Pulsed Field Gradient NMR Study of Poly(ethylene glycol) Diffusion in Whey Protein Solutions and Gels

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ABSTRACT: PEG self-diffusion coefficients of poly(ethylene glycol)s (PEGs) (1080, 8500, and 82 250 g/mol) were measured by PFG-NMR spectroscopy in whey protein solutions and gels in relation to whey protein concentration effects (from 6.49 to 40.45 g/100 g) and whey protein heat denaturation effects (30 min at 70 °C). A strong dependency of diffusion on probe size was observed in both whey protein solutions and gels: as PEG size increased, diffusion was reduced. This effect was more pronounced for higher protein concentrations. Changes in whey structure after thermal aggregation increased the diffusion coefficient for all PEGs, particularly for the 8500 g/mol PEG. The PEG self-diffusion coefficients in whey protein gels were compared to the gel structures characterized by scanning electron microscopy. The results are discussed in relation to a reptation model and compared to PEG diffusion in casein micelle suspensions and gels.

Introduction

Whey proteins are the second most abundant group of milk proteins after caseins. Whey proteins are globular proteins which are highly soluble in water and mainly consist of β-lactoglobulin (β-lg), α-lactalbumin (α-la), immunoglobulin (Ig), and bovine serum albumin (BSA). Kinsella et al.[1] have published a comprehensive review of the chemical, physical, and functional properties of whey proteins. Whey proteins are highly functional food proteins that are extensively used as basic ingredients. Gel formation following heating of these proteins is an important function that has been widely explored by the food industry. Three phenomena are involved (often simultaneously) in the aggregation of globular proteins: i.e., conformational changes, chemical reactions, and physical interactions.[2,3] The heat-induced denaturation of whey proteins takes place in two steps. First there is reversible unfolding of the protein molecules and exposure of hydrophobic molecular groups (and in the case of β-lg and BSA the free sulfhydryl groups become reactive), and then the rearrangement of denatured molecules leads to irreversible aggregation and network formation.

There is a relative abundance of information in the literature concerning factors affecting the denaturation steps and gel properties of whey proteins.[4−7] From these studies it can be clearly seen that structures and properties of formed gels can be affected by various factors, including protein concentration, pH, ionic strength, ion type, and time and temperature of heating. The usual methods used for gel characterization are principally rheology and microscopy. Oscillating linear strain rheological measurements are often employed to investigate a gelling system. This technique can distinguish a solid from a liquid on the basis of the frequency dependence of the storage and loss modulus of the system.[8−12] The mechanical properties of gels are manifestations of their microstructure. Microscopy techniques can be used to determine aggregate size, gel porosity, and homogeneity of the network according to protein concentration, pH, denaturation time, NaCl concentration, etc.[3,13−15]

Transport properties are important in food products because physicochemical modifications in these systems depend on them. In the domain of pharmacy and cosmetic, they control the diffusion of microorganisms.[16−18]

Rheology and microscopy approaches make it possible to establish correlations between the spatial organization and the mechanical properties of gels, but they do not report on the influence of transport properties inside these gels.

Numerous authors have demonstrated that the obstruction effects induced by a polymeric system (gel or solution) is highly sensitive to the size of the diffusing molecule. Therefore, additional information is obtained by varying the size of the diffusing probe molecule.

Also, to extend the scope of this study, we chose to introduce probes into matrices of structure and variable composition and to study the consequences on the diffusion of the probe molecules. Thus, both the size of the diffusing particle and the porosity of the obstructing network can be investigated.

As a nondestructive method, NMR is a tool of choice for a vast number of diffusion investigations in solutions and gels. Several variations of the method have been explored since the development by Stejskal and Tanner[19] of the pulsed gradient spin-echo sequence (PGSE), but the principle remains the same, with the application of two gradient pulses separated by a diffusion delay of a few tens of milliseconds in order to determine the molecular root-mean-square displacements over distances on the micrometer length scale.

The water self-diffusion coefficients in whey protein solutions and gels have already been measured by PFG-NMR in previous studies.[20,21] The results showed that water diffusion was sensitive to gelation via decreases in self-diffusion coefficients. The reduction was discussed in terms of changes in the physical structure of the local environment through which water molecules diffuse, i.e., the 3D gel network formed by gelation.

The diffusion of some solutes has been studied in milk proteins.[22,23] PEG diffusion in casein solutions and gels was measured[24] in a previous study, and effects of protein concentra-

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Table 1. Composition of Whey Protein Powder for 100 g of Powder

	g for 100 g of whey protein powder
total solids	95.22
total nitrogen matter	14.65
total protein matter	91.56
lactose	0.5
calcium	0.509
potassium	0.234
magnesium	0.067
phosphorus	0.192
Sulfur	1.329
β-lactoglobulin	54.1
α-lactalbumin	12.4
pure proteins in total solids	*96.16*

Table 2. Percentage of β-lg and α-la Denaturation in Whey Protein Solutions with Thermal Denaturation in a Water Bath at 70 °C for 30 min (NaCl 0.1 M)

whey protein concn (g/100 g)	% of β-lg denaturation	% of α-la denaturation
9.09	46.00	96.00
14.53	47.9	100
18.70	53.6	100
19.35	57.2	100
22.48	50.2	100

tion, PEG size, and gelation were investigated. Croguennoc et al.[25] measured dextran diffusion (1 700 000, 250 000, and 62 000 g/mol) in β-lg gels. Decreases in dextran self-diffusion coefficients occurred for each molecular mass during the sol/gel transition and were explained by friction between dextran and aggregated β-lg. It was found that the β-lg self-diffusion coefficient increased with sol/gel transition. These authors argued that the friction caused by the immobile structure of the gel was less than that caused by the mobile native proteins.

In the study reported here, poly(ethylene glycol)s (PEGs) were selected as probes offering a series of key advantages: a broad range of molecular weights can be covered, and they have a polydispersity factor close to one which prevents complications arising from molecular weight distribution effects. Weak interactions with proteins make it possible to observe the obstruction effects induced by proteins in various porous structures. Moreover, PEGs have been successfully used to probe various systems.[24,26−34]

The aims of this study are to investigate the self-diffusion coefficient of molecular probes in whey protein solutions and gels, in particular with regard to the effects of the size of the probe, the whey protein concentration, and the change in structure induced by gelation. The self-diffusion coefficient measurements are related to scanning electron microscopy (SEM) images in order to understand the effects of the gel matrix structure on PEG diffusion. Finally, the PEG diffusion in whey protein solutions and gels is compared with PEG diffusion in casein micelle suspensions and gels.

Materials and Methods

Materials. Whey protein powder from INRA (Rennes, France) was used (powder composition summarized in Table 1). In Table 2 the percentages of β-lg and α-la denaturation are given for a protein gel of 29.35 g/100 g. Powders were analyzed by SOREDAB (Bongrain, France).

PEG polymers $[H(OCH_2CH_2)]_nOH$ were supplied from Polymer Laboratories (Marseille, France) with different average molecular weights (M_w = 1080, 8500, and 82 250 g/mol) and low polydispersity indices (1.01, 1.02, and 1.02, respectively, as indicated by the suppliers). All polymers were used without further purification. The D_2O (purity above 99.8%) used for mixing the samples was purchased from Dr. Glaser (Basel, Switzerland). Sodium azide (NaN_3) (Merck, Darmstadt, Germany) and NaCl were used without purification.

Table 3. Hydrodynamic Radius of PEG Calculated with the Stokes−Einstein Equation from PEG Diffusion in D_2O at 20 °C

M_w (g/mol)	M_w/M_n	D_0 ($m^2\ s^{-1}$)	R_h* (nm)
1080	1.01	1.82×10^{-10}	1.18
8500	1.02	5.82×10^{-11}	3.68
82250	1.02	1.60×10^{-11}	13.39
634000	1.07	5.45×10^{-12}	39.30

Sample Preparation. Rehydration of the whey protein powder was performed at room temperature with a D_2O/NaCl solution (0.1 M). Sodium azide was added (0.02% w/w) to each solution to prevent bacterial growth. PEG was added at solutions for each average molecular weight (0.1% w/w). pH was measured, and no differences were observed between the samples (pH = 6.8 for whey protein solution of 32 g/100 g with PEG 82 250 g/mol).

Whey protein gels were prepared from whey protein solutions by thermal aggregation. The solutions were transferred to 5 mm NMR tubes (∼1 mL) and were kept at 70 °C in a water bath for 30 min and then cooled to 20 °C. Water evaporation was checked by weighing NMR tubes before and after gelation. No significant loss of water content occurred from the gel. NMR measurements were performed at least 2 days after sample preparation.

Determination of Dry Matter. The dry matter of all whey protein solutions was determined by measuring variations in weight after drying in an oven for 24 h at 100 °C. Protein concentrations were calculated from dry matter values and pure protein percentages (96.16% for whey proteins) (see Table 1). The protein concentrations ranged between 6.49 g/100 g and 40.45 g/100 g.

NMR Self-Diffusion Measurements. The majority of measurements were performed on a 200 MHz Bruker spectrometer, and some complementary measurements were performed on a 400 MHz Bruker spectrometer, both equipped with a field gradient probe.

All the measurements were conducted at 20 ± 0.1 °C. The gradient strength g used in this study ranged between 1.07 and 9.63 T/m. The diffusion coefficients were obtained using

$$\frac{I(\delta,\Delta,g)}{I_0} = \exp[-kD] \qquad (1)$$

with $k = \gamma^2 g^2 \delta^2 (\Delta - \delta/3)$. $I(\delta,\Delta,g)$ and I_0 are the echo intensities in the presence and absence of pulse gradients, respectively. The length of the gradient pulse is δ, Δ is the distance between the leading edges of gradient pulses (named diffusion time), γ is the gyromagnetic ratio (for protons, $\gamma = 26.7520 \times 10^7$ rad T^{-1} s^{-1}), and D is the self-diffusion coefficient.

A semilogarithmic plot of echo intensities vs k is known as a Stejskal−Tanner plot.

A total of 16 or 32 scans were collected with pulsed field gradient sequences with a recycle delay of 1 s. Δ was adjusted to have the same diffusion pathway for each PEG, in accordance with the Einstein equation $z = (2D_{PEG}\Delta)^{1/2}$ with $z \sim 2$ μm. In this case the distance covered by the PEG was greater than the whey protein diameter. For example, the value of the hydrodynamic radius of β-lg, which represents 54.1% of the whey protein powder, ranges between 2 and 4 nm.[23,35−37]

All the data processing was performed with Matlab or Tablecurve software. Monte Carlo simulations were used for error calculations with 100 iterations, following procedures described in another work.[38]

The PEG self-diffusion coefficient in pure D_2O (D_0) for each PEG was measured in a precedent work.[24] These values were used to calculate the PEG hydrodynamic radius R_h with the Stokes−Einstein equation (Table 3) and to normalize the self-diffusion coefficients in whey protein solutions and gels (D_{PEG}). The reduced diffusion coefficient is defined as $D_r = D_{PEG}/D_0$.

Scanning Electron Microscopy (SEM). Small cubes of the gels (5 × 5 × 5 mm) were cut out and immersed in 2.5% v/v glutaraldehyde at room temperature for 48 h and then rinsed

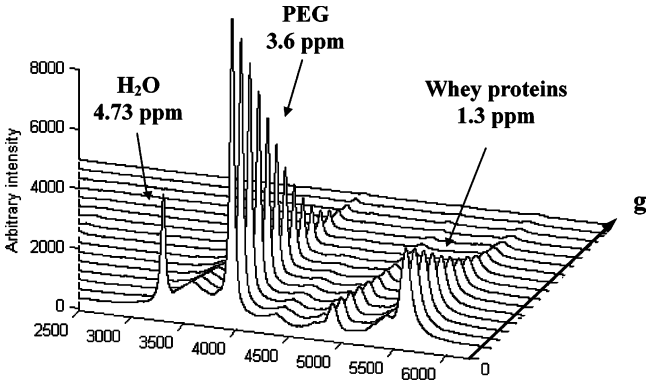

Figure 1. 8500 g/mol PEG diffusion in whey protein solution (34.81 g/100 g) at 20 °C measured with 400 MHz spectrometer and PFG NMR sequence. The abscissa is given as channel number in the 8K spectrum, with 500 points equal to about 0.7 ppm. Intensities are given in arbitrary units.

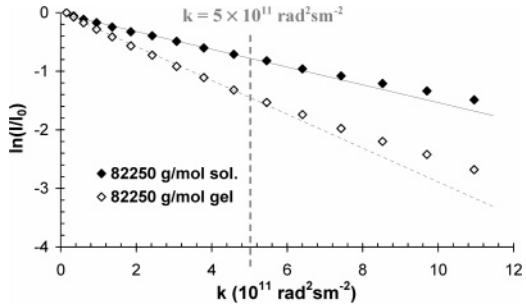

Figure 2. Stejskal−Tanner plots of 82250 g/mol PEG in whey protein solution and gel (34.81 g/100 g) at 20 °C measured with 400 MHz spectrometer and PFG NMR sequence. Dashed and solid lines are linear regressions of the first part of the data.

thoroughly three times for 10 min with distilled water, after which they were immersed in 0.2% v/v OsO_4 overnight at room temperature. The samples were rinsed several times with distilled water before being dehydrated in a graded ethanol series (10−30−50−70−80−90−95−100% (v/v)) in 20 min steps. Samples were then critical point dried through CO_2 in a critical point drier (CPD 010, Balzers Union Ltd., Liechtenstein). Dried samples were fractured, mounted onto specimen stubs, gold-coated, and analyzed microscopically using a scanning electron microscope (JEOL JSM 6301F) operating at an acceleration voltage of 9 kV. The images were produced by CMEBA (France, Rennes).

Results

1.1. Effects of Protein Signals. Whey protein protons were severely attenuated compared to PEG protons in the Hahn echo spectra due to their very much shorter spin−spin relaxation times (T_2). Nevertheless, the protein signal could not be totally attenuated for the most highly concentrated sample. An example of a proton spectrum obtained with the PFG NMR sequence for a diffusion measurement of 8500 g/mol PEG in whey protein gel (34.81 g/100 g) is presented in Figure 1. The protein signals overlap with the PEG signals at a chemical shift of ∼3.6 ppm, which induce a curvature in the Stejskal−Tanner plots. These curvatures were only observed for the highest k above 5×10^{11} **(**Figure 2).

The PEG self-diffusion coefficients were therefore calculated in all experiments with eq 1 for k values between 0 and 5×10^{11}. In this range the contribution of protein signals was negligible and linear attenuation was observed, regardless of PEG size or protein concentration in solutions and gel states.

1.2. PEG Self-Diffusion Coefficients in Whey Protein Solutions and Gels. When the denatured protein molecules were included in the network structure during aggregation, the aqueous phase was depleted of proteins and some whey proteins stayed in the aqueous phase. Using chromatography, Durand et al.[5] showed a clear separation between a narrow peak corresponding to residual native proteins and a broad peak that corresponds to the aggregates. With the thermal treatment used in this study (30 min at 70 °C) denaturation of α-la was complete and denaturation of β-lg was around 50%, independent of the concentration (Table 2).

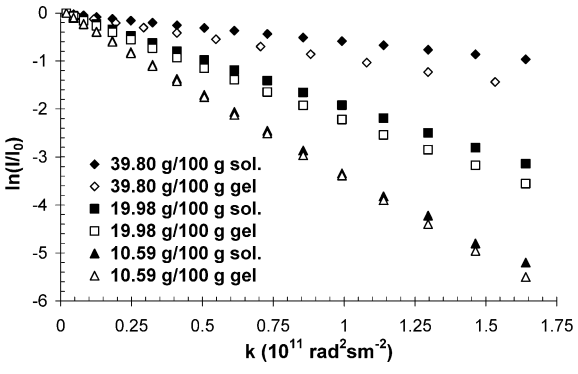

Figure 3. Stejskal−Tanner plots for 8500 g/mol PEG in whey protein solutions and gels at different concentrations at 20 °C measured with a 200 MHz spectrometer and PFG NMR sequence.

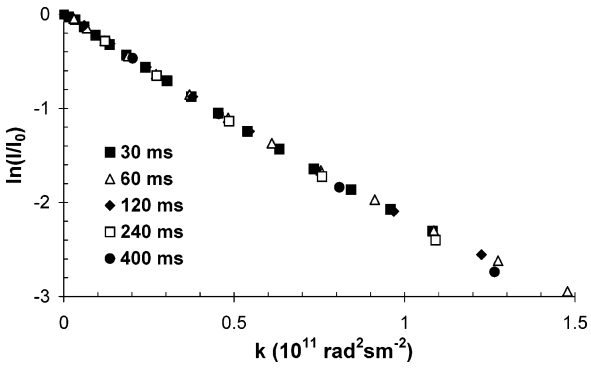

Figure 4. Stejskal−Tanner plots for 8500 g/mol PEG in whey protein gel (25 g/100 g) with various diffusion times Δ between 30 and 400 ms, $\delta = 1.2$ ms, and g between 0.7 and 5.95 T/m at 20 °C measured with a 400 MHz spectrometer and PFG NMR sequence.

The self-diffusion coefficients of all the PEGs in whey protein solutions and gels at concentrations between 6.89 and 37.88 g/100 g of water were measured at 20 °C. Examples of Stejskal−Tanner plots are given for 8500 g/mol PEG in solutions and gels for different whey protein concentrations **(**Figure 3). All the Stejskal−Tanner plots were linear with the range of k values used (see discussion above). This shows that PEGs have a single diffusion component in the observation time (Δ), corresponding to a diffusion path of around 2 μm.

The self-diffusion coefficient of 8500 g/mol PEG was measured in whey protein gel (25 g/100 g) with the PFG NMR sequence for Δ values of 30, 60, 120, 240, and 400 ms. The Stejskal−Tanner plots are given in Figure 4. The PEG self-diffusion coefficients were similar at each Δ value ($D_{PEG} \sim 2.26 \times 10^{-11}$ m^2 s^{-1}), indicating that no restricted diffusions were present and that the gel was homogeneous with respect to diffusion on a length scale of 4.2 μm.

Figure 5 shows D_r for all PEGs plotted as a function of whey protein concentrations in solutions and gels at 20 °C. As the whey protein concentration increased, the D_r decreased in both solutions and gels. Moreover, for a given whey protein concentration, the reduction in D_r was more pronounced for larger PEGs. It therefore appeared from these findings that

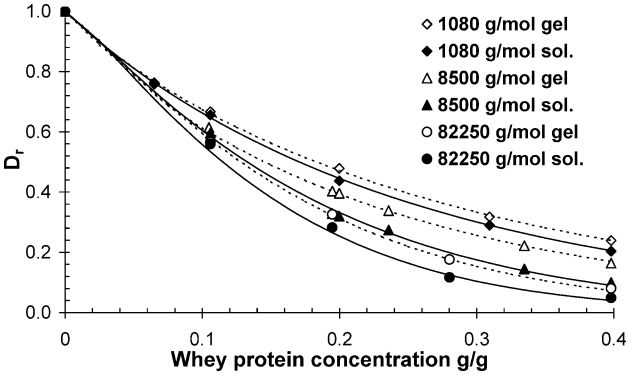

Figure 5. D_r (D_{PEG}/D_0) in whey protein solutions and gels at various concentrations. Solid lines and dashed lines are fits using the Phillies equation (eq 3) for solutions and gels, respectively.

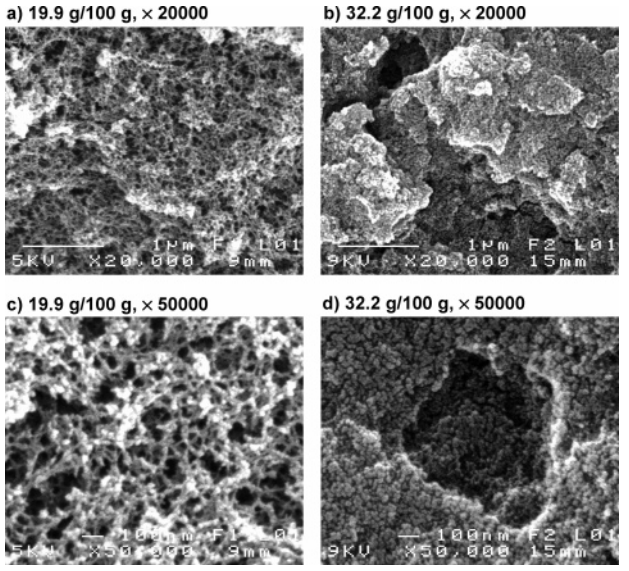

Figure 6. SEM images of whey protein gels (with $C_{NaCl} = 0.1$ M, $t_D = 30$ min at 70 °C) at two different whey protein concentrations (19.9 g/100g and 32.2 g/100 g) and two different magnifications (×20 000 and ×50 000).

PEG diffusion was sensitive to protein concentration and PEG size.

A slight increase in 1080 g/mol PEG diffusion occurred in whey protein gels compared to solutions after gelation. The difference became greater at 19.98 g/100 g, where the PEG self-diffusion coefficient changed from 8.14×10^{-11} m^2 s^{-1} in solution to 8.90×10^{-11} m^2 s^{-1} in the gel, an increase of 9%. At 39.80 g/100 g the corresponding increase was 18%. The phenomenon was accentuated for 8500 g/mol PEG; at 19.98 g/100 g the increase in D_{PEG} was 30% and reached 118% at 39.80 g/100 g. For 82 250 g/mol PEG the tendency started to reverse, in that diffusion was always faster in the gel but the difference was smaller. The increase was 15% at 19.98 g/100 g and 87% at 39.80 g/100 g.

SEM images of whey protein gels were made at different magnifications for two different whey protein concentrations (19.9 and 32.2 g/100 g) (Figure 6). The gels formed had a homogeneous structure and were composed of almost spherical aggregates linked together and forming the thread of the 3D network of chains and clusters. The whey protein formed two types of gel structure, depending on concentration: fine-stranded gels at 19.9 g/100 g and particulate gels at 32.2 g/100 g. The fine-stranded gel consisted of more or less associated linear aggregates, with very small pores between 20 and 100 nm. The particulate gels showed dense coarse domain aggregates, and the pores could no longer be distinguished on the nanometer scale. This can be clearly seen in all figures at different magnifications.

1.3. Analysis of the Diffusion Data. Numerous models have been proposed to explain the diffusion of solutes in polymer solutions, including obstruction models and hydrodynamic models. In diffusion models based on obstruction effects, the polymer is presented as fixed and impenetrable segments. In hydrodynamic theories, the frictional interactions between the solute and the polymer are taken into account. Such considerations allow the description of diffusion in more concentrated regimes where the polymer chains start to overlap, making obstruction theories less applicable. In this study, obstruction models were not appropriate for solute diffusion because the whey proteins were not immobile obstacles.[23,25] In addition, the whey protein concentrations are too high. The experimental findings are therefore discussed in terms of hydrodynamic models.

Phillies Model. This model has been proposed to describe the self-diffusion of macromolecules (polymers and proteins) in a wide range of concentrations. The stretched exponential equation proposed by Phillies is written as

$$D = D_0 \exp(-\beta c^{\nu}) \tag{2}$$

where c is the protein concentration in g/g and β and ν are scaling parameters.

Figure 5 shows the fit on PEG diffusion in protein solutions and gels with eq 2. Good agreement was observed with the experimental data.

Power Law Dependence. A simple power law gives a description of the solute diffusion coefficient vs molecular weight:

$$D_{PEG} = A M_w^{-\alpha} \tag{3}$$

with A being a preexponential factor and α a characteristic exponent. In fact, this equation is often used to describe the self-diffusion of polymer chains with α varying from 0.55 for dilute systems[39] to 2 in concentrated systems.[40]

In polymer theory, the Rouse model is a well-established model for nonentangled polymer chains.[41] The diffusion of a high molecular weight polymer in an unentangled system or a diluted solution is described by the Rouse model: $D = M_w^{-1}$.

The reptation theory was first introduced by de Gennes, who discussed the self-diffusion of a polymer chain of molecular weight M_w moving in a three-dimensional network, which is considered as a gel. The lateral movements of the chain are limited by obstacles formed by the gels. This theory was complementary to the works of Rouse. The reptation model is $D = M_w^{-2}$.

Nevertheless, the observation of α exponent near 2 is not sufficient to establish a reptation regime.[42] An excess of $\alpha = 2$ represents the operation of additional mechanisms such as constraint release.[40]

Computational studies[43] have shown that an increase in obstacle density shifts the chain diffusion dependence to a Rouse-like behavior from a reptational behavior. At the intermediate stage, the chain changes from a spherical conformation to an ellipsoidal conformation, which occurs when network mesh size approaches the solute radius.

To apply the eq 3 to our data, D_{PEG} should be corrected from changes of protein concentration. Therefore, in the following, the D_{PEG} was estimated using the Phillies model for whey

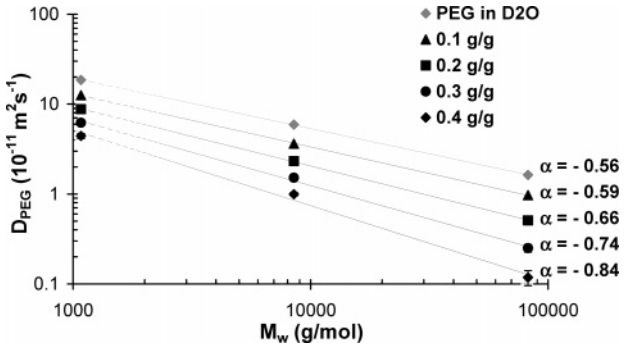

Figure 7. Power law representation of PEG self-diffusion coefficients in D_2O and whey protein gels vs PEG molecular mass for various whey protein concentrations.

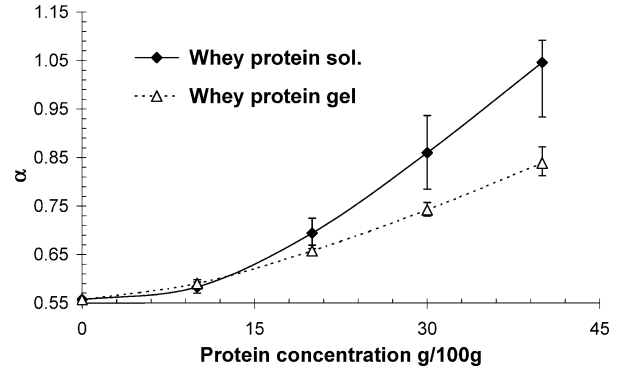

Figure 8. Exponent α extracted from eq 4 vs whey protein concentrations in solutions and gels. Solid and dashed lines are provided for easier reading.

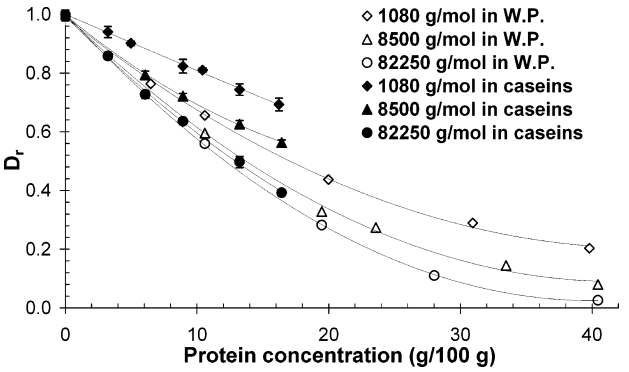

Figure 9. D_r (D_{PEG}/D_0) of PEGs in whey protein (W.P.) and casein solutions vs protein concentration. Dashed and solid lines are provided for easier reading. Casein data come from a previous study.[24]

protein concentrations between 0 and 0.4 g/g. The confidence interval included the error obtained for the fitting.

The plots of D_{PEG} vs PEG molecular mass (M_w) in whey protein gels for each concentration are given in Figure 7. Power law expression (eq 3) was used on all data and values of α were extracted for each concentration. Figure 8 shows α vs protein concentrations in whey protein solutions and gels.

Discussion

1. PEG Diffusion in Protein Solutions. In a previous study,[24] PEG self-diffusion was measured in casein suspensions and gels. Figure 9 shows D_r in casein and whey protein solutions and gels compared to protein concentrations. D_{PEG} decreased with increasing protein concentrations for both whey protein and casein. However, the type of protein affected the intensity of the decrease. For example, at a concentration of 10 g/100 g the decrease in D_r for 1080 g/mol PEG was 0.81 in a casein solution

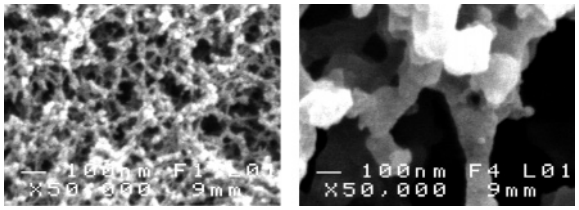

Figure 10. SEM images of whey protein gels (19.90 g/100 g) and casein gels (15.6 g/100 g).

and 0.66 in a whey protein solution. Thus, there is dependence on the protein size, which is around 2 nm for whey protein radius[23,35−37] and 100 nm for casein radius.[24,44]

Monte Carlo simulations have shown that obstacle size can have a considerable effect on how much an obstacle can hinder diffusion.[45,46] Ratto et al.[47] measured lipid diffusion in bilayers containing obstacles with radii ranging between 40 and 130 nm. In keeping with previously reported Monte Carlo simulations of diffusion,[48,49] extended obstacles were more effective than larger obstacles at hindering diffusion at a constant area fraction of 33%.

This is in agreement with our results, as caseins are larger obstacles for PEG than whey proteins. Although PEG can diffuse inside casein micelles (and not inside whey proteins because of their small size) and reduce diffusion, the mechanism was not strong enough to reduce PEG diffusion as much as in whey protein solutions. Thus, it appears that the dominant effect of the influence of protein concentration on the PEG diffusion is the interparticle distance.

Diffusion was more attenuated with increasing probe size for a given whey protein concentration. This behavior was consistent with that observed with PEG diffusion in casein suspensions and gels and must be correlated with the interparticle distance. Probe and obstacle size are thus important, and changes in obstacle size therefore have an effect on diffusion.

2. Gelation Effects. The protein reorganization after heat treatment led to an increase in interparticle distance. In agreement with SEM images, PEG diffusion in gels was enhanced by the formation of a space-spanning structure compared to solutions and thus provided less hindrance for the PEG. As seen in SEM images, the space in whey protein gels was visibly reduced compared to casein gels at the same protein concentration (Figure 10) and was in agreement with a faster PEG self-diffusion measured in casein gels.

The sol/gel transition of whey proteins had a weak effect on 1080 g/mol PEG diffusion because the size of this PEG (R_h = 1.18 nm) was sufficiently small not to be affected by structural modifications at larger distances. This was similar to 1080 g/mol PEG in casein solutions and gels.

The increase in diffusion in whey protein gels was accentuated for 8500 g/mol PEG. Gelation, and consequently creation of a less dense network and less hindrance in the aqueous phase, enhanced the 8500 g/mol PEG diffusion. Diffusion was more hindered with this PEG than the 1080 g/mol PEG in solution because of its larger size (R_h = 3.68 nm), explaining why the increase in diffusion between the solution and the gel is greater for this PEG.

The increase was smaller with 82250 g/mol PEG, diffusion being slightly faster in gels. The increase in space after thermal treatment was not sufficient to induce an equivalent effect on the diffusion coefficient.

In ι-carrageenan,[50] self-diffusion of water and aroma ($R_h \sim$ 2 nm) was found to increase in the gelled state compared to solutions. This effect was attributed to a gain in space between helices compared to the solutions containing randomly distrib-

uted chains that hinder the pathways of small molecules by obstruction effects. Croguennoc et al.[25] measured an increase in β-lg diffusion with the sol/gel transition: (2.3×10^{-11} m^2 s^{-1} and 3.3×10^{-11} m^2 s^{-1} for a solution and gel at 17.9 g/100 g, respectively). This was in agreement with the whey protein self-diffusion coefficients measured in this study for concentrations of 24.5 g/100 g: 1.45×10^{-11} m^2 s^{-1} and 2.25×10^{-11} m^2 s^{-1} in a solution and a gel, respectively.

3. Diffusion Models. In the case of small probes and in weakly concentrated systems obstruction models generally explain the diffusion. For example, PEG diffusion in casein solutions was well-defined with the obstruction model of Jönsson.[24] This model could not be applied for PEG diffusion in whey protein solutions because it assumes that the obstacle is fixed. Although large molecules such as casein micelles could be considered as immobile, this was not the case for whey proteins.

Hydrodynamic models could thus be used on PEG diffusion in whey protein solutions and gels. The Phillies hydrodynamic model showed good correlation with the experimental findings, but the physical significance of the scaling parameters β and ν in relation to the network structure or solute size remains uncertain.

Phillies suggested that β varies with R_h/a_0 for polymer probes, where a_0 is defined as the distance of closest approach between the solute and the polymer. The Phillies model was used by Masaro et al.[28,31] to analyze PEG diffusion data in poly(vinyl alcohol) (PVA) solutions and gels and by Petit et al.[51] in PVA solutions. They provided good fitting to the experimental data, but attempts to relate the scaling parameters to the physical properties of the system (such as probe size) were not successful for Masaro et al.[28,31] Petit et al.[51] proposed as a first approximation:

$$a_0 = R_h + \rho \tag{4}$$

where ρ is the monomer radius and found $\rho = 0.27$ nm for the monomer radius of PVA.

For PEG diffusion in whey protein solutions in this study, fitting of the results yielded $\beta = 11.8R_h/(R_h + 2.5)$, with $\rho = 2.5$ nm in good accordance with the hydrodynamic radius of whey proteins. In agreement with the appearance of aggregates of various sizes and no constant value for ρ, eq 4 did not fit the results in gels.

In contrast, other hydrodynamic theories have predicted a power law or linear dependence of β on R_h. Gibbs and Jonsson[52] reported β being equal to $10.8R_h^{0.52}$ for diffusion of various probes (with R_h between 0.1 and 3.7 nm) in polyacrylamide gels, whereas Russo et al.[53] suggested a linear dependence between β and R_h for the diffusion of latex spheres in aqueous solutions of hydroxypropylcellulose.

According to Phillies,[54,55] ν varied between 1 and 0.5. In this study ν values ranged between 0.97 and 1.23 in solutions and 0.93 and 1.10 in gels of whey proteins with increasing M_w. Gibbs and Jonsson[52] found ν to be approximately equal to unity for all the probes, and Masaro et al.[28,31] found ν close to 0.70 for PEG between 400 and 4000 g/mol and ν equal to 0.84 for 10 000 g/mol PEG. In the view of these authors ν seemed to be less sensitive than β to the hydrodynamic radius of the probe.

From the equation of the Phillies model a power law could be applied to PEG diffusion in whey protein solutions and gels. With the absence of entanglement between polymer chains, the diffusion could be described by the Zimm and Rouse model. The reptation model of de Gennes explained the diffusion for more concentrated systems with entangled polymer chains.

As expected, α was equal to 0.55 in pure water and shifted toward higher values with increasing whey protein concentration. An intermediate situation between the diffusion of an ideal sphere ($\alpha = 0.55$) and the wormlike displacement of a linear molecule in a network of fixed obstacles ($\alpha = 2$) was observed from our results. PEG is a highly flexible molecule, able to accommodate its size and shape to the details of the network in which it is enmeshed.[51] Our results showed that the reptation theory was not effective in demonstrating that the PEG chains were not entangled in the network.

At a given volume fraction of whey protein, α was always higher in solutions than in gels. For example, at 40 g/100 g α was equal to 1.046 in whey protein solution and 0.839 in whey protein gel. A space structure was created in the gels, and intraparticle distances increased. The PEG could thus conserve a more spherical shape to diffuse between the strands of the gels.

Favre et al.[56] showed a variation in α for PEG diffusion (4000−35 000 g/mol) in calcium alginate hydrogels (between 0 and 28.5 g L^{-1}) between 0.62 and 1.01. They concluded that the alginate network was not dense enough to trigger the reptation mechanism, but dense enough for the solute conformations corresponding to elongated shapes required for the diffusion step to be effective. The same conclusions were applicable in this study.

Conclusions

PEG self-diffusion coefficients in whey protein solutions and gels are sensitive to protein concentrations, PEG size, and gelation. Increase in PEG diffusion during the gelification of whey proteins was observed for all PEGs and was more pronounced for 8500 g/mol PEG. These results are explained by the creation of a network with more space, with the aggregation of proteins as observed on SEM images. The Phillies model was used to describe PEG diffusion in whey protein solutions and gels, but the scaling parameters α and ν lacked physical significance.

The diffusion findings were in accordance with the Rouse model involving a deformation of the PEG chain in an ellipsoidal conformation. This deformation was more pronounced in protein solutions compared to protein gels due to the creation of large space with gelation. The decrease in PEG diffusion with increasing protein concentration and PEG size was higher in whey proteins than in casein solutions and gels for a given protein concentrations. PEG diffusion was more hindered in the whey network than in the casein network for the same protein concentration. The particle sizes of whey proteins were smaller, but the interparticle distances which dominated the diffusion were shorter.

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