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Effects of Casein and Fat Content on Water Self-Diffusion Coefficients in Casein Systems: A Pulsed Field Gradient Nuclear Magnetic Resonance Study

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The water self-diffusion coefficients in casein matrixes were measured using a pulsed field gradient spin−echo nuclear magnetic resonance technique (PFG-SE NMR). The dependence of the water self-diffusion coefficient on the casein concentration and the aqueous phase composition is reported in both a rehydrated native phosphocaseinate dispersion and a concentrated casein retentate. A model has been proposed to explain the different behavior of the water self-diffusion coefficient in the two casein systems. This model demonstrates that the water self-diffusion cannot be simply explained by the water content only. So, taking into account the specific effect of each constituent of the aqueous dispersing phase, the water self-diffusion reduction induced by the casein micelle can be modeled. The effect of fat on the water self-diffusion coefficients was investigated. Anhydrous milk fat-reconstituted retentate samples were used in order to estimate the obstruction effect of fat globules in the modeling process. The dependence of the self-diffusion coefficient of water on the fat and casein content is reported. A general model included the effect of the aqueous phase composition, and the obstruction effects of casein micelles and fat globules were proposed. This model was validated for water self-diffusion coefficients in industrial fatty retentates.

KEYWORDS: Casein; fat; ultrafiltrated retentate; aqueous phase composition; ^{1}H NMR; self-diffusion; diffusion model

INTRODUCTION

Characterization of the transport phenomena in food science represents a considerable challenge for the design and optimization of food processes. A quantitative description of water diffusion or solute diffusion leads to a better knowledge of biomolecular interactions (*1*), porous media structure, or organization (*2*−*5*). In dairy gels, water diffusion is central in many steps of cheese manufacture (*6*, *7*). Indeed, diffusion mechanisms are involved in enzymatic reactions during renneting or ripening and they are responsible for food stability during storage.

Among the techniques that allow the determination of the water diffusion coefficient, the pulsed field gradient spin−echo nuclear magnetic resonance (PFG-SE NMR) technique is particularly interesting (*8*). Indeed, in a pulsed field NMR experiment, the observation time can be varied from a few milliseconds up to several seconds and the dependence of the diffusion coefficient on the observation time allows the distinction to be made between transport mechanisms. For example, if the self-diffusion is independent to the observation time for a porous system, then the system exhibits no restriction to diffusion. This situation was observed in starch gels (*9*−*12*), in sugar solutions (*11*), in pectin gels (*13*), in cheeses (*14*, *15*), in casein suspensions (*16*), and in bovine serum albumin gels (*17*, *18*). In 1983, Callaghan et al. (*15*) compared water self-diffusion in Cheddar and Swiss cheeses. Their results have shown that the water molecules were not confined in water droplets but had the freedom to move over distances much larger than the size of the fat droplets and that the magnitude of the diffusion coefficient was consistent with a migration along the surface of the protein chains. On the contrary, when the diffusion coefficient depends on the observation time, restriction to diffusion should be suspected as for emulsion (*19*−*21*) or diffusional anisotropy as, for example, in starch (*9*, *10*, *22*, *23*), gelatin (*24*, *25*) and pulp cellulose fibers (*23*, *26*, *27*).

Various theoretical descriptions of the water diffusion processes have been proposed (*28*−*31*), and the theoretical analysis of the concentration dependence of the self-diffusion coefficient is still the subject of debate (*32*). From these models, it is very difficult to distinguish the concentration effect from the structural

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Table 1. Chemical Composition of the NPC Powder

	g kg^{-1}
DM	907.1
TPM	797.34
noncasein nitrogen (NCN)	40.82
nonprotein nitrogen (NPN)	2.72
lactose	31.75
ashes	78.01
calcium	26.31

Table 2. Chemical Composition of the Different UF Retentates (with or without Fat) and UF Permeates

	DM[b]	TPM[b]	NCM[b]	fat
without AMF				
UF[a] retentate	26.4	19.3	2.8	
UF[a] permeate	6			
with AMF				
AMF-reconstituted fatty retentate				
UF[a] retentate	18.7	12.4	2.2	
UF[a] permeate	5.9			
reconstituted fatty retentate[c]	51.4	7.6	0.7	39.6
fatty industrial retentate:				
nondiluted	36.1	14.7	2.1	16
diluted	27.4	10.8	1.8	9.3
UF[a] permeate	5.9			

[a] UF: fractions obtained by ultrafiltration. [b] DM, TPM, NCM (noncasein matter), and concentrations are expressed in g per 100 g of total product. [c] Only the higher concentrated reconstituted fatty retentate is described here as an example.

effect on the diffusion mechanism. Moreover, to our knowledge, the models have been evaluated on a binary food system composed of water as the diffusing molecule in a porous media such as a gel or an emulsion. In a recently published paper, water self-diffusion variation with respect to casein concentration was investigated (*16*). The model used is based on Fick's first law and allows us to consider two water fluxes in the casein system: one around in the micelle and one inside the casein micelle.

The objective of this paper is to propose a model for water self-diffusion in complex dairy products including the bulk water composition, the casein, and the fat concentration. In the present paper, water self-diffusion in dairy casein systems studied with PFG-NMR was investigated. The paper is organized in three major parts: (i) effects of casein concentration and bulk water composition and modeling of water self-diffusion in fat free dairy casein systems, (ii) effect of fat content and modeling of the water self-diffusion in fatty casein systems, and (iii) validation of the model with experimental data from industrial retentates obtained by ultrafiltration (UF).

MATERIALS AND METHODS

Materials. Native phosphocaseinate powder (NPC) (INRA, Rennes, France) was used throughout, without any purification. The composition of NPC is summarized in **Table 1**. Sodium azide (Merck-Schuchardt, France) was used, also without any purification. The French company, Les Fromageries Bel (Vendôme), concentrated milk by UF up to a 5 volume concentration factor, to obtain UF retentate and UF permeate fractions (**Table 2**). These latter also supplied fatty retentates, referred to as "fatty industrial retentate" (**Table 2**). Anhydrous milk fat (AMF) was provided by Fléchard (La Chapelle d'Andaine, France). Rennet (CHR Hansen, Arpajon, France), an enzyme preparation provided by Les Fromageries Bel, was used for the casein coagulation.

Preparation of Fat Free Samples. Three series of fat free products were used as follows: NPC rehydrated in NaCl/water and in UF permeate solution and UF retentate in UF permeate solution. Rehydration of the NPC powders was performed with a 80 mM NaCl/water solution at room temperature and then heated to 30 °C for complete dissolution. The solutions were studied without pH adjustment. So, the pH of the NPC dispersion varied from 7.27 to 6.85 for concentrations ranging from 0.03 to 0.17 g/g of the TPM (total protein matter). The UF retentate fraction was combined with the UF permeate, to obtain different casein concentrations from 1 to 17 g/100 g of product. The pH of these solutions varied from 6.79 to 6.68.

Another solution was prepared as follows: 13.7 g of NPC powder was dispersed with magnetic agitation in 100 g of UF permeate and was referred to later as the NPC permeate dispersion. The pH of the micellar casein dispersion was 6.85. Sodium azide was added (0.02% v/v) to each solution to prevent any bacterial development.

Preparation of Fatty Samples. *Fatty Reconstituted Retentates from AMF*. To study the influence of fat, reconstitution of the fatty retentate was performed by mixing UF retentate and AMF at 60 °C using a commercial laboratory blender (Waring blender, Bioblock). The AMF was heated to 60 °C for 20 min in order to melt all of the existing fat crystals. Samples containing from 5 to 40 g of AMF/100 g of total product were thus prepared (i.e., concentrations of 5, 10, 15, 20, 30, and 40% of fat). For example, 200 g of AMF and 300 g of UF retentate were mixed to give 40% fat in the final product. Each sample was characterized by an identical TPM-to-water ratio equal to about 15 g of total proteins/100 g of water (**Table 2**). Sodium azide was added (0.02% v/v) to each solution to prevent any bacterial development. The freshly prepared samples were stored at 5 °C overnight. The following day, they were warmed to 20 °C before further analysis. These reconstituted fatty dairy solutions were referred to as AMF-reconstituted fatty retentates.

Industrial Fatty Retentates. The fatty industrial retentate was divided into two batches. The first was used throughout without any dilution and referred to as nondiluted fatty industrial retentate, and the second one was diluted with UF permeate to obtain the same TPM:water ratio as that of the AMF-reconstituted fatty retentate described above, i.e., ~15 g of TPM/100 g of water (**Table 2**). Sodium azide was added (0.02% v/v) to each solution to prevent any bacterial development. This latter was referred to as diluted fatty industrial retentate.

Gel Preparation and Whey Separation by Gel Cutting. All of the samples were preheated at 30 °C, and rennet was added at a concentration of 0.3 μL/mL, after a previous 1:100 dilution of concentrated rennet with distilled water. Fifty millimeters were gelled for extraction of the whey phase. After the rennet was added, the dispersions were vigorously shaken for 2 min and transferred to sealed bottles to prevent evaporation during coagulation. These samples were placed in an oven maintained at 30 °C for 2 h. After this renneting period, the gels were kept at ambient temperature overnight. The gels were cut cleanly into fine slices to initiate whey syneresis. The gel and the expelled whey were separated, and a small amount of whey could be collected. The whey phase analysis is summarized in **Table 3**. This experimentation was performed on both fatty industrial retentates, whereas for fat free products, this procedure was only performed on the most concentrated solution of rehydrated NPC dispersion and of UF retentate.

Dry Matter (DM) Determination. The DM of all of the samples was estimated by measuring the weight variation after drying in an oven at 103 °C for 24 h.

Table 3. Experimental Characteristics of Whey Phases Expelled from Gels of NPC, UF Retentate, and Fatty Industrial Retentate Samples

	DM (g/100 g of product)	$D^{water}_{aqueous\ phase}$ (10^{-9} m^2 s^{-1})
without AMF		
whey from NPC gel$_{DM\ 17.5\%}$	2.90 (±0.04)	1.83 (±0.01)
whey from NPC gel$_{DM\ 19.5\%}$	3.25 (±0.03)	1.816 (±0.005)
whey from UF retentate gel$_{DM\ 26\%}$	10.95 (±0.04)	1.52 (±0.01)
with AMF		
whey from gelled fatty industrial retentate:		
nondiluted	10.05 (±0.09)	1.522 (±0.002)
diluted	8.15 (±0.03)	1.60 (±0.02)

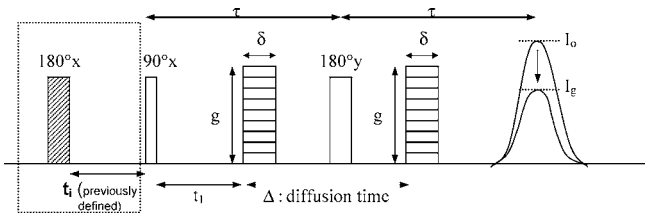

Figure 1. Standard PFG-SE sequence and the T_1-weighted SE sequence. A SE NMR signal is generated from a sequence consisting of 90°*x* and 180°*y* radio frequency pulses, and its intensity is modulated by two field gradient pulses *g*. TE is the echo time and corresponds to 2τ. Recuperation time, $T_R = 5$ s; interpulse spacing time, $\tau = 7.5$ ms; diffusion time, $\Delta = 7.5$ ms; width of the field gradient pulses, $\delta = 0.5$ ms; and the delay (t_1) between the first pulse rf and the first gradient pulse was fixed at 1 ms. In the experiments, *g* was incremented from 0.5 to 2.5 Tm^{-1}. For the T_1-weighted SE sequence, an additional 180°*x* radio frequency pulse was included (diagonally shaded) and the parameters were identical to the SE sequence. The predelay t_i is experimentally defined and equal to 35 ms at 20 °C.

Protein Determination. The protein content was determined using the Kjeldahl method (FIL20B, ISO 8968-1). The total protein content was deduced from the total nitrogen matter multiplied by the conversion factor 6.38.

Fat Determination. The fat content in fatty industrial retentates was determined by Les Fromageries Bel using the Heiss's technique (NF V04-287). The extraction of the fat in reconstituted fatty products with a view to their characterization was performed by INRA according to the method described by the norm NF V03-030 (1991).

Droplet Size Distribution Measurements. A Saturn Digisizer 5200 (Micromeritics, Creil, France) was used to determine and control the globule size distribution in fatty retentates. The mean droplet size ($d_{43} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3$, when n_i is the number of droplets with diameter d_i) varied from $d_{43} = 3.0$ (± 0.1) μm for reconstituted fatty retentates with 5−15% fat content to $d_{43} = 5.0$ (± 0.1) μm for reconstituted fatty retentates with 20−40% fat content. The droplet size measurements obtained for both diluted and nondiluted fatty industrial retentate were estimated as being 3.6 and 3.7 μm (± 0.1 μm), respectively.

NMR Measurements. Samples of 0.5 mL were placed in sealed NMR tubes (0.5 mL equivalent to 10 mm in height, corresponding to the homogeneity area of the probe, 8 mm diameter). All NMR measurements were performed on a 0.47 T NMR spectrometer (The Minispec; Bruker Spectroscopy, F-67166 Wissembourg, France) operating at 20 MHz for protons, equipped with a pulsed gradient unit [NMS GU200 ($G \leq 4$ T m^{-1})]. The NMR probe was heated or cooled by constant gas flow (air or liquid nitrogen) by means of a variable temperature control unit B-VT3000 managed by computer. Before the NMR measurements, the tubes were placed in a cryostat (Julabo FP50-HP, Julabo Labortechnik GmbH, Germany). Then, the tube was placed in the NMR probe for thermal equilibration. The time needed for thermal equilibrium (10 min) and the temperature were controlled with a series of samples equipped with a copper−constantan thermocouple placed at the center of the sample. The sample temperature was monitored periodically by inserting a copper−constantan thermocouple in the NMR tube filled with the same product. This sample was not included in the NMR experimental planning. The measurements were conducted at 20 ± 0.5 °C.

Determination of Diffusion Coefficients Using PFG-NMR. The strengths of the gradient pulses were calibrated with pure water at 20 °C (1.98×10^{-9} m^2 s^{-1}). The echo intensity was the average of three repetitive scans with a recuperation time of 5 s. For fat free samples, the self-diffusion experiments were performed using the standard PFG-SE sequence described by Stejskal and Tanner (*8*) (**Figure 1**). For fatty samples, the T_1-weighted SE sequence was used (**Figure 1**) (*33*). This T_1-weighted method allowed us to measure selectively the water NMR signal in fatty products without interference from the fat NMR signal. Thus, the water self-diffusion coefficient in complex food products could be accurately performed.

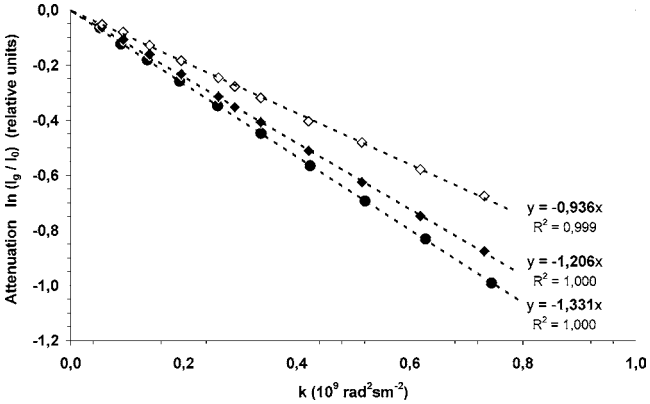

Figure 2. Echo attenuation for water vs *k* at 20 °C for fat free UF retentate (●) obtained from the standard SE sequence and for diluted (◆) and nondiluted (◇) fatty industrial retentate obtained from the T_1-weighted SE sequence. Standard errors were less than 1% for all of the measurements. The solid lines are the results of the fit of eq 3 to the data.

In both cases, diffusion coefficients were obtained using

$$I(\delta,\Delta,g) = I_0 \times \exp\left[-\gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right) D\right] \quad (1)$$

where $I(\delta, \Delta, g)$ and I_0 are the echo intensities of the NMR signal in the presence of gradient pulses of strength *g* and in the absence of gradient pulses, respectively. γ is the gyromagnetic constant for ^1H ($\gamma = 2.6752 \times 10^8$ rad T^{-1} s^{-1} for protons), δ is the duration of the *z* gradient pulse, and Δ is the time interval between the gradient pulses. The values of the delays Δ and δ used in the water self-diffusion measurements were 7.5 and 0.5 ms, respectively. The delay between the first 90° pulse and the first gradient pulse t_1 was fixed at 1 ms. τ is the time interval between the successive 90 and 180° rf pulses and is equal to 7.5 ms.

In the case of the T_1-weighted SE sequence, we need to define first the delay t_i, which is equal to 35 ms at 20 °C. To eliminate the effect of spin relaxation, the diffusion coefficient determination was performed by keeping δ and Δ constant and varying *g*. In our experiments, *g* was incremented from 0.5 to 2.5 T m^{-1}.

For the water self-diffusion experiments, the fitting equation was simplified as:

$$I(\delta,\Delta,g) = I_0 \times \exp[-kD] \quad (2)$$

where *k* is defined as

$$k = \gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)$$

This equation became

$$\ln\frac{I_g}{I_0} = -kD \quad (3)$$

Therefore, $D^{\text{water}}_{\text{experimental}}$, the water self-diffusion coefficient, was obtained from the slope of the logarithm plot of the echo attenuation I_g/I_0 vs *k* ($k = [\gamma^2\delta^2 g^2(\Delta - \delta/3)]$) using eq 3.

As an example, the logarithm of the echo attenuation vs *k* is given in **Figure 2** for UF retentate with and without fat content at 20 °C.

The measurements were done in triplicate. Standard errors on experimental water self-diffusion coefficients were estimated for all samples and were less than 1% (i.e., $D^{\text{water}}_{\text{experimental}} \pm 0.01 \times 10^{-9}$ m^2 s^{-1}).

RESULTS AND DISCUSSION

Water Self-Diffusion in UF Retentate. The water self-diffusion coefficients for rehydrated NPC dispersions and UF retentate samples according to the DM-to-water ratio (expressed

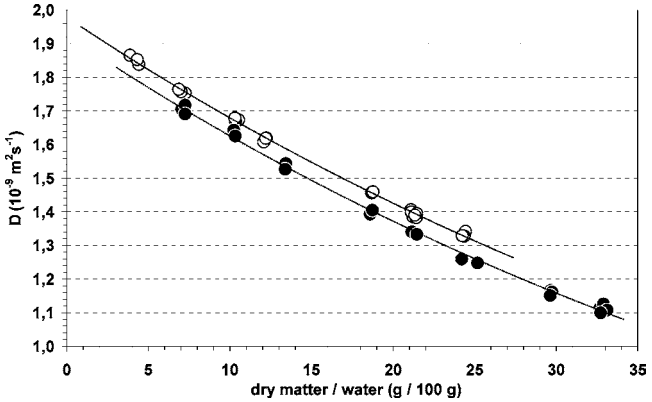

Figure 3. Observed water self-diffusion coefficients vs the DM-to-water ratio (g/100 g) for rehydrated NPC dispersions (○) and UF retentate samples (●). Standard errors were less than 1% for all of the measurements. The solid lines are guides for the eye.

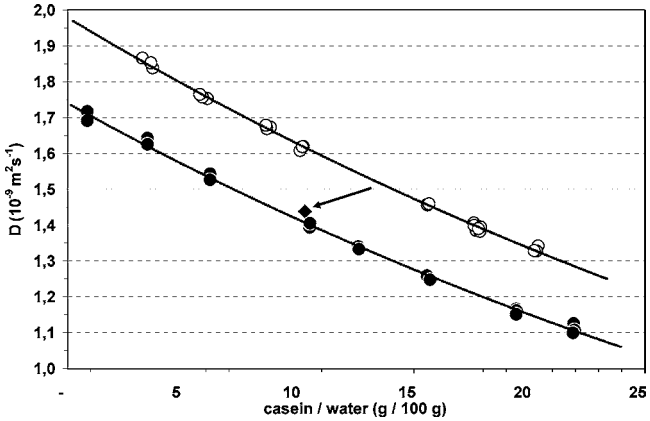

Figure 4. Observed water self-diffusion coefficients vs the casein-to-water ratio (g/100 g) for rehydrated NPC dispersions (○), UF retentate samples (●), and NPC permeate dispersions (◆). Standard errors were less than 1% for all of the measurements. The solid lines are guides for the eye.

in g per 100 g of water) are shown in **Figure 3**. As expected, the water self-diffusion coefficient decreased when the DM concentration increased. The water diffusion decrease in the rehydrated NPC dispersion is in agreement with previously published results (*16*). The authors have shown the important influence of casein concentration on the water self-diffusion coefficient reduction. They have also demonstrated that the majority of the water molecules are not confined in compartments or affected by the presence of barriers and can diffuse freely over a length scale of 24 μm for a micellar casein concentration of 0.19 g/g. Consequently, in their casein systems, no restriction in the diffusion of the water molecule was detected.

However, a difference is observed in **Figure 3** between water self-diffusion in rehydrated caseins and native caseins of the retentate throughout the DM concentration range investigated. To explain this particular behavior, we could assume, as a first approximation, a change of the bulk water composition. The powders of NPC are rehydrated in a NaCl/water solution. Conversely, the UF retentate is diluted with UF permeate. So, as compared to the bulk water phase in the rehydrated NPC dispersion, the one in the UF retentate sample was richer in whey proteins, lactose, and mineral salts. This soluble fraction has a significant effect on water diffusion and so must be taken into consideration.

To validate this assumption, we compared the water self-diffusion coefficient values obtained for both NPC dispersions rehydrated in a NaCl/water solution and in UF permeate (**Figure 4**). It appears that the water self-diffusion coefficient value of NPC powder rehydrated in permeate is superimposed with the main diffusion decay curve obtained for native caseins of UF retentate. This result allows us to verify the absence of structural effect of casein micelle between native casein in retentate and casein micelle from NPC powder. Despite little changes in the pH between the two systems, the water self-diffusion coefficient was not modified. This observation is supported by the results of Mariette et al. (*16*). These authors have observed no difference between the water self-diffusion in a Na−caseinate solution and that in a micellar casein dispersion, despite the size difference of the colloidal particles in the two systems.

Thus, to correctly compare the two casein systems, we need to determine the water self-diffusion coefficient of their corresponding aqueous phase. Casein micelles, when they are coagulated by the addition of rennet solution, are known to spontaneously shrink, which induces the release of the whey phase (*34*). Moreover, a minor variation in the water phase composition was observed during the shrinkage of the gel and whey expulsion.

So, to establish the effect of the aqueous phase on water diffusion in these two casein systems, the water self-diffusion coefficient of the whey expelled from the NPC and retentate gels was estimated. The determination of the water self-diffusion coefficient in the whey phase was performed on the most concentrated solution for both NPC and UF retentate gels. The results corresponding to the whey analysis were summarized in **Table 3**. The water self-diffusion coefficient for the whey phase extracted from NPC gel with 17.5% of DM ($D^{\text{water}}_{(\text{aqueous phase})_{\text{NPC}}} = 1.83 \pm 0.01 \times 10^{-9}$ m^2 s^{-1}) differs from that of the NaCl/water solution ($D^{\text{water}}_{\text{NaCl-water}} = 1.96 \pm 0.02 \times 10^{-9}$ m^2 s^{-1}). The NPC powders are not completely pure and contain a small amount of whey proteins, salts, and sugars; these "impurities" in rehydrated NPC dispersions cannot be neglected when the casein concentration is high. The same trends were observed for the whey phase extracted from the UF retentate gel. The water self-diffusion coefficient ($D^{\text{water}}_{(\text{aqueous phase})_{\text{retentate}}} = 1.52 \pm 0.01 \times 10^{-9}$ m^2 s^{-1}) differs from that of the permeate solution ($D^{\text{water}}_{\text{permeate}} = 1.72 \pm 0.01 \times 10^{-9}$ m^2 s^{-1}). This difference is simply explained because the whey proteins expelled during the drainage process are taken into consideration. Because of the DM dependence on water self-diffusion in the aqueous phase, $D^{\text{water}}_{\text{aqueous phase}}$, the normalization of the experimental self-diffusion coefficient in the casein systems was carried out by taking into account the exact water phase concentration for the estimation of $D^{\text{water}}_{\text{aqueous phase}}$ of each whey phase. The diffusion coefficient values of the other solutions were deduced from a linear regression analysis of the data sets ($D^{\text{water}}_{\text{aqueous phase}}$, DM). This leads to $D^{\text{water}}_{\text{aqueous phase}} = -0.0493$ ($\pm$0.002) $\times$ $\text{DM}_{\text{aqueous phase}} + 1.974$ ($\pm$0.006).

The specific effect of casein molecules on water diffusion vs the casein-to-water ratio is presented in **Figure 5**. The reduced self-diffusion coefficient decreases with the casein:water ratio for both cases. With our constructed model, we note that the water diffusion coefficient is remarkably comparable for rehydrated caseins and for native caseins of UF retentate whatever the concentration in the range of the present study. Thus, our results clearly indicate that to explain water diffusion in protein solutions, several effects should be considered: the casein and/or whey protein content, the composition of the DM, and more particularly, the composition of the aqueous phase.

As a consequence, when models are used to explain the variation of water self-diffusion coefficient vs DM content,

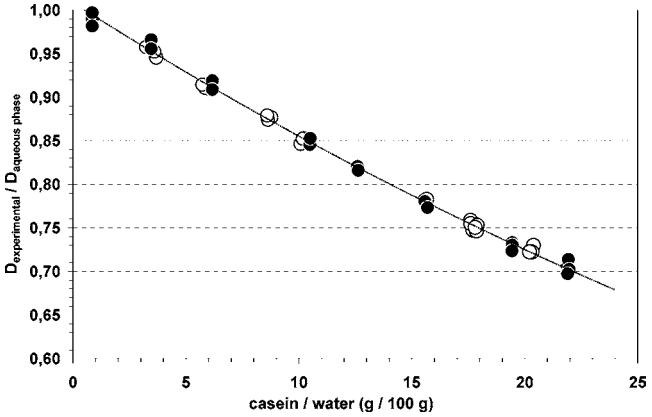

Figure 5. Water self-diffusion coefficients ($D^{water}_{experimental}$), normalized with respect to water diffusion in the aqueous phase ($D^{water}_{aqueous\ phase}$), vs the casein-to-water ratio (g/100 g) for rehydrated NPC dispersions (○) and UF retentate samples (●). Standard errors were less than 1% for all of the measurements. The solid line corresponds to the best fit from eq 4a with $K = 1.60$.

careful attention should be paid to the exact value for the bulk water diffusion coefficient of the aqueous phase. Generally, $D^{water}_{aqueous\ phase}$ is assimilated to the pure water self-diffusion coefficient, D_0. So, the effect of the obstructing molecule under study, on water diffusion, is probably overestimated. Therefore, a general model of variation of the water self-diffusion as a function of the casein content could be computed.

Water Diffusion Model for the Water−Casein System. In the literature, various models have been proposed to describe the reduction of water mobility (*29*−*31*). These models are based on different physical concepts such as obstruction effects, free volume concepts, and hydrodynamic interactions. As examples for obstruction effects, the authors have cited the Maxwell−Fricke model and the model of Mackie and Meares. They also exposed Cukier's model developed to describe the diffusion of Brownian spheres in semidilute polymer solutions based upon hydrodynamic interactions. Several of them require numerous physical parameters relating to the system under study or are based on scaling concepts. Other theories are based on the solution of Fick's first law for different geometries (*35*) or are based on a random walk simulation from digitized two-dimensional images (*36*). Recently, a model has been proposed for water self-diffusion in a casein−water system based on the formalism of the cell model framework (*16*). This model included both the obstruction effect induced by the proteins and the hydration effect, i.e., the lowering of the water diffusion on account of water−protein interactions.

According to this model, the water self-diffusion in the casein system $D^{water}_{expected}$ is given by the following:

$$D^{water}_{expected} = D^{water}_{aqueous\ phase} \cdot \left(1 + \upsilon^{casein} \cdot \frac{m^{casein}}{m^{water}}\right) \cdot \frac{\left(1 + \upsilon^{casein} \cdot \frac{m^{casein}}{m^{water}} - K \cdot \frac{m^{casein}}{m^{water}}\right)}{\left(1 + \upsilon^{casein} \cdot \frac{m^{casein}}{m^{water}} + K \cdot 0.5 \cdot \frac{m^{casein}}{m^{water}}\right)} \quad (4a)$$

where

$$K = (\upsilon^{casein}/\upsilon^{water} + H) \cdot \beta \quad (4b)$$

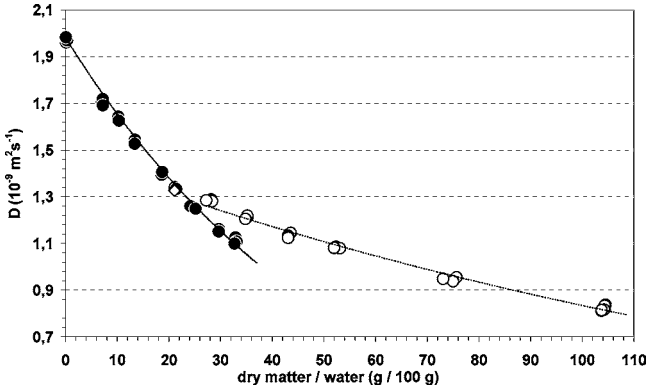

Figure 6. Observed water self-diffusion coefficients vs the DM-to-water ratio (g/100 g) for fat free UF retentate (●) and AMF-reconstituted fatty UF retentate (○) (◇ corresponds to the UF retentate before fat addition). Standard errors were less than 1% for all of the measurements. The solid lines are guides for the eye.

β is a constant parameter, which depends on both the water concentration and the water self-diffusion in two water compartments, i.e., the casein micelle and the bulk water compartments (*16*). υ^{casein} and υ^{water} are the specific volume of casein, 0.75 $cm^3\ g^{-1}$, and of water, 1 $cm^3\ g^{-1}$, respectively. $D^{water}_{aqueous\ phase}$ corresponds to the water self-diffusion coefficient obtained in whey expelled from the gel. H corresponds to the water hydration number in the casein system. The (m^{casein}/m^{water}) ratio is calculated from the chemical composition of the samples and is expressed in g/g. K is the only unknown parameter, and this value can be obtained by fitting eq 4a to the experimental data. Using this model, we assumed that there is "free" water surrounding the casein particles for the casein concentration range studied.

The result of the fitting process is presented in **Figure 5**. The K value obtained for the best fitting is $K = 1.60 \pm 0.01$. This value is quite similar to the value obtained by Mariette et al. (*16*), i.e., $K = 2.07$. The small difference is explained because, in our case, the variation of the water self-diffusion of the bulk water according to the casein concentration was taken into account. In the previously mentioned work, the water self-diffusion coefficient was assumed as a constant and equal to the water self-diffusion of pure water (e.g., for pure water at 20 °C, $D_0 = 1.98 \times 10^{-9}\ m^2\ s^{-1}$).

Effect of the Fat on Water Diffusion in UF Retentate. The water self-diffusion coefficient for the AMF-reconstituted fatty retentates vs the DM-to-water ratio is shown in **Figure 6**. The addition of fat to a fat free retentate induced a strong effect on the water self-diffusion coefficient. As expected, an increase in the amount of fat globules to the retentate induced a decrease of the water self-diffusion coefficient. However, it is important to notice that the water diffusion followed a particular pattern in the presence of fat. Indeed, the water self-diffusion coefficient for retentate containing fat is higher than that of fat free retentate, with an equal DM-to-water ratio (**Figure 6**). This is in accordance with the results found in cheese, obtained by Geurts et al. for salt diffusion (*7*). Their results show the importance of the composition of the cheese matrix; as in some cases, the moisture content of cheese was equal, but the self-diffusion coefficient values were completely different, and in others, the self-diffusion was found equal, but the moisture content was different. According to Guinee and Fox (*37*), the increase of the self-diffusion coefficient with fat content and with equal moisture content is not due to fat, per se. Normally, a reduced self-diffusion coefficient due to the tortuosity effect of fat

globules should be observed. So, the increase of the water self-diffusion coefficient would be explained rather by the concomitant decrease in the protein volume fraction. The water mobility reduction caused by the effect of the protein matrix overrides the increased obstruction caused by increasing the fat content, and so, the water self-diffusion coefficient increases.

From our results, it appears that the obstruction effects induced by fat and by protein micelle were different. The decrease of the water self-diffusion coefficient according to the DM content was then different if the DM was composed of fat matter or composed of casein micelles only. When fat is added to the fat free retentate, the expected reduction of self-diffusion based on the DM content is lower. Consequently, the same water self-diffusion coefficient could be obtained despite a change in the DM content. For example, two retentates containing 0 and 20% fat (g/g) and, respectively, defined by a DM:water ratio of 33 and 52% (corresponding to 75 and 65% moisture) were characterized by the same water self-diffusion coefficient of 1.11 $\pm$ 0.01 $\times$ 10^{-9} m^2 s^{-1} and one of 1.082 $\pm$ 0.003 $\times$ 10^{-9} m^2 s^{-1}, respectively.

Water Diffusion Model for the AMF−Casein System. To propose a model, which explained the water hindrance by both the casein molecules and the fat globule droplets, we assume that the addition of the amount of fat to UF retentate has no effect on the hindrance of the protein. In other words, the addition of fat induces no modification of the effect of the casein on the water mobility. The hindrance effect of both systems was considered as independent of each other. Thus, the water diffusion in fatty products can be characterized by the following equation:

$$D^{\text{water}}_{\text{AMF-casein system}} = D^{\text{water}}_{\text{aqueous phase}} \times f\left(\frac{m^{\text{casein}}}{m^{\text{water}}}\right) \times f\left(\frac{m^{\text{fat}}}{m^{\text{water}}}\right) \quad (5a)$$

Then, from eq 5, the pure hindered effect from the fat globule on the water self-diffusion coefficient for the AMF-reconstituted fatty retentate can be estimated from:

$$f\left(\frac{m^{\text{fat}}}{m^{\text{water}}}\right) = \frac{D^{\text{water}}_{\text{AMF-casein system}}}{D^{\text{water}}_{\text{aqueous phase}} \times f\left(\frac{m^{\text{casein}}}{m^{\text{water}}}\right)} = \frac{D^{\text{water}}_{\text{AMF-casein system}}}{D^{\text{water}}_{\text{fat-free retentate}}} \quad (5b)$$

This normalized $D^{\text{water}}_{\text{AMF-casein system}}/D^{\text{water}}_{\text{fat-free retentate}}$ vs the $m^{\text{fat}}/m^{\text{water}}$ ratio using eq 5b described only the fat obstruction effect as observed for a simple fat-in-water emulsion. The decrease of the water self-diffusion coefficient, according to the $m^{\text{fat}}/m^{\text{water}}$ ratio could be described by a model, assuming spherical obstructing particles. If we supposed that the effect of water−fat interactions is negligible and the obstruction effect by the fat droplet is the dominant effect on the water diffusion, then the model is given according to Jönsson et al. (*35*) by:

$$D^{\text{water}}_{\text{AMF-casein system}} = \frac{1}{1 + 0.5 \times \phi} \times D^{\text{water}}_{\text{fat-free retentate}} \quad (6)$$

where $\phi = m^{\text{fat}}\upsilon^{\text{fat}}/m^{\text{fat}}\upsilon^{\text{fat}} + m^{\text{water}}\upsilon^{\text{water}}$, the volume fraction of fat globule in the AMF model.

This model required no adjustment parameter and can be directly computed on the data (**Figure 7**). As can be seen from this obstruction model, the water mobility is overestimated. The assumption that the interactions between fat and water molecules have no effect on the water mobility is a too severe simplification, and a pool of water molecules, having a lower mobility in the vicinity of the fat interface, should be considered to explain the global decrease of the water mobility (*21*).

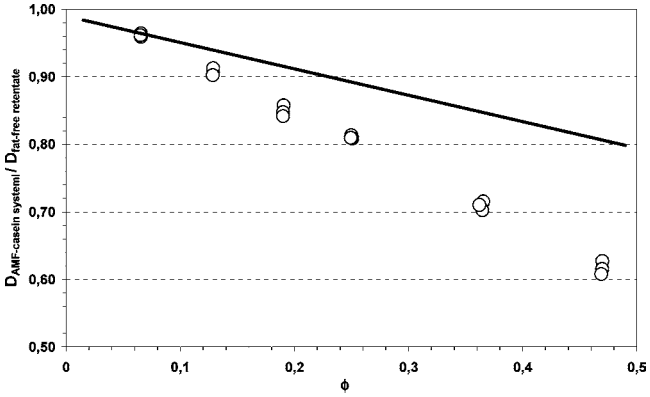

Figure 7. Water self-diffusion coefficient in the AMF-reconstituted fatty models normalized with respect to the water self-diffusion coefficient obtained in its respective retentate vs fat droplet volume fraction ϕ for the experimental data (○) and for the simple obstruction model (solid line).

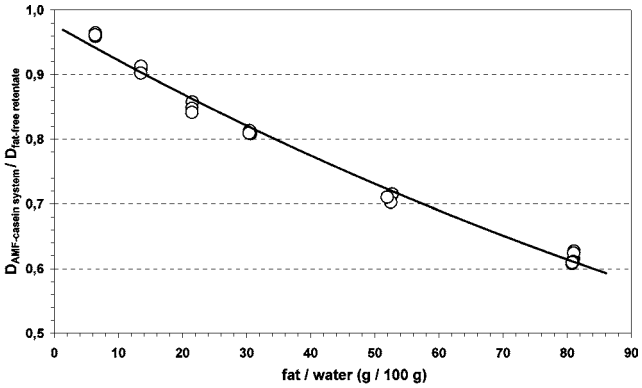

Figure 8. Water self-diffusion coefficients ($D^{\text{water}}_{\text{AMF-casein system}}$), normalized with respect to water diffusion in UF retentate without AMF added ($D^{\text{water}}_{\text{fat-free retentate}}$), vs the fat-to-water ratio (g/100 g) for the AMF-reconstituted fatty retentate samples. Standard errors were less than 1% for all of the measurements. The solid line corresponds to the best fit from eq 8 with $K = 1.34$.

Therefore, we propose to test the cell model for spherical obstructing particles, i.e., the fat globules, as already above described by replacing in eq 4a $m^{\text{casein}}/m^{\text{water}}$ with $m^{\text{fat}}/m^{\text{water}}$) and υ^{casein} with υ^{fat} (eq 7).

The ratio $m^{\text{fat}}/m^{\text{water}}$ is known according to the composition of the products and expressed in g/g. υ^{fat} and υ^{water} are the specific volume of the fat droplet, i.e., 1.093 cm^3 g^{-1}, and of water, i.e., 1 cm^3 g^{-1}, at 20 °C.

Now the two water compartments are the water from the bulk phase and the water in interaction with the hydrophilic headgroup of the triacylglycerols of the AMF. Equation 7 was used to fit the experimental data. The result of the fitting process is presented in **Figure 8**, and a new value for K was estimated. The best fit was obtained with $K = 1.34 \pm 0.01$. As expected, the K values obtained for these two systems, i.e., the water−casein system and the AMF−casein system, were different. This difference reflected the specific behavior of the two systems on the water mobility already pointed out. As K depends on hydration number and on self-diffusion of the solvated water molecule, we could not go further into the interpretation without knowing at least one of the parameters, i.e., H^{water} or D^{water}.

Validation of the Model on Industrial Retentate. A general equation including the effect of the bulk water composition,

Table 4. Experimental Water Self-Diffusion Coefficient $D^{water}_{experimental}$ Obtained from the Fit of Eq 3 to the Data as Compared to Expected Values $D^{water}_{expected}$ from the Model Using Eq 8 for the Fatty Industrial Products in Solution at 20 °C

	$D^{water}_{experimental}$ (10^{-9} m^2 s^{-1})	$D^{water}_{expected}$ (10^{-9} m^2 s^{-1})
nondiluted fatty industrial retentate	0.94 (±0.01)	0.93 (±0.01)
diluted fatty industrial retentate	1.21 (±0.01)	1.19 (±0.03)

the effect of the casein concentration, and the effect of the fat concentration on the water mobility could be proposed by combining eqs 4a and 7. This leads to

$$D^{water}_{expected} = D^{water}_{aqueous\ phase} \times f\left(K^{casein}, \frac{m^{casein}}{m^{water}}\right) \times f\left(K^{fat}, \frac{m^{fat}}{m^{water}}\right) \quad (8)$$

This model equation was validated with industrial fatty retentate at two concentrations. The validation required the determination of the water self-diffusion in the aqueous water phase corresponding to each industrial retentate. To extract whey, the two fatty industrial retentates were gelled with rennet and a small amount of expelled water phase was collected after the gel contracted. The water self-diffusion coefficient of each extracted water phase was determined and reported in **Table 3**.

The expected self-diffusion for each retentate could now be calculated from eq 8 using their respective casein, fat, and water contents. The expected self-diffusion coefficient was compared with the experimental one determined by the T_1-weighted SE-NMR sequence (**Table 4**). No difference was observed between the self-diffusion coefficients predicted from the model using eq 8 and the self-diffusion coefficients experimentally measured. So, this model, which takes into account the global contribution of each component, i.e., fat globules, casein, and/or whey proteins, lactose, salt minerals, etc..., can be used to predict the effect of the compositional effect on the water self-diffusion for industrial retentate.

In conclusion, our results demonstrate that the water self-diffusion coefficient, in complex products, could not be explained by the water content only. When caseins, fat globules, and soluble fractions are mixed in order to obtain cheese models, the effect of each constituent should be determined to explain the water self-diffusion correctly. From the model including the water phase concentration, the effect of fat content, and that of casein content on water diffusion, we demonstrate that the self-diffusion coefficient in industrial cheese products could be predicted. Moreover, we show that the addition of fat does not modify the water hindrance caused by casein micelles. The two obstruction effects, relative to fat globules and casein micelles, seem to be independent. This result was in agreement with the observation of Geurts et al. (*7*) despite the fact that the measurement methods and the diffusing molecules considered were different. In our case, the self-diffusion of water was measured for a distance probed by the water molecules of 7−8 μm, while Geurts et al. (*7*) calculated salt diffusion over a distance of 1 mm. Consequently, we could assume that despite the length scale considered and the type of cheese, the mechanisms involved in molecular transport are comparable.

It should be noticed that the model proposed was only established on liquid products in order to minimize the structural effect as can be observed in the gel. This previously constructed model should be very robust and powerful in order to investigate structural changes on water self-diffusion coefficients induced either by renneting or by ripening. So, in future work, we will use this model to predict the water diffusion in renneted products in order to quantify the structural effect after correction of the compositional effect.

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