term velocity correlation but discourage the use of Hertz cross-term velocity cross-correlation standards for systems in which a large size mismatch of solvent and solute is present. A more extended set of systems to apply the new references will be presented elsewhere.

VII. Future Perspectives

The velocity correlation approach above applied to the translation motion might be extended to an analysis of the internal motion as well. Recently, much attention has been devoted to concerted (highly correlated) internal motions associated with enzymatic reaction and allosteric effect. The fluctuation in position of α -carbon in protein chains, derived by the crystallographic atomic displacement parameter, molecular dynamics analyses, and time-resolved spectroscopy, can be quantified by self- and cross-correlation integrals similar to ones in eq 2. The integrals in eq 2 are averaged with respect to the angular value, but they can be modified to take into account the anisotropic correlation, which is often expected because of salt bridges, hydrogen bonding, and coordination sphere in the active site. The comparison of velocity or position fluctuations between amino acidic residues with an uncorrelated system can be misleading because of the reasons given in this paper and in ref 2. A more penetrating comparison can be done considering some nonspecific interaction such as a hard core or a more complex potential. This kind of procedure might be applied to NMR⁶⁶ or crystallographic⁶⁷ analysis. The comparison of the

experimental correlation and some model systems such as hard spheres may offer another quantitative criterion to detect local cooperative motions.

The above analysis of the translation motion could be extended to rodlike proteins and viruses or wormlike polymers, once the theory about the mutual diffusion, intradiffusion, and chemical potential of these systems were fully accepted.

VIII. Conclusion

New tools for analyzing interaction in real systems have been presented, coupling experimental data and theory of hard spheres. A number of aqueous solutions with different hydrodynamic and thermodynamic behaviors have been tested, ranging from conventional solute to polymer solutions.

A new procedure for reducing real systems to hard-sphere-like systems reveals the extent of the excluded volume effect on thermodynamic and hydrodynamic properties in mutual diffusion. The deviation of protein solutions from the hard-sphere model is due essentially to thermodynamic reason, rather than hydrodynamic ones.

Main- and cross-term velocity cross-correlation coefficients have been derived for hard-sphere–solvent mixtures. These VCCs, proposed as new standard coefficients in velocity correlation analysis, are evaluated for the two most accepted hydrodynamic theories and compared to the corresponding Hertz references for hard spheres.

Finally the new standards have been applied as new reference state in a preliminary VCC analysis of real binary systems, and their usefulness is compared to the Hertz ones. The new procedure allows scaling the effect of excluded volume for real systems, unmasking the specific attractive and repulsive effects. To maximize the quality of the information conveyed by the VCC analysis of real systems, we suggest to use both (Hertz and hard-sphere) references for the self-term VCCs but definitively to prefer the hard-sphere reference for analyzing the cross-term VCCs.

The limiting step in this kind of analysis is related to the accuracy of the diffusion and thermodynamic data. For protein solutions, we are limited by the lack of solvent intradiffusion coefficients.

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