NMR Study of the Water Diffusion in Casein Systems. Effect of the Micellar Casein Concentration

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Objectives

- To study the effect of casein micelle concentration on the water diffusion coefficient
- To study the effect of coagulation by rennet on the water diffusion coefficient
- To find a model to explain the water diffusion in casein systems
How to measure the water diffusion by NMR?

- To obtain a NMR signal, the sample is introduced into a magnetic field.

  If the magnetic field is **homogenous**, the NMR signal is **insensitive** to molecular diffusion.

  But, if the magnetic field is **inhomogeneous**, then the NMR signal become **sensitive** to molecular diffusion.

  So, a linear field gradient is applied in order to measure the Diffusion coefficient.
How to measure the water diffusion by NMR?

NMR signal without gradient (g=0)

NMR signal with gradient
How to measure the water diffusion by NMR?

\[ d = \sqrt{6D\Delta} \]

\[ 90^\circ x \quad 180^\circ y \]

RF Pulse

NMR signal with gradient

\[ \Delta \]

\[ g \]

\[ \Delta \]

\[ g \]
How to measure the water diffusion by NMR?

\[ \frac{I(g, t)}{I_0(g = 0, t)} = \exp \left[ -Dk \right] \]

\[ K = F(g, \Delta) \]

\[ \text{g, the gradient strength and } \Delta, \text{ the diffusion time} \]
How to measure the water diffusion by NMR?

- by changing $g$ with $\Delta = \text{cte}$

  
  \[
  \ln\left(\frac{I}{I_0}\right) \quad g
  \]

  No restriction, $D$ is the self-diffusion coefficient

- by changing $g$ for different $\Delta$ values

  
  \[
  \ln\left(\frac{I}{I_0}\right) \quad g
  \]

  Homogeneous diffusion process
  Self diffusion coefficient

  \[
  \ln\left(\frac{I}{I_0}\right) \quad g
  \]

  Heterogeneous diffusion process
  Apparent diffusion coefficient
Why using NMR to measure the water diffusion coefficient?

- Non invasive technique,
  - So the measurements could be done without any perturbation of the diffusion process.
- no tracer,
  - the measurements could be done without any specific chemical preparation.
- the diffusion could be measured in any direction
  - If $D_x = D_y = D_z$ then the diffusion is isotropic
  - If $D_x \neq D_y \neq D_z$ then the diffusion is anisotropic
- the diffusion could be measured for different travelling distances,
  - $2^r \gg d \Rightarrow D_{\text{eff}} = D_0$
  - $2^r \leq d \Rightarrow D_{\text{eff}} < D_0$
Why using NMR to measure the water diffusion coefficient?

- Non invasive technique,
  - So the measurements could be done without any perturbation of the diffusion process.
- no tracer,
  - the measurements could be done without any specific chemical preparation.
- the diffusion could be measured in any direction,
  - so diffusion anisotropy could be detected.
- the diffusion could be measured for different travelling distances,
  - so “restricted diffusion” from impermeable barrier could be observed.
- as NMR is molecular specific
  - so the diffusion coefficient from several molecules could be measured simultaneously.
Milk composition and structure

Water: 87%

Milk is an o/w emulsion:
Fat: 3-4%

Ash: 0.7%

Protein: 3.2-3.4%

Lactose: 4.6%

Casein micelle plays a major role in the functional properties of the milk.
Casein micelles

Caseins are organised as "micelles"

- Large mean diameter: 300 nm
- Roughly spherical
- The integrity involves many interactions such as hydrophobic bonding, hydrogen bonding, electrostatic interaction, disulphide bonding and calcium bonding.
- Highly hydrated with about 4 – 6 g water/g protein.

Schmidt’s model
Materials and methods

- The micellar casein solutions were prepared:
  - with a native micellar casein powder (INRA, Rennes)
  - Rehydrated in a water – NaCl solution (80 mM),
  - from 1 to 22 g/100g of water

- The gel was obtained:
  - After renneting at 30°C during 120 minutes

- The NMR measurements were performed at 20°C
  - A low field NMR (0.47 Tesla) “The Minispec” (Bruker)
  - With a pulsed field gradient unit with a maximum strength of 4 T/m
Attenuation of the NMR signal according to the pulsed field gradient strength

A linear variation was observed for all the samples for both protein solutions and gels.

So the diffusion coefficient is the self-diffusion coefficient.
Effect of the observation time $D$ on the water diffusion coefficient

Whatever the diffusion time $D$, a straight variation of the echo attenuation was observed. The self-diffusion is constant, the diffusion mechanism is the same.
Effect of the observation time $D$ on the water diffusion coefficient

- From the diffusion time, the distance over which the water molecules diffused could be deduced from:
  \[
  (6D\Delta)^{1/2}
  \]
- So the distance probed by the water molecules was between:
  - $8 \, \mu m$ ($\Delta = 7.5 \, ms$)
  - $43 \, \mu m$ ($\Delta = 210 \, ms$)
- so the self-diffusion is measured on a micrometer length-scale “micro-diffusion”
Effect of the casein concentration on the water self-diffusion coefficient

More the casein concentration increase more the water diffusion decrease.
The water diffusion is not modified after the aggregation of the casein induced by the rennet.
How to explain the water mobility reduction in casein dispersion?

First case: we suppose an obstruction effect induced by the impenetrable slow moving casein micelles.
How to explain the water mobility reduction in casein dispersion?

First case: we suppose an obstruction effect induced by the impenetrable slow moving casein micelles.

The self-diffusion can be described by:

\[ D_{\text{eff}} = D_0 \left( \frac{1}{1 + \phi} \right) \]

- Pure water self-diffusion coefficient
- Effective self-diffusion coefficient
- Volume fraction occupied by the micelles
Obstruction effect induced by impermeable micelle?

The model do not explained the experimental results
So another effect should be considered
How to explain the water mobility reduction in casein dispersion?

Second case: we suppose two fluxes, one around the micelle and one through the micelle.
Obstruction model included two water fluxes

According to the model proposed by Jönsson et al*, the self-diffusion can be decomposed in:

- **D1** is the water self-diffusion inside the micelle
- **C1** is the water content inside the micelle
- **D2** is the water self-diffusion outside the micelle
- **C2** is the water content outside the micelle

The water diffusion according to the cell-model is given by:

\[
D_{\text{eff}}^{\text{water}} = D_{0}^{\text{water}} \cdot \left(1 + \nu^{\text{cas}} \cdot \frac{m^{\text{cas}}}{m^{\text{water}}} \right) \cdot \left(\frac{1 + \nu^{\text{cas}} \cdot \frac{m^{\text{cas}}}{m^{\text{water}}} - K \cdot \frac{m^{\text{cas}}}{m^{\text{water}}}}{1 + \nu^{\text{cas}} \cdot \frac{m^{\text{cas}}}{m^{\text{water}}} + K \cdot 0.5 \cdot \frac{m^{\text{cas}}}{m^{\text{water}}}}\right)
\]
Obstruction effect induced by permeable micelle?

where

\[ K = \beta \times \left( \frac{\nu^{\text{cas}}}{\nu^{\text{water}}} + V^{\text{cas}} \right) \]

with

\[ \beta = \frac{D_2 C_2 - D_1 C_1}{D_2 C_2 + 0.5 D_1 C_1} \]

- The water diffusion inside the casein micelle \( D_1 \)?
- The micelle hydration \( C_1 \)?
- The water diffusion outside the micelle \( D_2 \)
- The water concentration outside the micelle \( C_2 \)
Obstruction effect induced by permeable micelle?

The best fit is obtained with $K = 1.60 \pm 0.01$
Obstruction effect induced by permeable micelle?

From k and if a casein voluminosity of \( V = 5 \) g water/g casein was assumed *, the water self-diffusion inside the micelle \( D_1 \) could be estimated

\[
D_1 = \frac{D_2 C_2}{C_1} \left( \frac{\frac{\nu\text{ cas}}{\nu\text{ eau}} + \nu\text{ cas} - k}{\frac{\nu\text{ cas}}{\nu\text{ eau}} + \nu\text{ cas} + 0.5k} \right)
\]

with \( C_2 = 1 \text{ g.cm}^{-3}, \ D_2 = D_0 \)
\( \nu\text{ casein} = 0.75 \text{ cm}^3\text{.g}^{-1}, \)
\( \nu\text{ water} = 1 \text{ cm}^3\text{.g}^{-1} \)
Obstruction effect induced by permeable micelle?

The water diffusion coefficient inside the micelle was $1.45 \times 10^{-9} \text{ m}^2 \text{ S}^{-1}$

If we compare to the self-diffusion of protein $(3 \times 10^{-12} \text{ m}^2 \text{ S}^{-1})$
  - the water diffusion is very high,
  - the water diffusion reduction could not be explained by strong water-protein interaction

If we compare to the self-diffusion of pure water $(1.99 \times 10^{-9} \text{ m}^2 \text{ S}^{-1})$
  - the water diffusion is slightly reduced
  - the reduction could be explained by the obstruction induced by the protein molecules
Conclusion

- The self-diffusion coefficient could be measured and the water molecules are free to diffuse for large distances (micrometer scale)
  - in solutions
  - in gels

- No restriction from impermeable barriers was involved in the hindrance of the water diffusion

- The water self-diffusion coefficients were not modified by the coagulation with rennet $D_{\text{sol}} = D_{\text{gel}}$

- The water diffusion in casein micelle could be explained by two water fluxes
  - One around the casein micelles
  - One through the casein micelles
Could we generalize this approach to other protein systems (globular protein) ?

Could we include in the model other effects ?
- Amount of soluble compounds
- Amount of fat

Could we describe the water diffusion in real product (cheese...) ?

Could we relate the water diffusion in micro-length scale to macroscopic length scale ?

Work in progress....