

## NMR relaxometry as a non-invasive tool to characterize milk powders

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**Abstract** – The ability to quickly solubilize powders in aqueous medium without forming insoluble material is of primary importance for the quality of many food powders. The NMR method developed gives a complete picture of the reconstitution process of milk powders in water. It allows the quantification of the water absorption by powder particles, their reconstitution rate and the detection of the presence of insoluble materials. Furthermore, the NMR transverse relaxation rate of the reconstituted product is proved to be an excellent indicator of the structural state of milk proteins. It is shown that the poor reconstitutability of native cow phosphocasein micelles concentrated by tangential microfiltration and powdered by spray-drying can be significantly improved by addition of whey proteins, suitable polydextroses or NaCl before spray-drying powder, without significantly affecting their micellar structure. On the other hand, the decrease of the relaxation rate showed an important modification of the casein structure when a citrate solution or, to a lesser extent, when a phosphate solution was added to the retentate. The addition of CaCl<sub>2</sub> strongly disturbed the micellar organization and led to the formation of insoluble structures during spray-drying.

**Milk powder / NMR relaxation / rehydration / solubilization / micellar casein**

**Résumé** – La relaxométrie RMN comme outil non invasif de caractérisation des poudres laitières. La capacité à se solubiliser rapidement en milieu aqueux est de première importance pour la qualité de nombreuses poudres alimentaires. La méthode RMN développée donne une description complète du processus de reconstitution des poudres dans l'eau. Elle permet de quantifier le phénomène d'absorption d'eau par les particules, la vitesse de reconstitution et de révéler la présence de matériaux insolubles. D'autre part, la vitesse de relaxation RMN transversale du produit reconstitué s'avère être un excellent indicateur de l'état structural des protéines laitières. Il est montré que la

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Communication at the 1st International Symposium on Spray Drying of Milk Products, Rennes, France, October 16–18, 2001.

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médiocre reconstituabilité des micelles de caséines bovines natives concentrées par microfiltration tangentielle puis séchées par atomisation peut être significativement améliorée par l'addition préalable au séchage de protéines laitières solubles, de certaines maltodextrines ou de NaCl avant atomisation sans affecter significativement la structure des micelles. D'autre part, la décroissance des vitesses de relaxation de l'eau en interaction avec les macromolécules traduit d'importants changements de la structure des protéines quand une solution citrate ou, dans une moindre mesure, quand une solution phosphate est ajoutée. L'addition de  $\text{CaCl}_2$  perturbe fortement l'organisation micellaire et conduit à la formation de matériaux insolubles en cours de séchage.

## **Poudre laitière / relaxation RMN / réhydratation / solubilisation / caséine micellaire**

### **1. INTRODUCTION**

More and more food ingredients are commercialized as dehydrated powders obtained by spray-drying or freeze-drying to reduce transport costs and greatly improve their preservation. Rapid and complete reconstitution of these dehydrated products in water is essential for practical use. Developments in membrane microfiltration processing of skimmed milk have allowed the preparation of native phosphocaseinate suspension (NPCS) [3, 8]. NPCS has excellent rennet-coagulating properties such as a greatly reduced coagulation time and a good gel firmness compared to that of raw milk [8]. The apparent high insolubility (ISI) of freshly dried NPCS powders [5], determined according to the IDF standard [4], does not result from insoluble material formed during spray-drying [13] or ageing but from a decreased water transfer in pure micellar casein in comparison with the water transfer of low heat milk powder (ISI < 0.5 mL) during rehydration [9, 10]. The reconstitutability of these powders, such as native micellar caseins, can be very significantly improved if the concentrates are enriched with suitable co-ingredients, such as soluble proteins, some suitable carbohydrates or salts before drying. Numerous studies have demonstrated the high sensitivity of the water NMR relaxation rate to micellar casein structure [6]. For example, addition of EDTA disrupts the micelle structure and induces a decrease in the relaxation rate. Furthermore, the water relaxation rate decreases with pH because of the modification of the micellar structure

following removal of micellar calcium phosphate. In this presentation, a kinetic pulse NMR technique for determining both the rate of solution, the time required for complete reconstitution of powders and the transverse relaxation rate of reconstituted solution is described. The method, potentially available for many food or non-food powders, is presented in the case of the reconstitution of native casein micelles from cow, ewe and goat, enriched or not with soluble whey proteins, suitable carbohydrates or salts.

### **2. MATERIALS AND METHODS**

#### **2.1. Milk powders**

The native phosphocaseinate suspension (NPCS) was prepared from skim milks of cow, ewe and goat by microfiltration and diafiltration (pore diameter: 0.1  $\mu\text{m}$ ) on a MFS 19 unit (Tetra Laval, Århus, Denmark; 4.6  $\text{m}^2$ ) at 50 °C and  $200 \pm 5 \text{ g}\cdot\text{kg}^{-1}$  total solids according to Fauquant et al. [3] and Pierre et al. [8]. Whey protein concentrate (WPC) was separated from previous cow milk microfiltrate by tangential membrane ultrafiltration (cutoff: 10 KD) followed by purification through water diafiltration, and freeze-dried. Combined cow casein powders (CCP) were obtained by two techniques:

- WPC and Polydextrose Litesse (PDL) from Pfizer Co. (Orsay, France) were first mixed into cow NPCS retentate before

**Table 1.** Chemical and mineral composition of cow NPCS and CCP powders, TS = Total Solid, TP = Total Protein,  $\tau$ : Total,  $\tau_s$ : Soluble.

Co-ingredient added to NPCS	Co-ingredient added to TS	Drying technique	TS g·kg <sup>-1</sup>	TP g·kg <sup>-1</sup>	Ca <sub>T</sub> <sup>b</sup> mmol·L <sup>-1</sup>	Ca <sub>S</sub> <sup>b</sup> mmol·L <sup>-1</sup>	Na <sub>T</sub> <sup>b</sup> mmol·L <sup>-1</sup>	Na <sub>S</sub> <sup>b</sup> mmol·L <sup>-1</sup>	P <sub>T</sub> <sup>b</sup> mmol·L <sup>-1</sup>	P <sub>S</sub> <sup>b</sup> mmol·L <sup>-1</sup>
none	0	freezing	996	858						
WPC	12	freezing	996	887						
PDL	12	freezing	996	860						
none	0	spraying	920	823	160	2.9	1.5	1.3	119	4
NaCl	12	spraying	912	726	137	13.4	412	403	-	-
CaCl <sub>2</sub>	12	spraying	918	727	367	204	-	-	-	-
SPS	12	spraying	913	717	129	1.4	316	251	282	116
SCS	30	spraying	920	565	104	70	687	532	-	-

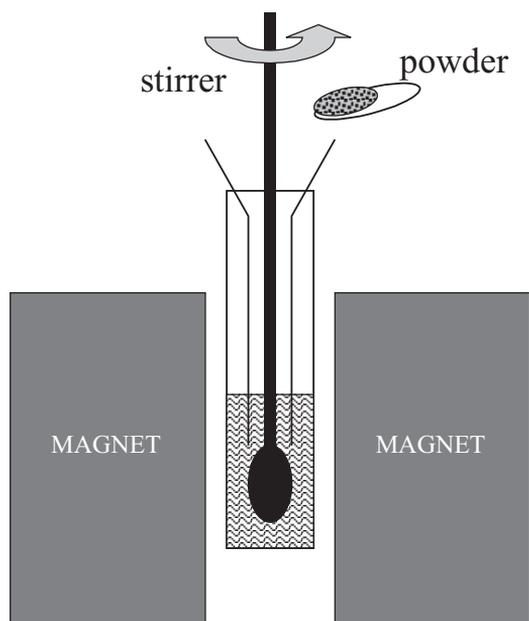
freeze-drying to obtain 4, 8 and 12% PDL/TS and 4, 8 and 12% WPC/TS powders (TS = Total Solid).

- Mineral salts were added to cow NPCS concentrate for 30 min at 50 °C before spray co-drying. Solutions of NaCl, sodium citrate (SCS) (Grosseron SA, St Herblain, France), CaCl<sub>2</sub>, and sodium phosphate (SPS) (Panreac Quimica SA, Barcelona, Spain), all at 205 ± 5 g·kg<sup>-1</sup> total solids, were added to cow NPCS to obtain different concentrates of about 12% salt (w/w) on total solids for the addition of NaCl, CaCl<sub>2</sub>, SPS and a concentrate of 30% salt (w/w) on total solids for the addition of SCS. After addition of salt, the pH was adjusted to 7.1 with 1N KOH (NaCl, CaCl<sub>2</sub> and sodium phosphate solution) or with 1N HCl (sodium citrate solution) at 20 °C. The exact concentrations obtained during the experiments are reported in Table I.

The spray-drying of concentrates was performed at Bionov (Rennes, France) in a 3-stage pilot-plant spray dryer (GEA, Niro Atomizer, St-Quentin-en-Yvelines, France) following the recommendations of Schuck et al. [9].

## 2.2. NMR method for characterizing powder rehydration

A glass tube, 40 mm in diameter and filled with 20 mL of water at 40 °C, was put into the gap of the magnet of a Minispec Bruker PC 10 NMR spectrometer operating at the resonance frequency of 10 MHz. A suitably designed funnel and an electric stirrer (glass spatula) were inserted into the tube (Fig. 1). The method was first described by Davenel et al. [2]: they showed that the solubilization rate was independent of the quantity of powder poured, up to 20 g powder / 100 mL water, and increased with the stirring rate. In the following experiments, after starting, the stirrer was adjusted to a 1500 rpm rotating rate for freeze-dried powders and 1150 rpm for spray-dried powders, and 1 g of powder was poured into the water. The NMR measurements were generally continued until the solution was completely reconstituted, except if insoluble materials were formed. Each decay curve was obtained by sampling the maximal of 845 spin echoes of a Carr-Purcell-Meiboom-Gill (CPMG) sequence every 20 seconds during the reconstitution



**Figure 1.** Device for controlling the reconstitution process of powders by NMR.

period. The interpulse spacing between 180° pulses was fixed at 2 ms to limit the effect of diffusion caused by stirring. The NMR kinetic method was used in triplicate.

The CPMG curves were approximated well by a sum of two exponential curves of the form:

$$S(t) = A_p \exp(-t.R_{2p}) + A_s \exp(-t.R_{2s}). \quad (1)$$

$A_p$  and  $R_{2p}$  are, respectively, the amount of protons (% of total proton population) and the relaxation rate of a fast decay component attributed to water protons in fast exchange with exchangeable protons of non-dissolved powder particles, and  $A_s$  and  $R_{2s}$  the amount of protons and the relaxation rate of a slow decay component attributed to water protons and exchangeable protons in the reconstituted phase [2].

### 3. RESULTS AND DISCUSSION

#### 3.1. Description of the rehydration of the NPCPS powders by NMR

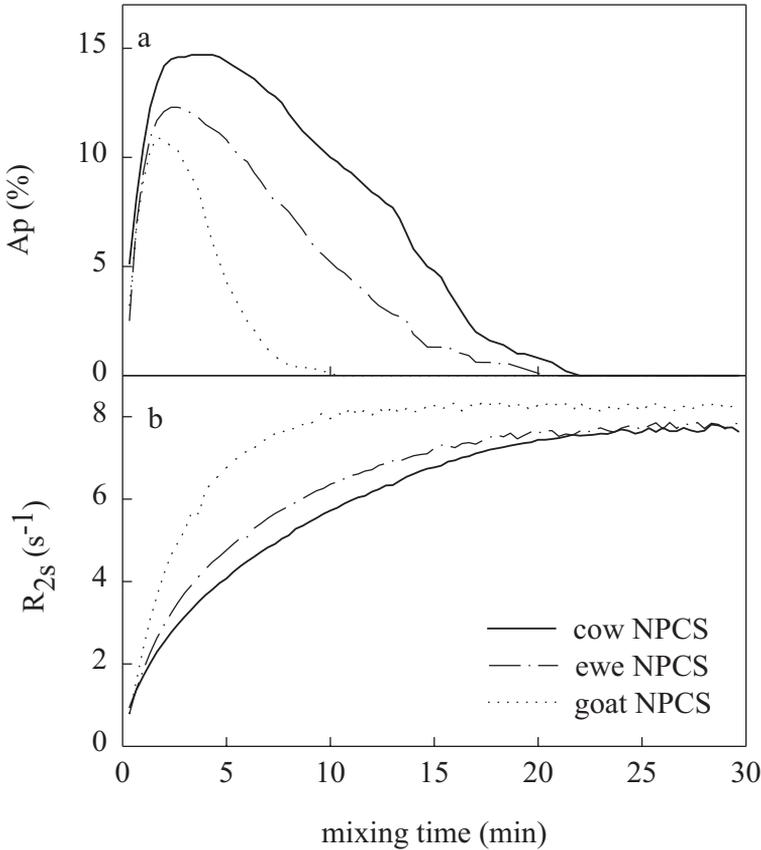
The changes measured in the two proton populations of pure NPCPS show two phenomena occurring during pure NPCPS rehydration (Figs. 2, 3 and 4): water absorption by powder particles and solubilization of particles. The water absorption is

described by the variation of the intensity of the  $A_p$  value during the powder rehydration kinetic. During the first two minutes, the population  $A_p$  increases greatly because of the water absorption and, after reaching a maximum, this population decreases continuously because of the solubilization of the particles. The solubilization of the particles explains the evolution of the relaxation rate  $R_{2s}$  of the second component which, in the case of rehydration of pure NPCPS powder, is approximately proportional to the concentration  $A_s$  of the solubilized phase. The high value of  $R_{2s}$  (Tab. II) observed after reconstitution of freeze-dried and spray-dried pure NPCPS powders is related to the interactions of water protons with the casein protons, the mobility of which is greatly reduced by their structure in micelles.

Pure cow NPCPS powders with a high ISI of 14.4 mL are generally considered to be poorly soluble powders in which rehydration of the micelle remains incomplete [5]. Actually, the present NMR method showed that reconstitution was fully achieved after a long Reconstitution Period (RP) after which the  $A_p$  proton population was no longer observable: the RP was about 30 min and 22 min for freeze-dried and spray-dried cow NPCPS powders, respectively (Tab. II). The difference observed

**Table II.** Reconstitution period (RP), final relaxation rate  $R_{2s}$  and insolubility index ISI of pure cow NPCPS and CCP powders.

Co-ingredient added to NPCPS	Co-ingredient added g·100g <sup>-1</sup> TS	Drying technique	RP (min)	$R_{2s}$ (s <sup>-1</sup> )	ISI (mL)
None	0	freezing	32	8.4 (± 0.2)	
WPC	12	freezing	13	8.1 (± 0.2)	
PDL	12	freezing	18	8 (± 0.2)	
None		spraying	22	8.7 (± 0.07)	14.4
NaCl	12	spraying	9.5	8.4 (± 0.13)	9
CaCl <sub>2</sub>	12	spraying	∞	3.1 (± 0.2)	14.6
SPS	12	spraying	6	4.4 (± 0.14)	0
SCS	30	spraying	5	3.1 (± 0.06)	0



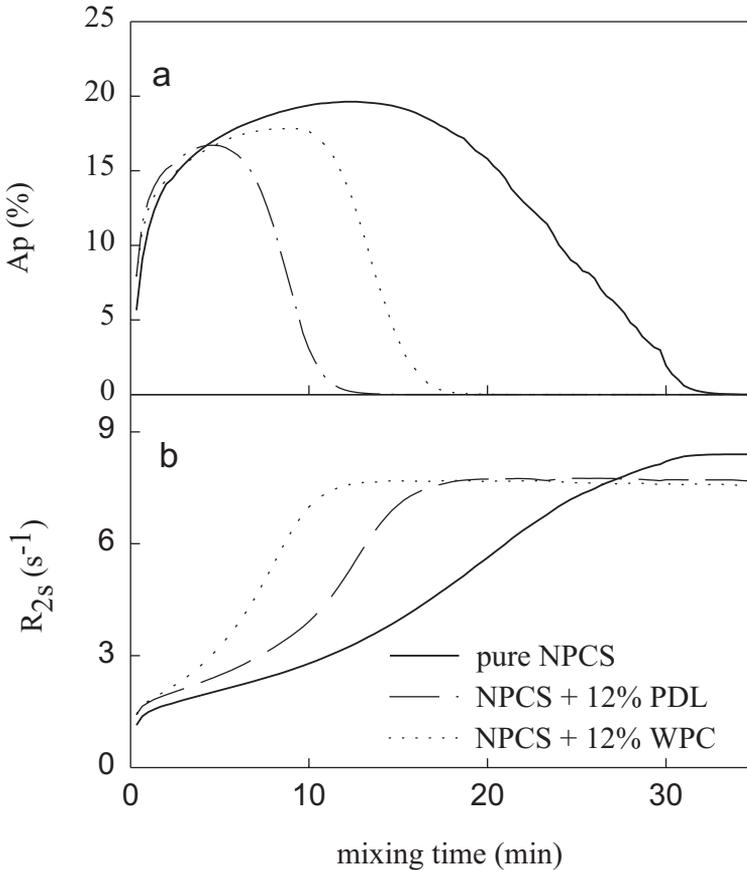
**Figure 2.** Effect of animal species on (a) reconstitution period RP and (b) final relaxation rate  $R_{2s}$  (b) of pure NPCS powders.

between RP values could be related to the difference in the size of the particles resulting from the two drying techniques: the freeze-drying process produced pellets larger than the particles obtained by spray-drying and probably more difficult to rehydrate. Despite no significant  $R_2$  differences between the three species, the RP value appeared to be much lower for goat NPCS powder (10 min) than for ewe and cow NPCS powders, 20 and 22 min, respectively (Fig. 2).

### 3.2. Effect of the addition of whey proteins or polydextrose

The NMR method showed that the addition of suitable macromolecules such as

whey protein or polydextrose before drying led to a significant improvement in the solubilization rate when the RP was reduced to 13 min and 18 min when 12% WPC and 12% PDL, respectively, were added (Tab. II). The decrease in the relaxation rate could be explained mainly by the decrease in the NPCS concentration. When this NPCS concentration effect on the relaxation was corrected, no significant difference was any longer observed. These co-ingredients did not seem to modify the structure of the casein micelles. Because the reconstitution rate of NPCS was only faster when these additives were added before drying, the hypothesis is proposed that the steric hindrance of these large molecules could have reduced



**Figure 3.** Effect of the addition of polydextrose PDL or Whey protein WPC on (a) reconstitution period RP and (b) final relaxation rate  $R_{2s}$  (b) of CCP powders.

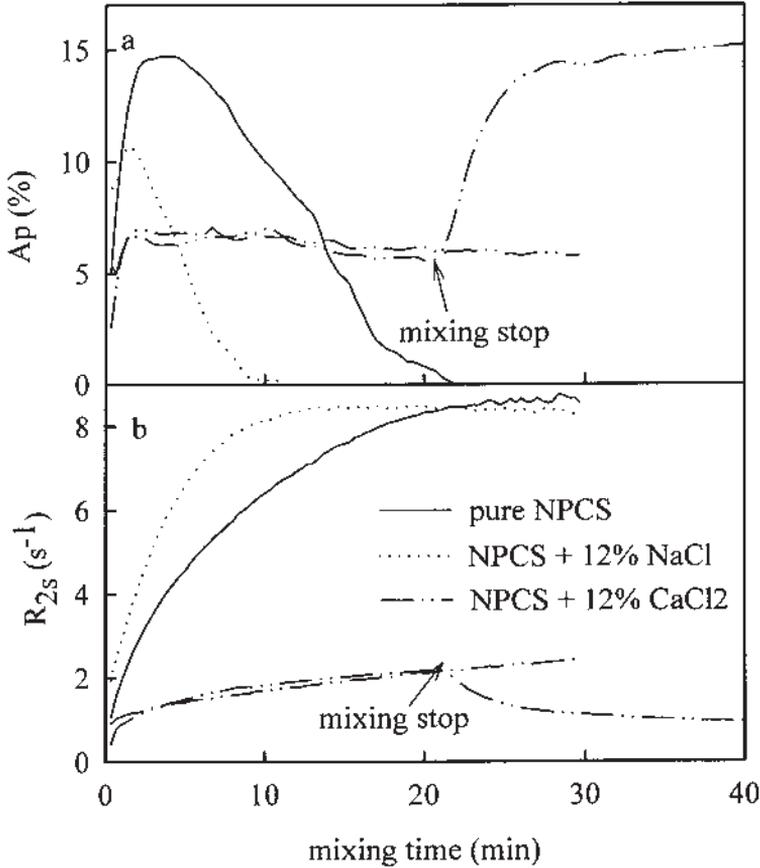
the folding of caseins during drying, making later rehydrations easier.

### 3.3. Effect of the addition of salts

Addition of NaCl to NPCS concentrate before spray-drying strongly decreased the ISI and RP values (ISI: 0.9 mL; RP: 9.5 min). In spite of this significant improvement in the reconstitution rate (Fig. 4) and slight solubilization of the micellar calcium (Tab. I), the relaxation rate  $R_{2s}$  of the reconstituted solution in the presence of NaCl was not significantly different from the pure NPCS relaxation rate (Tab. II); it is hypothesized that NaCl would exert little

influence on micelle structure [7] and that the big decrease in the RP value is more probably related to the hygroscopic strength of NaCl.

Addition of citrate ions increased the soluble calcium ( $Ca_S/Ca_T = 67.1\%$  against 1.8% without addition of citrate), in agreement with Shalabi and Fox [12] and Schuck et al. [11]. The addition of sodium citrate solution (SCS) resulted in fast solubilization, as shown by the very low RP value and by the ISI value lower than 0.5 mL. The resulting solution consisted of casein micelles in the form of sodium caseinates associated with the occurrence of a single proton population, characterized



**Figure 4.** Effect of the adding of different salts on (a) reconstitution period RP and (b) final relaxation rate  $R_{2s}$  (b) of CCP powders.

by NMR relaxation rates which were much lower than the relaxation rate measured with reconstituted NPCS. This could be attributed to a decrease in the amount of hydration water induced by the change in micelle structure. The transparency of the solution indicates the formation of soluble caseins related to greater quantities of calcium complexes [6, 8].

The addition of phosphate ions to the cow NPCS resulted in phosphorus binding to the micelle (bound P/P added = 36.2% for the same TP content), without changing the soluble calcium content [11]. This addition of SPS also led to a strong decrease in the RP value associated with the occurrence

of a single proton population. However, the RP values and  $R_{2s}$  values were midway between those of the reconstitution of pure NPCS and the reconstitution of NPCS in the presence of SCS: the  $R_{2s}$  value suggests that the SPS solution could destructure casein micelles less than the SCS solution.

Reconstitution of CCP powder in the presence of  $\text{CaCl}_2$  salt led to considerable changes in protein structure associated with instability of the casein micelles which began to precipitate just after mixing stopped (Fig. 4) as shown by the high ISI and the non-measurable RP value due to experimental delay. Moreover, the mathematical adjustment of the NMR relaxation decay

signals measured with or without mixing (Fig. 4) revealed two NMR relaxation components. In the case of continuous mixing, the two relaxation rates correspond to the water in interaction with particles and the water in the reconstituted phase. When mixing was stopped, the mixture underwent a fast sedimentation and the two relaxation components could be respectively attributed to the sedimentation phase and the supernatant phase. This precipitate probably resulted from aggregation of casein micelles or submicelles through a decreasingly negative charge on the protein by additional Ca binding, leading to a reduction in electrostatic repulsion [1]. In this case, the high ISI of these solutions was related to the presence of insoluble substances, whereas in the case of rehydration of pure NPCS powder, the high ISI only represented the low water transfer rate in casein.

#### 4. CONCLUSION

The various applications of the NMR relaxometry presented in this paper show the ability of this non-invasive technique to deliver novel and useful information about the reconstitution process of milk powders, close to industrial conditions. The insolubility index and NMR relaxometry gave compatible information on powder rehydration. However, a high ISI did not indicate whether the powder could be totally reconstituted in water even after a long reconstitution period or whether the powder contained insoluble substances. Though it was more time-consuming, the NMR method clearly differentiated between the two phenomena. The method could measure long reconstitution periods in solution where water transfers were slow, or detect the presence of insoluble structures in some other solutions where rehydration was only partial. Moreover, a decrease in the NMR transverse relaxation rate of the reconstituted solution indicated more or less important changes in the micellar

structures, resulting from the effect of some additives on protein-protein interactions.

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