Macromolecules **2001,** *34,* 991−1000

Multicomponent Diffusion in Crowded Solutions. 1. Mutual Diffusion in the Ternary System Poly(ethylene glycol) 400−NaCl−Water

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Received July 7, 2000; Revised Manuscript Received October 16, 2000

ABSTRACT: The multicomponent approach in the description of molecular diffusion takes into account the correlation of motion between solutes. Here it is shown that this approach is necessary to describe correctly the flows of each component in concentrated solutions of macromolecules, defined "crowded solutions". Microscopic intuitive considerations make clear this necessity also for hypothetical uncharged hard particles. We present precise measurements of mutual diffusion coefficients relative to five compositions of the ternary system poly(ethylene glycol) (PEG) 400−NaCl−water, changing both the PEG and salt concentrations. The values of the experimental diffusion coefficients for this system seem to be dominated by an excluded volume effect. Our recent predictive equations, proposed to evaluate the diffusion coefficients in a ternary systems of hard sphere solutes, have been tested on the experimental data with reasonable success. The thermodynamic and gravitational stability analysis on the collected data is also reported. By changing the choice of solvent constituent from water to PEG or NaCl, it is clear that the counter-flow related to the polymer flux in crowded solutions is due essentially to the water and not to other constituents.

I. Introduction

The phenomenological description of the diffusion in *n*-component systems is based on the generalized Fick's equation:[1]

$$J_i = -\sum_{j=1}^{n-1} D_{ij} \nabla C_j \quad i = 1, 2, ..., n-1 \tag{1}$$

which includes the main-term, D_{ii}, and the cross-term, D_{ij}, with $i \neq j$, diffusion coefficients.

Very often in studying the diffusive evolution of real boundaries, the so-called pseudobinary approximation is used. In this approach, the cross-term diffusion coefficients are neglected and the main term diffusion coefficients are usually approximated by the diffusion coefficients of the corresponding binary systems. These binary diffusion coefficients are often determined by dynamic light scattering technique.[2] Although the experimental techniques to determine the $(n-1)^2$ diffusion coefficients are well developed,[3] the pseudobinary approach is predominant. In the past decade, this approximation has been widely used in describing the diffusive processes in cytoplasmatic system[4] and to model crystal growth of macromolecules as protein,[5,6] virus,[7] DNA,[8] etc. We have shown some possible erroneous consequences of this approximation in a work by some of us on the simulation of hydrodynamics relevant in crystal growth.[9]

In this paper, we emphasize that this approach is seriously wrong in many cases and focus attention on systems containing macromolecular solutes that "crowd" the solution, as in cytoplasmatic solutions,[10] in solutions from which proteins precipitate in the presence of nonionic polymers,[11] and generally in the phase behavior of colloidal solutions.[12]

Why make the diffusion description more difficult? Because many ternary systems have been investigated in the last 50 years, and a number of them show cross terms that are significant or are larger than the main terms. Large cross-diffusion coefficients have been found in electrolytes mixtures,[13] in the presence of a chemical equilibrium between the solutes,[14] and in systems containing large size solutes, such as macromolecules,[15] polyelectrolytes,[16,17] and proteins.[18−23] While the first two classes of ternary systems have been extensively discussed from the theoretical and quantitative point of view, multicomponent diffusion in systems containing macromolecules is a current field of research.

Considering the effect of molecular exclusion on the diffusion motion, we recently obtained intuitive predictive equations for the main and cross-term diffusion coefficients.[24,25] These equations have been tested on several systems.[24−27] Very encouraging was the good prediction of the D_{ij} of the NaCl−lysozyme−water system,[25] for which significant electrostatic interactions could be expected. The easy use of these equations and the small number of parameters involved in the D_{ij} prediction should encourage the more correct multicomponent approach, even if experimental data are not available.

The present study deals with a weakly interacting aqueous system, containing a low molecular weight polydisperse polymer, poly(ethylene glycol) (PEG) 400 (solute 1), and a simple salt, NaCl (solute 2). This system might be expected to have small cross-diffusion terms, and therefore, it is describable in principle by the pseudobinary approximation. However, the experimental cross-term diffusion coefficient for the motion of simple salt by the polymer concentration gradient, D_{21}, is positive and of same order of magnitude of the corresponding main-term, D_{22}. Moreover D_{22} is much smaller than the diffusion coefficient of the binary system NaCl−water.

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10.1021/ma0011800 CCC: $20.00

Table 1. Density Data, Partial Molar Volumes, and Results of the Gravitational Instability Analysis for the Ternary System PEG 400 (1)−NaCl (2)−Water at 25 °C

	c	a	b	e	a	d
$\bar{C}_1$ (mol dm^{-3})	0.1256	0.2578	0.3899	0.2579	0.2578	0.2623
$\bar{C}_2$ (mol dm^{-3})	0.7758	0.7735	0.7797	0.2501	0.7735	1.2718
H_1 (kg mol^{-1})	0.0602 ± 0.0001	0.0624 ± 0.0002	0.06449 ± 0.00005	0.06370 ± 0.00005	0.0624 ± 0.0002	0.062 42 ± 0.00004
H_2 (kg mol^{-1})	0.0392 ± 0.0001	0.0391 ± 0.0009	0.03838 ± 0.00002	0.03968 ± 0.00008	0.0391 ± 0.0009	0.038 42 ± 0.00002
$\bar{d}$ (kg dm^{-3})	1.036015 ± 0.000002	1.043955 ± 0.000003	1.052383 ± 0.000002	1.023349 ± 0.000002	1.043955 ± 0.000003	1.063324 ± 0.000001
$\bar{V}_1$ (cm^3 mol^{-1})	340.5	338.4	336.4	337.3	338.4	338.3
$\bar{V}_2$ (cm^3 mol^{-1})	19.28	19.39	19.50	18.8	19.39	20.08
$\bar{V}_0$ (cm^3 mol^{-1})	18.05	18.06	18.07	18.06	18.06	18.05
overstability[a]	−0.61	−0.64	−0.98	−0.32	−0.64	−0.97
isodensimetric[a]	−1.7	−1.8	−1.7	−1.5	−1.8	−1.6
fingers[a]	−7.8	−9.2	−6.0	−8.3	−9.2	−6.1

[a] Ratios of ($\Delta C_2/\Delta C_1$) describing the onset of gravitational instability.

The mutual diffusion properties of systems PEG−salt−water are of interest in protein crystallization.[28−31] The recent possibility of growing crystals on space platforms, has enhanced the rule of the diffusive transport in crystallization process, since gravity convection and sedimentation are negligible.[32−34] Furthermore, recent studies show an anomalous salt effect on the second virial coefficient of proteins in the presence of PEG at low ionic strength; the second virial coefficient of the protein is strongly dependent on PEG concentration,[35,36] whereas at high ionic strength it is practically independent of the PEG concentration.

Systems PEG−salt−water are widely described in the literature from different points of view: phase diagrams,[37,38] heats of dilution,[39] cloud-point measurements,[40] self-diffusion coefficients,[41] conducibility,[42] activity coefficients,[43] densities and viscosity,[44] alkali metal ions complexation.[45] However mutual diffusion studies are completely lacking.

This is the first of a series of studies dealing with the mutual diffusion properties of aqueous ternary systems containing PEG (of different molecular weights and polydispersities) and sodium chloride.[46]

The experimental D_{ij} values of the investigated system have been compared with those obtained by the predictive equation we recently proposed for a mixture of hard sphere solutes in a continuum solvent. The efficiency of the our predictive analysis is compared with other literature predictive approaches.[47,48]

Some viscosity measurements were performed at the compositions of interest, since they are needed for the diffusion analysis.

II. Experimental Section

Materials. Sodium chloride 99.99% was purchased from Aldrich. Poly(ethylene glycol) (PEG) with numerical average molecular weight 400 Da has been purchased from Aldrich and used without further purification. The sample has a mild polydispersity, described by the Poisson distribution and discussed elsewhere from a diffusive point of view.[49] All solutions were prepared by weight using double-distilled water.

Density Measurements. The densities, d, of all solutions have been measured at 25.00 ± 0.01 °C using an Anton PAAR densimeter, model 602. The instrument was calibrated with double-distilled water and with air whose density was based on the ambient humidity and pressure where the humidity was corrected to 25.00 °C. The following equation was fitted to the experimental data

$$d = \bar{d} + \sum_{i=1}^{n-1} H_i(C_i - \bar{C}_i) \qquad (2)$$

where

$$H_i = \left(\frac{\partial d}{\partial C_i}\right)_{\bar{C}_j,\bar{C}_i} \qquad (3)$$

and $\bar{d}$ is the density at the average concentration, $\bar{C}_i$. The volumetric results are reported in Table 1.

Mutual Diffusion Measurements. Diffusion experiments were performed at 25.00 ± 0.01 °C using Gouy interferometry,[3] whose experimental details and theory are widely described elsewhere.[50] Five compositions with different salt or polymer composition were analyzed. Each composition is investigated measuring four interferometric runs, with different composition of the total refractive index gradient. For each run we record 20 scans of the interferometric pattern (positions of the interferometric minima), generated by the refractive index gradient along the diffusion cell. The four diffusion coefficients, D_{ij}, at each composition have been obtained using literature programs for Gouy data analysis.[51,52] A single channel cell (a = 2.5000 cm) was used, and the initial boundary was set up with the siphoning technique. A He−Ne laser source was used (λ = 632.8 nm), and the Gouy minima were recorded in real time with a photodiode array. The interferometric data are reported in Table 2 (a−e). All the mutual diffusion coefficients in Table 3 are expressed in the volume fixed reference frame.[53] The mild polydispersity of PEG makes the errors in the D_{ij} larger than usual. The effect of polydispersity (very mild for the case of a living polymer as PEG) on the analysis of interferometric data in ternary systems containing a polydisperse sample will be the subject of a future paper.

Viscosity Measurements. The viscosity measurements on the binary systems PEG 400−water and the ternary system PEG 400−NaCl−water have been performed at 25.00 ± 0.01 °C, using an Ubellhode viscometer and double-distilled water as reference liquid. The experimental relative viscosity (viscosity of the solution divided by viscosity of water) are reported in Table 4.

III. Theory

The Predictive Procedure. The concentration dependence of the mutual diffusion coefficient, D_i, and viscosity, η_i, in monodisperse noninteracting hard sphere suspensions was object of several theoretical studies.[24,54−61] In dilute binary solutions, the main results are expressed by the Carter−Phillies[61] and Einstein[3] equations

$$D_i = D_i^{\infty}(1 - 0.898\phi_i) \qquad (4)$$

$$\eta_i = 1 + 2.5\phi_i \qquad (5)$$

where D_i^{∞} is the diffusion coefficient at infinite dilution, ϕ_i is the solute volume fraction, and η_i is the relative viscosity of the system.

To extend the hard sphere theory to ternary systems, we have used a very simple and intuitive considerations, based on the jump model.[62] We start by analyzing this

Table 2. Gouy Interferometric Parameters Relative to Five Compositions (a−e) of the System PEG 400 (1)−NaCl (2)−Water at 25 °C[a]

(a)

	A1	A2	A3	A4
$\bar{C}_1$ (mol dm^{-3})	0.2578	0.2578	0.2578	0.2578
$\bar{C}_2$ (mol dm^{-3})	0.7735	0.7735	0.7735	0.7735
ΔC_1 (mol dm^{-3})	0.0001	0.0159	0.0241	0.06340
ΔC_2 (mol dm^{-3})	0.0799	0.0864	0.0412	0.0001
α_1	0.0054	0.4990	0.7602	0.9996
J_m	28.82	62.96	62.26	125.90
$D_A \times 10^5$ (cm^2 s^{-1})	1.577	0.750	0.553	0.434
$Q_o \times 10^4$	−256.2	184.9	198.8	77.9

(b)

	B1	B2	B3	B4
$\bar{C}_1$ (mol dm^{-3})	0.3898	0.3899	0.3898	0.3898
$\bar{C}_2$ (mol dm^{-3})	0.7796	0.7797	0.7797	0.7796
ΔC_1 (mol dm^{-3})	0.0065	0.1283	0.0252	0.0318
ΔC_2 (mol dm^{-3})	0.1389	0.1040	0.0350	−0.0002
α_1	0.2007	0.3997	0.7953	1.0010
J_m	62.47	63.07	61.82	61.80
$D_A \times 10^5$(cm^2 s^{-1})	1.239	0.886	0.518	0.411
$Q_o \times 10^4$	−158.4	89.7	173.5	112.3

(c)

	C1	C2	C3	C4
$\bar{C}_1$ (mol dm^{-3})	0.2623	0.2623	0.2624	0.2623
$\bar{C}_2$ (mol dm^{-3})	1.2718	1.2718	1.2718	1.2718
ΔC_1 (mol dm^{-3})	0.0001	0.0062	0.0245	0.0248
ΔC_2 (mol dm^{-3})	0.1672	0.1335	0.0332	0.0017
α_1	0.0024	0.2074	0.8064	0.9879
J_m	60.29	60.94	61.68	51.33
$D_A \times 10^5$(cm^2 s^{-1})	1.667	1.168	0.556	0.458
$Q_o \times 10^4$	−182.2	93.9	166.2	81.9

(d)

	D1	D2	D3	D4	D5
$\bar{C}_1$ (mol dm^{-3})	0.2623	0.2623	0.2623	0.2624	0.2623
$\bar{C}_2$ (mol dm^{-3})	1.2718	1.2718	1.2718	1.2718	1.2718
ΔC_1 (mol dm^{-3})	0.0000	0.006 2	0.015 6	0.024 9	0.031 2
ΔC_2 (mol dm^{-3})	0.2551	0.1362	0.085 0	0.033 9	−0.0000
α_1	0.000 8	0.2046	0.5084	0.8054	1.0002
J_m	89.85	60.67	61.23	61.63	61.59
$D_A \times 10^5$(cm^2 s^{-1})	1.761	1.187	0.763	0.518	0.415
$Q_o \times 10^4$	−385.2	3.6	221.6	212.1	140.1

(e)

	E1	E2	E3	E4
$\bar{C}_1$ (mol dm^{-3})	0.2579	0.2579	0.2579	0.2579
$\bar{C}_2$ (mol dm^{-3})	0.2501	0.2501	0.2500	0.2500
ΔC_1 (mol dm^{-3})	0.0002	0.0064	0.0246	0.0307
ΔC_2 (mol dm^{-3})	0.1669	0.1335	0.0334	0.0002
α_1	0.0050	0.2015	0.7961	0.9989
J_m	63.60	63.60	62.30	61.82
$D_A \times 10^5$(cm^2 s^{-1})	1.692	1.182	0.528	0.426
$Q_o \times 10^4$	−400.3	−34.6	120.8	30.7

[a] J_m is the total number of Gouy interference fringes. D_A: apparent diffusion coefficient, Q_0 is the "area under the deviation function".[31] α_i is the refractive index fraction of the species i.

model for a binary system. The solution is considered just as a lattice with a continuum solvent and a solute of finite volume that can occupy all the cells of the lattice. We will treat the solute as impenetrable particles subject to a hard core potential generating a molecular exclusion phenomenon, elsewhere defined by physicists as depletion phenomena[35,63] or by biologists as macromolecular crowding.[64,65]

The solute Brownian motion is described statistically by the Smoluchoswki equation.[66] The rigorous solution of this equation proves to be difficult, so alternative routes have been explored. We consider for simplicity just one-dimensional fluxes. At infinite dilution the single solute molecule in the lattice can statistically move in a time interval Δt to any cell at a distance Δx, which is related to the diffusion coefficient by Einstein's equation

$$D_i^{\infty} = \frac{(\Delta x)^2}{\alpha \Delta t} \tag{6}$$

where α depends on the boundary conditions.[67] At finite concentration, in the presence of a diffusion boundary between two solutions at different concentrations, the particle motion has different probability in the two directions. The number of molecules leaving the more crowded solution is larger than the number of molecules leaving the less crowded one. As a consequence, a net flux of solute molecules takes place from the more concentrated to the less concentrated solution; the diffusion boundary spreads out; and an homogeneous solution is obtained at $t = \infty$. Because of the presence of occupied cells, the distance Δx that a single molecule can statistically move in a time interval Δt is shorter than that moved at infinite dilution. Consequently the diffusion coefficient decreases. Because the fraction of occupied cells is represented by the solute volume fraction, eq 4 is qualitatively explained.

On extending previous considerations to a ternary system, (solute i−solute j−solvent 0), it is evident that the main diffusion coefficient D_{ii} must be affected by the volume fraction of both the solutes. We have proposed for the main terms the following general empirical expression[24]

$$D_{ii} = D_i \frac{\eta_i}{\eta_{ij}} \tag{7}$$

where η_{ij} is the viscosity of the ternary system, and D_i and η_i are respectively the diffusion coefficient and the viscosity of the corresponding binary system (solute i−solvent 0) considered at the same concentration C_i of component i. Equation 7 attributes the difference between D_{ii} and D_i simply to the different viscosity of the two systems (η_i and η_{ij}). Extending eq 5 to ternary systems

$$\eta_{ij} = 1 + 2.5(\phi_i + \phi_j) \tag{8}$$

and considering eq 4, eq 7 becomes

$$D_{ii} = D_i^{\infty}(1 - 0.898\phi_i)\frac{1 + 2.5\phi_i}{1 + 2.5(\phi_i + \phi_j)} \tag{9}$$

where the D_{ii} dependence on ϕ_i and ϕ_j is clearly shown. It is interesting to point out that in dilute solution eq 9 is equivalent to

$$D_{ii} = D_i^{\infty}(1 - 0.898\phi_i - 2.5\phi_i) \tag{10}$$

which clearly shows the different contribution of the two solutes in determining the value of the main diffusion coefficient D_{ii}.

The jump model can also provide a very simple picture for the cross fluxes. We start by considering an initial diffusion boundary with a finite concentration gradient of component j, $\nabla C_j \neq 0$, and no concentration gradient of component i, $\nabla C_i = 0$. Because the different crowding

Table 3. Mutual Diffusion Coefficient, D_{ij} Expressed as 10^5 cm^2 s^{-1}, for the Ternary System PEG 400 (1)−NaCl (2)−Water (0) at 25 °C at Five Compositions Expressed in mol dm^{-3}(a−e)[a]

	E	a	d	c	a	b
$\bar{C}_1$	0.2579	0.2578	0.2623	0.1256	0.2578	0.3899
$\bar{C}_2$	0.2501	0.7735	1.2718	0.7758	0.7735	0.7797
D^0_{11}	0.418 ± 0.001	0.399± 0.005	0.377 ± 0.003	0.420 ± 0.002	0.399± 0.005	0.386 ± 0.005
D^0_{12}	0.041 ± 0.001	0.031 ± 0.002	0.037 ± 0.001	0.023 ± 0.001	0.031 ± 0.002	0.053 ± 0.002
D^0_{21}	0.197 ± 0.012	0.548 ± 0.052	0.832 ± 0.025	0.566 ± 0.016	0.548 ± 0.052	0.559 ± 0.023
D^0_{22}	1.157 ± 0.006	1.160 ± 0.053	1.167 ± 0.015	1.337 ± 0.012	1.160 ± 0.053	0.976 ± 0.021
D^1_{00}	0.429	0.438	0.426	0.454	0.438	0.419
D^1_{02}	−1.5	−1.6	−1.5	−1.4	−1.6	−1.6
D^1_{20}	−0.010	−0.029	−0.044	−0.031	−0.029	−0.030
D^1_{22}	1.15	1.12	1.12	1.30	1.12	0.943
D^2_{11}	−0.318	−0.0320	−0.247	0.0350	−0.0320	−0.500
D^2_{10}	−0.039	−0.023	−0.033	−0.021	−0.023	−0.048
D^2_{01}	28	22	26	23	22	27
D^2_{00}	1.89	1.59	1.79	1.72	1.59	1.86
$R_1{*}\lambda/a$	2027.6	1965.8	1981.8	1990.8	1965.8	1956.5
$R_2{*}\lambda/a$	380.1	360.1	352.8	361.6	360.1	361.2
$\|\|D\|\|$	0.476	0.446	0.409	0.548	0.446	0.347

[a] Three solvent choices for the D_{ij} are reported, together with the refractive index increment R_i and the determinant value of the diffusion matrix.

Table 4. Relative Viscosity Data, η_{rel}, for the Binary System PEG 400 (1)−Water and the Ternary System PEG 400 (1)−NaCl (2)−Water at 25 °C

C_1	C_2	η_r
0	0	1
0.0500	0	1.081
0.1123	0	1.209
0.1535	0	1.291
0.2064	0	1.393
0.2674	0	1.555
0.3113	0	1.676
0.3822	0	1.898
0.6484	0	3.029
0.2579	0.2501	1.558
0.2578	0.7735	1.651
0.2623	1.2718	1.762
0.1256	0.7758	1.335
0.2578	0.7735	1.651
0.3899	0.7797	2.078

of the two solutions due to the different concentration of solute j, a net flux of solute i will take place on a statistical basis from the more crowded solution toward the less crowded solution. The magnitude of this cross flux depends essentially on the concentration of solute i and on the volume fraction of component j.

Considering the "effective" concentration gradient of each species instead of stoichiometric ones, we have proposed for the cross-diffusion coefficients the equation[24]

$$D_{ij} = D_{ii}\frac{C_i\phi_j}{C_j(1-\phi_j)^2} \quad \text{with } i \neq j \qquad (11)$$

We point out that eq 11 predicts, as the jump model does, that the cross-term diffusion coefficients are zero only in a physical model of uncharged points.

Because of nonzero solute volume in real systems, the volumetric contribution is always present, but it assumes a more important role in determining the values of the diffusion coefficients in systems containing large size molecules. Experimental diffusion studies on multicomponent solution of weak polyelectrolytes[16,17] and nonelectrolyte macromolecules[15,46] confirm this prediction. The use of eqs 9 and 11 is obviously limited to systems where only volumetric interactions are present.

The system analyzed in this paper is constituted by water (0), a nonelectrolyte highly hydrophilic polydisperse sample of PEG 400 (1), and NaCl (2), a simple electrolyte.

In the case of binary systems containing a simple electrolyte, the transport properties are essentially determined by the electrostatic interactions between the ions present in solution; consequently, eqs 4 and 5 do not hold.

In a ternary system containing a nonelectrolyte and a simple salt with no solute−solute interactions, the main diffusion coefficient of the electrolyte is determined by the interactions between its constituent ions and by the volumetric effect of the nonelectrolyte solute. Because the diffusion coefficient of the corresponding salt-solvent binary system, D_2, already takes into account the ion−ion interactions, the main diffusion coefficients of the ternary system, D_{22}, can be reasonably predicted using eq 7. Also for the nonelectrolyte components, PEG 400, the use of eq 9 is critical. Equations 4 and 5 are relative to binary systems of hard spheres, while PEG 400 is a polydisperse sample, constituted by k component, which behaves as a pseudobinary system. Therefore, the measured diffusion coefficient is an apparent diffusion coefficient D_A, which is a complex average of the k^2 diffusion coefficients D_{ij}.[49] For this reason, systematic deviation from the Phillies equation can be expected, and the use of eq 9 can lead to misleading results.

Regarding the cross-term diffusion coefficients, both D_{12} and D_{21} can be predicted by our eq 11 if the effective volumes of PEG 400 and NaCl can be evaluated.

The D_{ij} ($i \neq j$) values predicted by eq 11 have been compared with those obtained by the corresponding predictive Batchelor equation.[48] Batchelor analyzed a mixture of hard spheres in a continuum solvent, and obtained simple analytical expression for the diffusion coefficients to the first order of volume fraction

$$D_{ii} = D_i^{\infty}\left(1 + 1.45\phi_i - \sum_{k\neq i}\frac{2.5\phi_k}{1 + 0.6\lambda_{ik}}\right) \qquad (12)$$

Table 5. Comparison of the Predictive Approaches Described in the Paper: Experimental Data (E) and Batchelor (B) from Eq 13 and Sartorio (S) from Eqs 9 and 11, Applied to the System PEG 400 (1)−NaCl (2)−Water at 25 °C

	e	a	d	c	a	b
D_{11}^{E}	**0.418±0.001**	**0.399±0.005**	**0.377±0.003**	**0.420±0.002**	**0.399±0.005**	**0.386±0.005**
D_{11}^{S}	0.409	0.386	0.364	0.409	0.386	0.377
D_{12}^{E}	**0.041±0.001**	**0.031±0.002**	**0.037±0.001**	**0.023±0.001**	**0.031±0.002**	**0.053±0.002**
D_{12}^{S}	0.005	0.005	0.005	0.003	0.005	0.008
D_{12}^{B}	0.043	0.043	0.043	0.021	0.043	0.0646.
D_{21}^{E}	**0.197±0.012**	**0.548±0.0052**	**0.832±0.025**	**0.566±0.016**	**0.548±0.052**	**0.59 ± 0.023**
D_{21}^{S}	0.177	0.550	0.914	0.490	0.453	0.421
D_{21}^{B}	0.39	1.28	1.99	1.21	1.28	1.22
D_{22}^{E}	**1.157±0.006**	**1.160±0.053**	**1.167±0.015**	**1.337±0.012**	**1.16 ± 0.053**	**0.976±0.021**
D_{22}^{S}	0.967	0.956	0.953	1.183	0.956	0.76

$$D_{ij} = D_i^{\infty}\phi_i({\lambda_{ij}}^3 + 2{\lambda_{ij}}^2) \qquad (13)$$

where λ_{ij} is the ratio between the radii of j and i spheres. Equation 12, which, for binary systems, reduces to

$$D_i = D_i^{\infty}(1 + 1.45\phi_i) \qquad (14)$$

predicting an increase of the diffusion coefficients on the concentration, was recognized by Carter and Phillies[61] to be wrong (see eq 4) due to a numerical error. However the derivation of eq 13 is correct.

Another set of predictive equations for the D_{ij} was developed by Cussler,[47] but it can be applied only to mixtures of molecules belonging to the same homologous series.

We point out that the Sartorio and Batchelor approaches require just the effective volume and the diffusion coefficient at infinite dilution of the solutes. On the other hand, Cussler's approach requires also the second virial coefficients for each solute.

Sartorio's, Batchelor's, and Cussler's equations are the only ones in the literature for the dependence of the ternary diffusion coefficients on the solute concentrations. In fact the Carter and Phillies expression[61] for binary systems, eq 4, was not extended explicitly to multicomponent systems.[58,59] Similarly the velocity correlation function approach is well developed for binary systems,[68−70] but the ternary systems have not yet been investigated by this theoretical method.

Evaluation of the Molecular Volume. We emphasize that in predictive equations, the molecular volumes appear as introduced parameters. We discussed elsewhere the different possibilities for evaluating the "effective" molar volumes of the diffusing particles.[24] We concluded that the best way to obtain them, for solutes that can be assimilated to hard spheres, is to use eq 4, considering

$$\phi_i = C_i V_{\mathrm{eff},i} \qquad (15)$$

where C_i is the molar concentration and $V_{\mathrm{eff},i}$ is expressed in dm^3 mol^{-1}. This route to obtain the "effective" molar volume of a diffusing particle was first used by 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. Equation 4 was used for the aqueous solutions of the first PEG*i* oligomers, (from dimer, $i = 2$, to hexamer, $i = 6$)[24−27] to obtain reasonable values for the "effective" molar volumes. These values have been fitted to a straight line obtaining

$$V_{\mathrm{eff},i} = (14.9 \pm 8.9)10^{-3} + (52.7 \pm 2.1)10^{-3}\, i \quad \mathrm{dm^3/mol} \qquad (16)$$

However due to polydispersity, the use of the Phillies equation cannot be extended, as noted above, to the PEG 400−water system. Then the average effective molar volume of this solute has been evaluated by using eq 16 and its number-average degree of polymerization. Using $i = 8.7$ we obtained

$$V_{\mathrm{eff},i,\mathrm{PEG400}} = (0.4724 \pm 0.0066)\ \mathrm{dm^3\ mol^{-1}} \qquad (17)$$

For sodium chloride, a rough estimate of the effective volume can be obtained from the limiting ionic diffusion coefficients and the Stokes−Einstein equation

$$V_{\mathrm{NaCl}} = \frac{4}{3}\pi\left[\left(\frac{kT}{4\pi\eta^{\circ}D_{\mathrm{Na^+}}^{\infty}}\right)^3 + \left(\frac{kT}{4\pi\eta^{\circ}D_{\mathrm{Cl^-}}^{\infty}}\right)^3\right] \qquad (18)$$

which leads to the value $V_{\mathrm{NaCl}} = 0.0683$ dm^3 mol^{-1}.

IV. Results

Viscosity Data. The viscosity data relative to the binary system PEG 400 (1)−H_2O (0) are reported in Figure 1 as a function of the PEG 400 concentration. For comparison we also reported the trend predicted by the Einstein equation, eq 5, using the "effective" molar value of PEG 400 reported above. As can be seen, the agreement between the predicted and the experimental trend is very poor even in dilute solutions. It is worth remembering that we have found that on increasing the molecular weight of the polymeric sample, the range of validity of the Einstein equation becomes smaller and smaller.

The viscosity data relative to the ternary system PEG 400 (1)−NaCl (2)−H_2O (0) are reported in Figure 2. The viscosity data have been collected at the same mean compositions used for the diffusion experiments. The data at constant PEG 400 concentration are reported as a function of NaCl molar concentration, and vice versa. As expected, the viscosity increase is much larger when the polymeric component is added rather than the simple salt.

Diffusion Data. The experimental data reported in Table 2 are the classical parameters of Gouy interferometry defined elsewhere.[71,49] The main- and cross-term diffusion coefficients are reported in Table 3 and drawn in Figures 3−6 as a function of solute concentrations. The binary data for the salt[72] and PEG 400[73] are also reported for comparison.

Data at C_1 = Variable and C_2 = Constant. The diffusion coefficients D_{11} and D_{12} are reported in Figure 3, and the coefficients D_{21} and D_{22} are reported in Figure

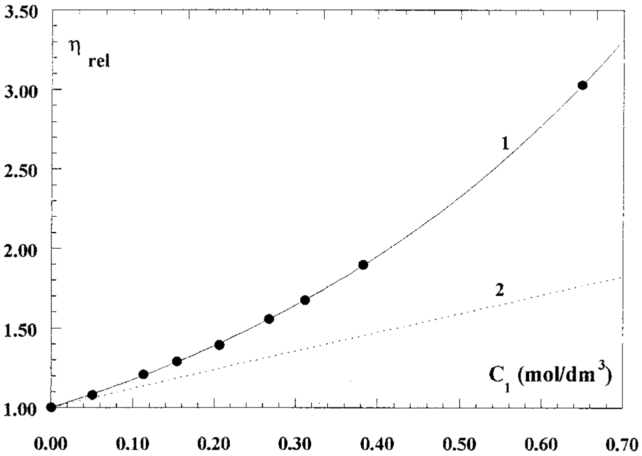

Figure 1. Relative viscosity of the binary system PEG 400 (1)−H_2O (0) as a function of PEG 400 molar concentration, C_1. Curve 1: experimental trend. Curve 2: trend predicted by the Einstein equation, eq 5, using $V_{\mathrm{eff,PEG400}} = (0.4724 \pm 0.0066)$ $\mathrm{dm^3\ mol^{-1}}$.

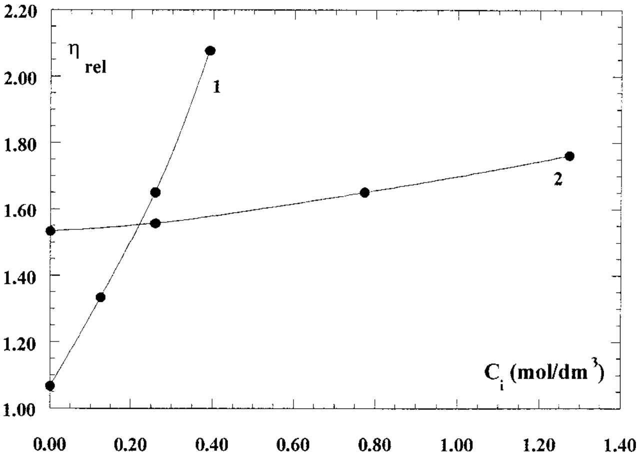

Figure 2. Relative viscosity of the ternary system PEG 400 (1)−NaCl (2)−H_2O (0). Curve 1: viscosity of the ternary system at $C_2 = 0.776$ mol/dm^3 as a function of the PEG 400 molar concentration. Curve 2: viscosity of the ternary system at $C_1 = 0.259$ mol/dm^3 as a function of the NaCl molar concentration.

4. The main diffusion coefficient D_{11} decreases at increasing PEG 400 concentration, having values smaller than the correspondent D_1; the differences are in all cases very small. The cross-term diffusion coefficient D_{12}, which is zero at $C_1 = 0$ mol/dm^3, increases at increasing C_1 and has very small values. The main diffusion coefficient D_{22} decreases very sharply at increasing PEG 400 concentration. Its limit at $C_1 \rightarrow 0$ corresponds to the binary diffusion coefficient D_2 at the same C_2 concentration. The D_{22} value obtained at the highest PEG 400 concentration is $D_{22} = 0.976 \times 10^{-5}$ cm^2/s in comparison with the corresponding binary value at the same NaCl concentration $D_2 = 1.480 \times 10^{-5}$ cm^2/s. The effect of adding the polymeric component is thus very large in determining the value of the diffusion coefficient of the simple salt. The effect of PEG 400 on the motion of the NaCl is also shown by the D_{21} diffusion coefficient. It is almost constant at varying C_1, with very large values of the same order of magnitude as D_{22}.

Data at C_1 = Constant and C_2 = Variable. The diffusion coefficients D_{11} and D_{12} are reported in Figure 5, and the coefficients D_{21} and D_{22} are reported in Figure 6. The main diffusion coefficient D_{11} decreases a little at increasing NaCl concentrations. Its limit value at $C_2 \rightarrow 0$ corresponds to the binary diffusion D_1 at the same PEG 400 concentration. The cross-term diffusion coefficient D_{12} is almost constant with increasing C_2, also having small values in this case. The main diffusion coefficient D_{22} is almost constant, with C_2 having values smaller than the corresponding value of the binary system D_2. The two curves as a function of C_2 are almost parallel, and the average difference $(D_2 - D_{22})$ is quite large, $(D_2 - D_{22}) = 0.32 \times 10^{-5}$ cm^2/s. The cross-diffusion coefficient D_{21} shows a large increase with increasing the NaCl molar concentration. It is zero at $C_2 = 0$ mol/dm^3 and has very large values at the highest NaCl concentration, at $C_2 = 1.2718$ mol/dm^3. Its value at that concentration is $D_{21} = 0.832 \times 10^{-5}$ cm^2/s and is significant compared to the corresponding main term $D_{22} = 1.167 \times 10^{-5}$ cm^2/s.

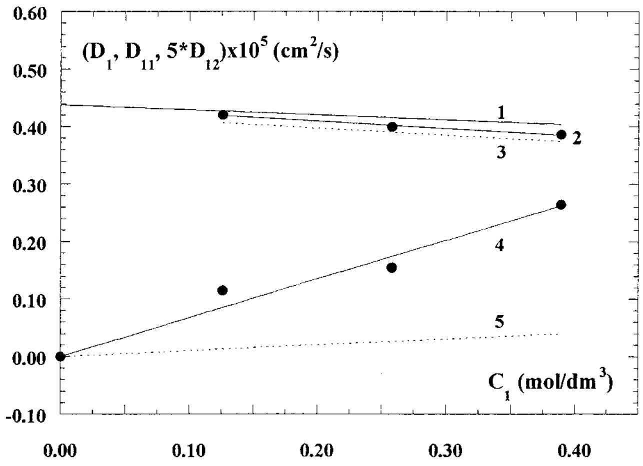

Figure 3. Diffusion coefficient of the ternary system PEG 400 (1)−NaCl (2)−H_2O (0) at constant NaCl concentration, $C_2 = 0.776$ mol/dm^3, as a function of PEG 400 molar concentration. Curve 1: D_1 trend. Curve 2: experimental D_{11}. Curve 3: D_{11} predicted trend by eq 7 using the experimental binary and ternary viscosities. Curve 4: experimental D_{12}. Curve 5: D_{12} predicted trend using eq 11.

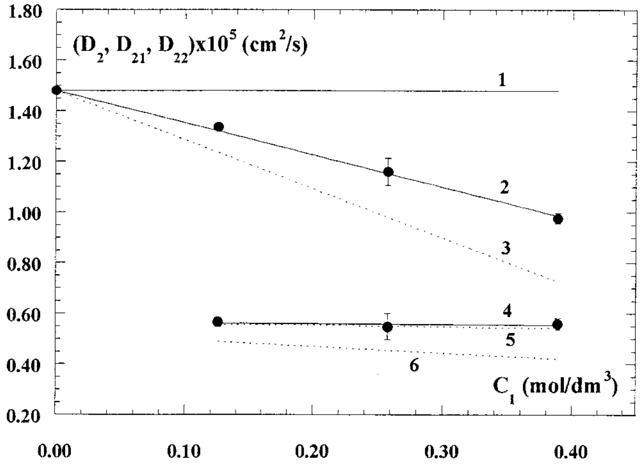

Figure 4. Diffusion coefficient of the ternary system PEG 400-(1)-NaCl(2)−H_2O(0) at constant NaCl concentration, $C_2 = 0.776$ mol/dm^3, as a function of PEG 400 molar concentration. Curve 1: D_2 trend. Curve 2: experimental D_{22}. Curve 3: D_{22} predicted trend by eq 7 using the experimental binary and ternary viscosities. Curve 4: experimental D_{21}. Curve 5: D_{12} predicted trend using eq 11 and the experimental D_{21} values. Curve 6: D_{21} predicted trend using eq 11 and the D_{22} values predicted by eq 7.

Change of Solvent Constituent. Because of the abundance of two components, the crowded solutions

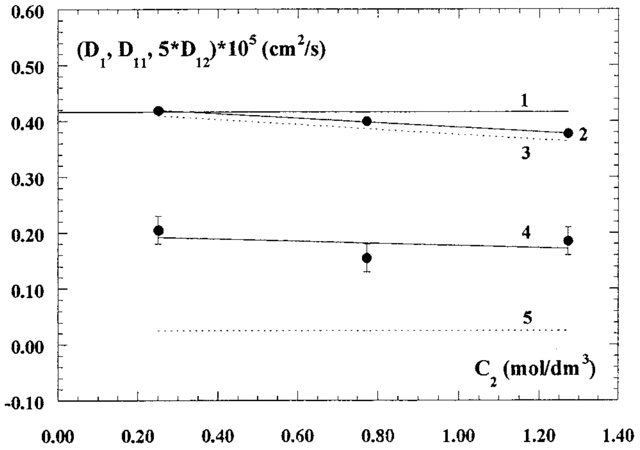

Figure 5. Diffusion coefficient of the ternary system PEG 400 (1)$-$NaCl (2)$-H_2O$ (0) at constant PEG 400 concentration, $C_1 = 0.259$ mol/dm^3, as a function of NaCl molar concentration. Curve 1: D_1 trend. Curve 2: experimental D_{11}. Curve 3: D_{11} predicted trend by eq 7 using the experimental binary and ternary viscosities. Curve 4: experimental D_{12}. Curve 5: D_{12} predicted trend using eq 11 and the experimental D_{11} values.

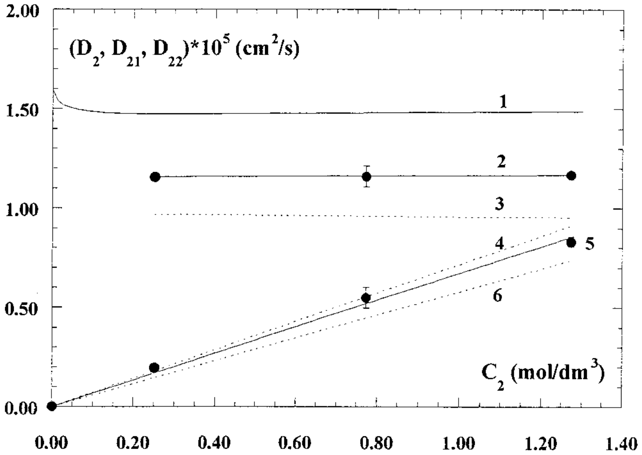

Figure 6. Diffusion coefficient of the ternary system PEG 400 (1)$-$NaCl (2)$-H_2O$ (0) at constant PEG concentration, $C_2 = 0.776$ mol/dm^3, as a function of NaCl molar concentration. Curve 1: D_2 trend. Curve 2: experimental D_{22}. Curve 3: D_{22} predicted trend by eq 7 using the experimental binary and ternary viscosities. Curve 4: experimental D_{21}. Curve 5: D_{21} predicted trend using eq 11 and the experimental D_{22} values. Curve 6: D_{21} predicted trend using eq 11 and the D_{22} values predicted by eq 7.

can be analyzed with different choice of the solvent constituent. To make more clear the presentation of results in our crowded solution, we have evaluated the D_{ij}^{γ} using alternatively water, PEG and NaCl as solvent, γ, where γ denotes the choice of solvent.[51] In Table 3 are reported the three different representations of the diffusion coefficients determined, as described in the literature by some of us, from the H_i, C_i, and D_{ij} values.[51] This different point of view is consistent with the strong volumetric effect of macromolecules, emphasizing that the counter-flow related to the PEG motion is due essentially to the water.

The high D_{21}^{0} and D_{01}^{2} values show clearly the volumetric effect of the PEG 400 (1). About the counter-flow a rough picture of the dragging effect of a component i under the flow of the component j can be obtained by the ratio $D_{ij}^{\gamma}/D_{jj}^{\gamma}$. Changing the solvent we can evaluate who takes the place of PEG or salt motion. If the NaCl is chosen as the solvent, the movement of PEG results in a large amount of water taking its place by moving in the opposite direction. That is because the cross-term D_{01}^{2} (movement of water due to PEG gradient) is large and positive compared to the main term D_{11}^{2} (movement of PEG due to PEG gradient) which is small and negative, as is shown in Table 3. We emphasize that the D_{11}^{2} is negative; this unusual, counter-intuitive case is perfectly possible,[53] provided the sum of the main terms is positive. Therefore, the ratio of D_{01}^{2}/D_{11}^{2} is about -80, so the PEG transports a lot of water in the opposite direction and no more than two salt molecules in the same direction. Furthermore, some water will be attached on the hydration shells of PEG, and thus the water molecules moved by PEG will be even higher than 80. Finally even if in our system the salt is very speedy and abundant, the counter-flow associated with the PEG motion is due to the water, at least for 98%. This can be considered a property of any macromolecule: the counter-flow associated with a macromolecule is essentially due to the most abundant component and the assumption used in eq 11 holds.

V. Discussion

Test of the Predictive Equation. The experimental D_{ij} values and the predicted ones from eqs 7, 11, and 16 are reported in Table 4. We start by analyzing the capability of eq 7 to predict the main diffusion coefficients D_{11} and D_{22}. As can be seen in Table 4 and Figures 3 and 5, the main diffusion coefficient of the polymeric component, D_{11}, can be predicted with a fairly good accuracy even if the experimental D_{11} values are always larger than the predicted ones. In contrast, in Table 4 and Figures 4 and 6, significant differences have been found between the experimental and predicted main-term diffusion coefficients of the simple salt component, D_{22}.

The inability of eq 7 to predict the simple salt main diffusion coefficient needs some comments. We recall that in lysozyme (1)$-$NaCl (2)$-$water (0) system, a good prediction of the simple salt main diffusion coefficient, based on eq 7, was obtained.[25] In the present case, several factors, not present in the lysozyme$-$NaCl$-$water system, can contribute to make the prediction unreliable. First the large decrease of the dielectric constant at increasing PEG 400 concentration can modify the ion$-$ion interaction.[74] This makes unrealistic the use in eq 7 of the D_2 diffusion coefficient of the corresponding binary aqueous salt solution. Moreover, it seems that the sodium ion can form a complex with the PEG 400, which is known as a poor crown ether.[75,76] Another factor could contribute to make the prediction of the D_{22} unreliable. We found that eq 7 gives the best results in dilute ternary solutions where the ternary viscosity is not very different from the two corresponding binary viscosities. In the present case, the viscosity of the ternary system is close to the viscosity of the polymeric binary system, but is systematically much larger than the viscosity of the simple salt binary system. The good prediction of D_{11} and the bad prediction of D_{22} seem to confirm that eq 7 gives worse results when the viscosity of binary and ternary systems are too different.

In considering the ability of eq 11 to predict the cross-term diffusion coefficients, some observations are necessary. The prediction of the D_{12} is very poor. The predicted values are always very small with respect to the experimental values; the ratio between the experi-

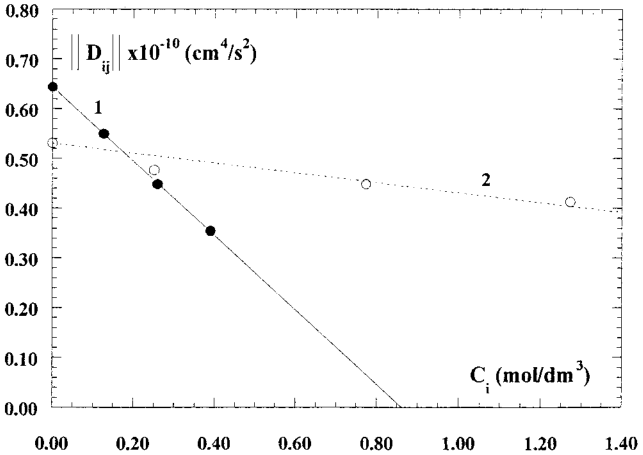

Figure 7. Determinant of the diffusion matrix for the ternary system PEG 400(1)-NaCl(2)$-H_2O$(0). Curve 1: at constant NaCl concentration, $C_2 = 0.776$ mol/dm^3, as a function of PEG molar concentration. Curve 2: at constant PEG concentration, $C_2 = 0.259$ mol/dm^3, as a function of NaCl molar concentration.

mental and predicted values is about 10. This cannot be imputed to a bad prediction of the corresponding main term, D_{11}, which is involved in computing D_{12}, because the D_{11} prediction is fairly good. On the other hand, the V_2 value necessary to obtain good agreement between experimental and predicted values is absolutely unrealistic. Consequently, some other effects must be present to invalidate the use of eq 11 in predicting the D_{12} values. A possible chemical or electrostatic interaction between PEG 400 and the NaCl could justify the difference between the experimental and predicted values.

Regarding the D_{21} diffusion coefficient, the prediction suffers from the inability of eq 7 to predict correctly the main diffusion coefficient D_{22} (see Table 4 and curves 6 of Figure 4 and Figure 6). If the experimental D_{22} is used, the prediction is quite good, considering the errors on the diffusion coefficients. A realistic estimate of errors reported in figures, two times the standard deviations, comes from the calculations using the four possible three experiments set at different refractive ratio, α_i, for each overall ternary solution composition.

Very strangely the D_{ij} ($i \neq j$) Batchelor prediction gives opposite results. A very good agreement between the experimental and predicted D_{12} is found while the D_{21} are greatly overestimated.

Determinant of the Diffusion Matrix. The polymer−salt−water systems can be subject to phase separation.[38] The diffusion matrix is very sensitive to the thermodynamic stability of the system. In fact the conditions for thermodynamic stability imply that the trace of the diffusion matrix must be positive and the determinant be positive.[77,78] This determinant is zero only along the spinodal curve, so the experimental value of determinant $||D_{ij}||$ is a measure of the system thermodynamic stability. The $||D_{ij}||$ values at the compositions investigated are reported in Table 3 and drawn in Figure 7. The $||D_{ij}||$ value decreases at increasing C_1 (C_2) at constant C_2 (C_1). The largest variation is observed changing C_1 at constant C_2. By extending the linear experimental trends of the $||D_{ij}||$ data as a function of C_1 outside the concentration range investigated, we can obtain a rough estimate for the spinodal composition [$C_1 = 0.85$ mol dm^{-3}; $C_2 = 0.776$ mol dm^{-3}]. Because of the very small variation of $||D_{ij}||$ as a function of C_2 and a slight scatter of points, we preferred not to extrapolate the observed trend to $||D_{ij}|| = 0$ to get the spinodal composition at constant C_1. We point out that this aspect is of great importance in crystallization phenomena. A similar analysis can be extended to systems containing proteins to get actual spinodal compositions of interest in obtaining protein crystals. This is one of the few examples in the literature of how nonequilibrium data can be useful to obtaining equilibrium data.[77,19]

Gravitational Behavior. Finally we want to comment briefly on the gravitational instability of diffusion boundaries in our experiments. In fact, in multicomponent systems, even starting from an initial gravitationally stable diffusion boundary, it is possible to have density inversion along the vertical axis of the diffusion cell, followed by gravitational instability. This aspect has been investigated by some of us,[79−81] assuming a linear relation between density and solute concentrations (eq 2). The instability occurs when the isodensimetric conditions are included between the region of “fingers” and “overstability”.[81] The analysis of the gravitational instability can be helpful to clarify how the addition of PEG 400 to protein-salt systems reduces the nucleation time,[28,36,82] thereby changing the time evolution of the large fluctuation in metastable systems.[83] In general the addition of PEG to a ternary aqueous system containing proteins and a simple salt can change the conditions for the appearance of convection. In Table 1 the ratios of the solute concentration gradients ($\Delta C_2/\Delta C_1$) defining the instability conditions are reported for the five compositions investigated. In our system an increase of PEG 400 concentration produces an increment of the gravitational stability composition range, between overstability and fingering.

The extension of this gravitational analysis to a quaternary system is already theoretically developed.[79] It will be tested experimentally on an aqueous quaternary system containing protein−PEG and a simple salt.

VI. Conclusion

The necessity to use the multicomponent approach in polymer diffusion description is stressed. The mutual diffusion coefficients for the ternary system PEG 400-NaCl−water have been measured. A recent procedure to predict the mutual diffusion coefficients for ternary systems containing hard sphere solutes has been tested on this system. We obtained a good result in predicting the main diffusion coefficients of the polymeric solute and the cross-diffusion coefficients relative to the motion of NaCl under the polymeric solute concentration gradient. The prediction of other two diffusion coefficients is poor even if their trends as a function of the solutes concentrations is qualitatively predicted. Our D_{ij} predictive analysis allows a better evaluation of the D_{ij} than Batchelor’s approach.

We stress that in some cases a predictive analysis is the only way to obtain some estimation of the D_{ij}’s in polydisperse samples. We mean a polydisperse sample from a diffusive point of view for synthetic polymers,[49,88] as well as for homo-association phenomena (micelles,[84] vescicles,[85] microemulsion,[86] nucleation[87]). In these cases, it is impossible to determine the D_{ij} because it is impossible to change independently the concentration gradient of each components as required by the current experimental techniques.

The analysis of the change of solvent constituent has emphasized that the counter-flow associated with the polymer motion in crowded solutions in general is essentially due to the constituent present in largest amount (usually called the solvent: here it is water), and not to solutes, even as fast and abundant as the salt in our system. The experimental diffusion coefficients have been used to analyze the thermodynamic and gravitational stability of the investigated system. This analysis allowed us to evaluate one composition of the spinodal curve, and to show that the thermodynamic stability of the system increases at increasing PEG concentration.

Acknowledgment. This research was supported by the ASI Contract CNR/ASI ARS-99-15. The authors thank also Pamela Bowman for her help in performing a literature search for this work.

References and Notes

(1) Vitagliano, V. *Pure Appl. Chem*. **1991**, *63*, 1441.
(2) Nesmelova, I.V.; Fedotov, V. D. *Biochim. Biophys. Acta* **1998**, *1383*, 311.
(3) Tyrrell, H. J. V.; Harris, K. R. *Diffusion in liquids*; Butterworths: London, 1984.
(4) Gershon, N. D.; Porter, K. R.; Trus, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5030.
(5) Otalora, F.; Garcia-Ruiz, J. M. *J. Cryst. Growth* **1997**, *182*, 141.
(6) Lin, H.; Rosenberger, F.; Alexander J. I. D.; Nadarajah, A. *J. Cryst. Growth* **1995**, *151*, 153.
(7) McPherson, A. *J. Phys. D: Appl. Phys*. **1993**, *26*, B104.
(8) Ducruix, A.; Giegè, R. In *Crystallization of Nucleic Acids and Proteins. A practical Approach*; Ducruix, A.; Giegè, R., Eds.; IRL Press and Oxford University Press: Oxford, England, 1992.
(9) Castagnolo, D.; Carotenuto, L; Vergara, A.; Sartorio, R.; Vitagliano, V. Numerical simulations of FID hydrodynamics of protein crystallization. *J. Cryst. Growth*, in press.
(10) Jacobson, K.; Wojcieszyn, J. *Proc. Natl. Sci. U.S.A.* **1984**, *81*, 6747.
(11) Patel, S.; Cudney, B.; McPherson, A. *Biochem. Biophys. Res. Commun*. **1995**, *207*, 819.
(12) Adams, M.; Fraden, S. *Biophys. J*. **1998**, *74*, 669.
(13) Paduano, L.; Sartorio, R.; Vitagliano, V.; Albright, J. G.; Miller, D. G.; Mitchell, J. *J. Phys. Chem*. **1990**, *94*, 6885.
(14) Paduano, L.; Sartorio, R.; Vitagliano, V. *J. Phys. Chem. B* **1998**, *102*, 5023.
(15) Albright, J. G.; Vergara, A.; Paduano, L.; Vitagliano, V.; Sartorio, R Multicomponent diffusion in systems containing molecules of different size. 1. Mutual diffusion in ternary system poly(ethylene glycol) 2000-poly(ethylene glycol) 200-water. Submitted to *Macromolecules.*
(16) Sartorio, R.; Vitagliano, V.; Guarino, G.; Ortona, O. *J. Solution Chem*. **1987**, *16*, 935.
(17) Leaist, D. G. *J. Solution Chem*. **1989**, *18*, 420.
(18) Albright, J. G.; Annunziata, O.; Miller, D. G.; Paduano, L.; Pearlstein, J. *J. Am. Chem. Soc.* **1999**, *121*, 3256.
(19) Annunziata, O.; Paduano, L.; Pealrstein, A. J.; Miller, D. G.; Albright, J. G. *J. Am. Chem. Soc.* **2000**, *122*, 5916.
(20) Leaist, D. G. *J. Phys. Chem*. **1986**, *90*, 6600.
(21) Leaist, D. G. *J. Phys. Chem*. **1989**, *93*, 474.
(22) Leaist, D. G. *J. Chem. Soc., Faraday Trans*. **1986**, *90*, 6600.
(23) Leaist, D. G. *J. Solution Chem*. **1987**, *16*, 805.
(24) Vergara, A.; Paduano, L.; Vitagliano, V.; Sartorio, R. *J. Phys. Chem. B* **2000**, *104(33)*, 8086.
(25) Vergara, A.; Paduano, L.; Vitagliano, V.; Sartorio, R. *Mater. Chem. Phys.* **2000**, *66*, 126.
(26) Vergara, A.; Paduano, L.; Vitagliano, V.; Sartorio, R, Multicomponent diffusion in systems containing molecules of different size. 3. Mutual diffusion in ternary system hexaethyleneglycol-diethyleneglycol-water. Submitted to *Phys. Chem. Chem. Phys.*
(27) Vergara, A.; Paduano, L.; Vitagliano, V.; Sartorio, R, *J. Phys. Chem. B*, **2001**, *105*, 328.
(28) Ray, W. J., Jr.; Bracker, C. E. *J. Cryst. Growth* **1986**, *76*, 562.
(29) Ray, W. J., Jr. *PROTEINS: Struct., Function, Genetics* **1992**, *14*, 300.
(30) Ray, W. J., Jr.; Puvathingal, J. M. *J. Biol. Chem*. **1986**, *261*, 11544.
(31) Arakali, S. V.; Luft, J. R.; De Titta, G. T. *Acta Crystallogr.* **1995**, *D51*, 772.
(32) DeLucas, L. J.; et al. *Science* **1989**, *246*, 651.
(33) Chayen, N.; Helliwell, J. R. *Nature* **1999**, *398*, 20.
(34) Vekilov, P. G.; Alexander, J. I. D.; Rosenberger, F. *Phys. Rev. E* **1996**, *54*, 6650.
(35) Kulkarni, A. M.; Chatterjee, A. P.; Schweitzer, K. S.; Zukoski, C. F. *Phys. Rev. Lett.* **1999**, *83*, 4554.
(36) Kulkarni, A.; Zukoski, C. Depletion interactions and protein crystallization. *J. Cryst. Growth*, in press.
(37) Bailey, G. T., Jr.; Callard, F. E. *J. Appl. Polym. Sci*. **1959**, *1*, 56.
(38) Kenkare, P. U.; Hall, C. K. *AIChE J*. **1996**, *42*, 3508.
(39) Garvey, M. J.; Robb, I. D. *Makrom. Chem. Rapid Commun.* **1978**, *1*, 993.
(40) Boucher, E. A.; Hines, P. M. *J. Polym. Sci.: Polym. Phys.* **1978**, *16*, 501.
(41) Philipps, H. O.; Marcinnkowsky, A. E.; Sachs, S. B.; Kraus, K. A. *J. Phys. Chem*. **1977**, *81*, 679.
(42) Bordi, F.; Cametti, C.; Di Biasio, A. *J. Phys. Chem***.** **1988**, *92*, 4772.
(43) Adamcova, Z. *Adv. Chem. Ser*. **1976**, *155*, 361.
(44) Snyder, S. M.; Cole, D. K.; Szlag, D. C. *J. Chem. Eng. Data* **1992**, *37*, 268.
(45) Honda, H.; Ono, K.; Murakami, K. *Macromolecules* **1990**, *23*, 515.
(46) Unpublished data corresponding to ternary systems PEG 2000−NaCl−water (University of Naples) and tetraethylene glycol−NaCl−water (in collaboration with J. G. Albright at the Texas Christian University).
(47) Cussler, E. R.; Lightfood, E. N. *J. Phys. Chem*. **1965**, *69*, 2875.
(48) Batchelor, G. K. *J. Fluid Mech*. **1983**, *131*, 155.
(49) Vergara, A.; Paduano, L.; Vitagliano, V.; Sartorio, R. *J. Phys. Chem. B* **1999**, *102*, 8756.
(50) Kegeles, G.; Gosting, L. J. *J. Am. Chem. Soc.* **1947**, *69*, 2516.
(51) Albright, J. G.; Miller, D. G. *J. Phys. Chem*. **1989**, *93*, 2170.
(52) Miller, D. G.; Sartorio, R.; Paduano, L. *J. Solution Chem.* **1992**, *21*, 459.
(53) Miller, D. G.; Vitagliano, V.; Sartorio, R. *J. Phys. Chem*. **1986**, *90*, 1509.
(54) Felderhof, B. U.; Jones, R. B. *Faraday Discuss. Chem. Soc.* **1983**, *76*, 179.
(55) Felderhof, B. U. *Physica* **1977**, *89A*, 373.
(56) Felderhof, B. U. *J. Phys. A: Math. Gen*. **1978**, *11*, 929.
(57) Phillies, G. D. J. *J. Colloid Interface Sci*. **1987**, *119*, 518.
(58) Phillies, G.; Stott, J.; Ren, S. Z. *J. Phys. Chem*. **1993**, *97*, 11563.
(59) Phillies, G. D. J. *J. Phys. Chem*. **1995**, *99*, 4265.
(60) Batchelor, G. K. *J. Fluid Mech*. **1976**, *74*, 1.
(61) Carter, J. M.; Phillies, G. D. J. *J. Phys. Chem*. **1985**, *89*, 5118.
(62) Han, J.; Herzfeld, J. *Biophys. J*. **1993**, *65*, 1155.
(63) Mao, Y.; Cates, M. E.; Lekkerkerker, H. N. W. *Physica A* **1995**, *222*, 10.
(64) Cuneo, P.; Magri, E.; A. Verzola, E. Grazi, *Biochem. J*. **1992**, *281*, 507.
(65) Herzfeld, J. *Acc. Chem. Res*. **1996**, *29*, 31.
(66) Hanna, S.; Hess, W.; Klein, R. *Physica* **1982**, *111A*, 181.
(67) Alder, B. J.; Gass, D. M.; Wainwright, T. E. *J. Chem. Phys.* **1970**, *53*, 3813.
(68) Sharma, R.; Tankeshwar, K. *J. Chem. Phys*. **1998**, *108*, 2601.
(69) Trullas, J.; Padro, J. A. *J. Chem. Phys*. **1993**, *99*, 3983.
(70) Ranieri, F. O.; Timmermann, E. O. *J. Chem. Soc., Faraday Trans*. **1986**, *82*, 1339.
(71) Fujita, H.; Gosting, L. J. *J. Phys. Chem*. **1960**, *64*, 1256.
(72) Lobo, V. M. M.; Quaresma, J. L. *Electrolyte solutions: Literature data on thermodynamic and transport properties*; Coimbra, 1981; Vols. I and II.
(73) Vergara, A.; Paduano, L.; Vitagliano, V.; Sartorio, R. *Phys. Chem. Chem. Phys* **1999**, *1*, 537.
(74) Arnold, K.; Herrmann, A.; Pratsch, L.; Gawricsh, K. *Biochim. Biophys. Acta* **1985**, *815*, 515.
(75) Kraus, S.; Roger, S. *J. Chromatogr*. **1983**, *257*, 237.
(76) Balasubramanian, D.; Chandani, B. *J. Chem. Educ.* **1983**, *60*, 77.
(77) Vitagliano, V.; Sartorio, R.; Spaduzzi, D.; Laurentino, R. *J. Solution Chem*. **1978**, *7*, 605.
(78) Izmailov, A. F.; Myerson, A. S. *J. Chem. Phys*. **2000**, *112*, 4357.
(79) Vitagliano, P. L.; Ambrosone, L.; Vitagliano, V. *J. Phys. Chem.* **1992**, *96*, 1431.
(80) Miller, D. G.; Vitagliano, V. *J. Phys. Chem*. **1986**, *90*, 1706.

(81) Vitagliano, V.; Sartorio, R.; Spaduzzi, D.; Laurentino, R. *J. Solution Chem*. **1977**, *6*, 671.
(82) Galkin, A.; Vekilov, P. Submitted to *Proc. Natl. Acad. Sci. U.S.A.*
(83) Valiati, A.; Giglio, M. *Entropie* **1999**, *35*, 33.
(84) Wennerström, H.; Lindmann, B. *Phys. Rep.* **1979**, *52*, 1.
(85) Bergströmem, M.; Eriksson, J. C. *Langmuir* **1998**, *14*, 288.
(86) Ambrosone, L.; Ceglie, A.; Colafemmina, G.; Palazzo, G. *J. Chem. Phys.* **1997**, *107*, 10756.
(87) Kadima, W.; McPherson, A.; Dunn, M. F.; Jurnak, F. A. *Biophys. J*. **1990**, *57*, 125.
(88) Harnau, L.; R.; Winkler; Reinehker, P. *Macromolecules* **1999**, *32*, 5956.

MA0011800