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# STRUCTURAL ORGANIZATION OF CASEIN MICELLES IN CONCENTRATED LAYER DURING CROSS-FLOW ULTRAFILTRATION

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## ABSTRACT

*Small-Angle X-Ray Scattering (SAXS) has been coupled to cross-flow ultrafiltration to determine the behaviour of casein micelles concentrated layers at the membrane surface. The concentrated layer formation and its reversibility was followed. We also studied the effect of temperature on the structural organization of casein micelles during cross-flow filtration. Concentration profiles have been deduced from the scattering intensities registered in-situ in the course of the cross-flow ultrafiltration of casein micelles dispersions. SAXS and filtration results for a reference experiment (110 kPa, 0.03 m.s<sup>-1</sup>, 3h of filtration, 25°C) show the formation of a thick fouling layer of 150µm and reaching 195 g/L near the membrane surface. Furthermore, results show some reversibility of the upper layer of those accumulated casein micelles layers and an irreversibility of fraction close to the membrane surface. A change of temperature (45°C or 12°C instead of 25°C) led to a different organization of concentrated layer with a thicker layer when increasing temperature. However, for the three different temperature, an irreversible fouling layer reaching 3.5 C0 close to the membrane is still lasting after a pressure relaxation. Those results are important for understanding the concentrated layer properties of casein micelles during cross-flow ultrafiltration and they could be used to adjust the filtration parameters in order to avoid the formation of an irreversible concentrated layer at the membrane surface.*

## INTRODUCTION

Ultra- and microfiltration of skim milk are widely used in the dairy industry as tools for the concentration and the fractionation of proteins but their performances are limited by formation of a concentrated layer of casein micelles at the membrane surface

Despite of some recent progress on casein fouling layer characterization at macro- and micro-scales (Qu *et al.*, 2012; Jin *et al.*, 2014), still little is known about its' properties and behaviour (layer formation and compression, sol-gel transition, and reversibility). The aim of the current work was to elucidate local structure and behaviour of casein micelles fouling layer during cross-flow ultrafiltration with the help of *in-situ* small angle X-ray scattering (SAXS) analysis. The analysis was performed during complete filtration cycle: fouling layer accumulation (membrane fouling), followed by pressure relaxation and shear-induced erosion (membrane cleaning) under various operating conditions (transmembrane pressure TMP, cross-flow velocity and temperature). This allowed to (i) distinguish the thickness and local solid concentration profiles in flowing (sol) and stagnant (gel) parts of the fouling layer; (ii) discriminate the effect of time, transmembrane pressure, shear, temperature on the sol-gel transition; (iii) study the reversibility of the sol-gel transition under the moderate laminar cross flow.

## EXPERIMENTAL

### Dispersions

All dispersions were prepared from casein powder (Table 1) (Promilk 802 B, Ingredia, Arras, France) dispersed in a solvent (UF permeate) obtained in our laboratory through membrane ultrafiltration (5 kD cutoff) of skimmed milk. The totality of the milk protein fraction, *i.e.* caseins and whey proteins, is eliminated through this operation. The UF permeate contains the milk minerals, lactose, and a few other low molar mass molecules. Sodium azide purchased from Sigma-Aldrich (St. Louis, MO, USA), was added to the UF permeate as preservative at  $0.5 \text{ g.L}^{-1}$ . Caseins micelles from the casein powder dispersed in UF permeate are similar to the “native” casein micelles found in fresh milk. The dispersions were prepared by thoroughly mixing the casein powder in UF permeate for 15h at  $35^\circ\text{C}$ . The initial casein concentration chosen is  $55 \text{ g.L}^{-1}$ , which is twice more than the casein concentration in milk.

Table 1. Composition of casein powder.

	TS (% w/w)	Caseins (% TS)	Noncasein nitrogen matter (% TS)	Nonprotein nitrogen matter (% TS)
Surface	95.0	75.9	8.0	0.8

### SAXS Cross-Flow coupled Filtration

A specific SAXS- cross-flow ultrafiltration cell previously developed by Jin *et al.* (2014) has been used at the ESRF (ID02 TRUSAXS Beamline, Grenoble) to access to the structural organization at nanometer length scales and concentration profiles at the vicinity of the membrane surface during the filtration process. This cell (Fig. 1.a.) is made of transparent polycarbonate and contains a flat polyethersulfone ultrafiltration membrane (PES 100kD, Pleiade, Orelis Environnement, France).

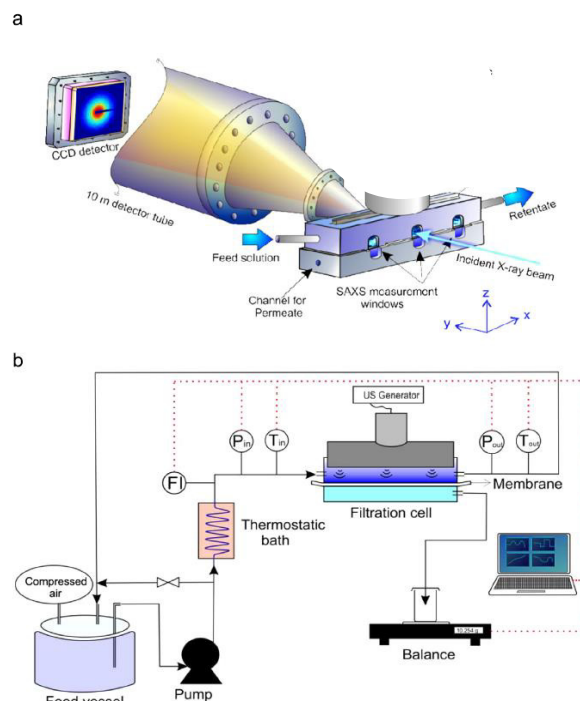


Figure 1. (a) SAXS Cross-flow coupled Filtration Cell during in situ SAXS measurement; (b) overview experimental setup of cross-flow ultrafiltration. Adapted from Jin *et al.* (2014).

As shown in Figure 1.b and described by Jin *et al.* (2014), the SAXS cross-flow cell is inserted in the filtration rig which allow to control the pressure, crossflow rate and temperature.

For several operating conditions (pressure, crossflow, temperature) the casein micelle concentration was deduced from the *in-situ* SAXS measurements as a function of the distance  $z$  from the membrane surface, with 20  $\mu\text{m}$  vertical resolution.

After each experiment, the membrane was cleaned by filtering a 0.1% (v/v) Ultrasil 25F (alkaline solution, Henkel-Ecolab SNC, Issy les Moulineaux, France) at 100 kPa and 45°C at 0.3  $\text{m}\cdot\text{s}^{-1}$ . The membrane was then rinsed with deionized water at 100 kPa until neutral pH was recovered in the permeate.

### SAXS measurement analysis

SAXS measurements were carried out at the ID02 TRUSAXS Beamline, at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). The incident beam passed through the sample and the two-dimensional scattered intensity patterns were recorded on a high-resolution fiber optically coupled CCD detector. Measured scattering patterns were normalized to an absolute intensity scale after applying standard detector corrections and then azimuthally averaged to obtain the one-dimensional intensity profiles denoted by  $I(q)$ .

In order to determine the casein micelle concentration within concentrated layer during filtration, a calibration curve was firstly established by static SAXS measurement in a temperature controlled (25°C) flow-through capillary cell (diameter of 2 mm). Ten dispersions of known casein micelle concentrations (Fig 2. a.) were measured and then the relation between their scattering intensities at  $q = 1 \text{ nm}^{-1}$  and their concentration in casein micelles was established (Fig 2. b.):  $I(q = 1 \text{ nm}^{-1}) = 0.013303 C/C_0$  ( $R^2 = 0.999$ ). With this calibration curve, and the scattered intensity measured *in-situ* in the filtration cell at a certain  $z$  distance from the membrane surface, the casein micelle concentration is deduced.

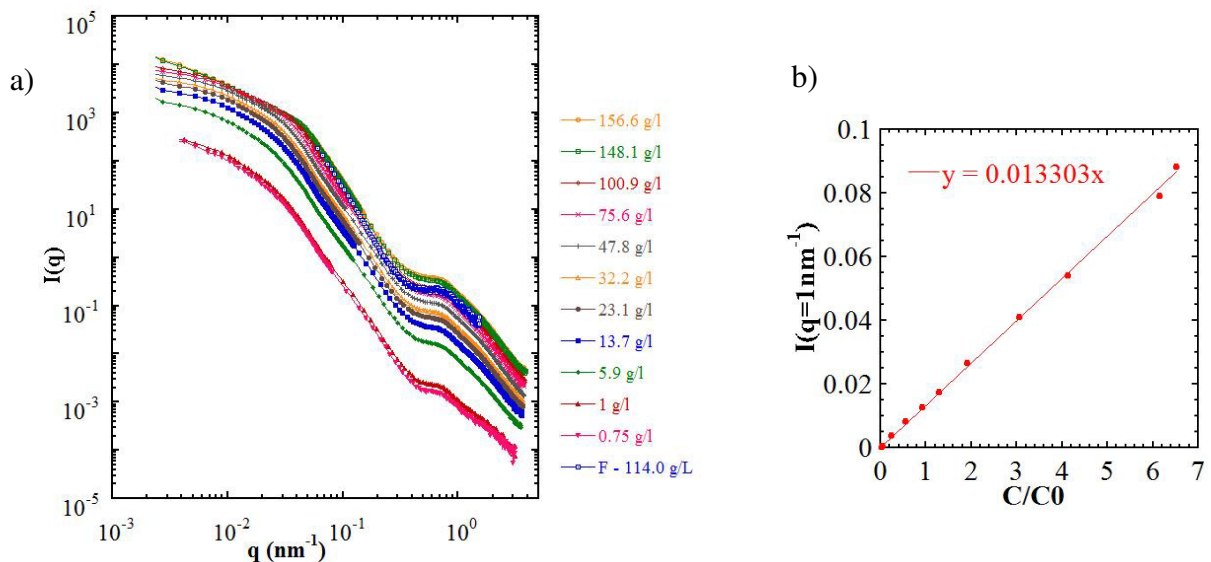


Figure 2. SAXS profiles of intensities scattered during *in-situ* measurements in a capillary cell for different casein micelle concentrations. For comparison a SAXS profile in the filtration cell at the deduced 114.0 g/L concentration is plotted ( $z = 70 \mu\text{m}$  after 194 min of cross-flow ultrafiltration at  $T = 25^\circ\text{C}$ ,  $v = 0.03 \text{ m}\cdot\text{s}^{-1}$  and  $\text{TMP} = 110 \text{ kPa}$ ). (b) Calibration curve resulting from the relation between concentration and scattering intensity at  $q = 1 \text{ nm}^{-1}$

This method allows to determine the concentration evolution of casein micelles dispersions as a function of the distance  $z$  from the membrane (Fig 4.), which corresponds to the concentration profiles of the suspensions in the accumulated layers during filtration.

## RESULTS AND DISCUSSION

### SAXS profiles

SAXS profiles of casein micelles dispersion measured *in-situ* are all similar from near the membrane ( $z = 30 \mu\text{m}$ ) to the bulk ( $z = 200 \mu\text{m}$ ) (Fig. 3.) and the curve is identical to the SAXS profile of skim milk (Jin *et al.*, 2014). SAXS profile is related to the structure casein micelles and figure 3 shows that there is no structure change of casein micelles within the distance to the membrane and so within the concentration.

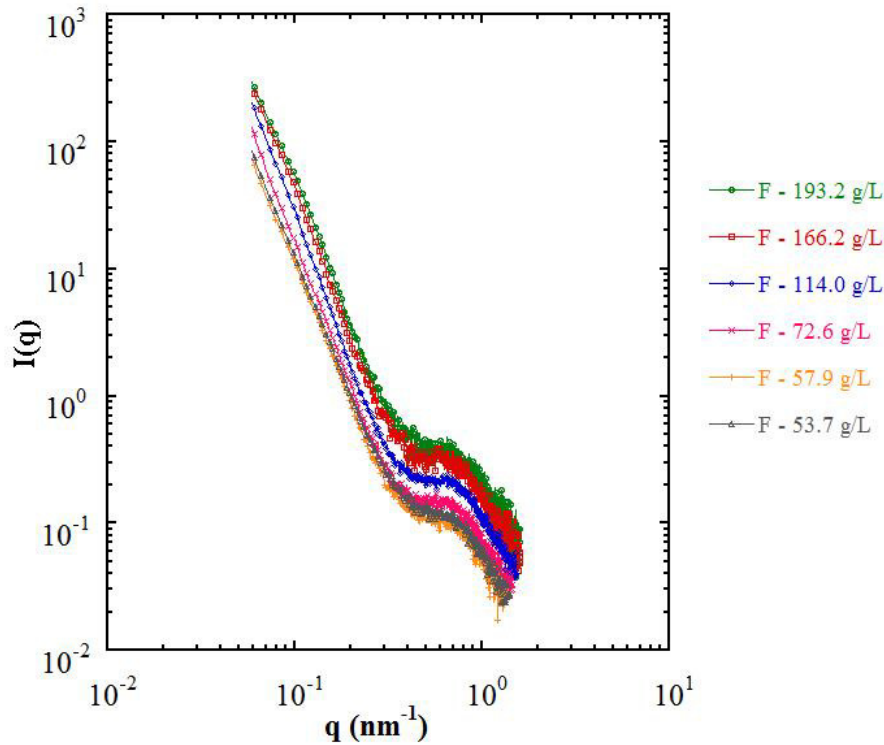


Figure 3. SAXS profiles of intensities scattered during *in-situ* measurements in the filtration cell after 194 min of cross-flow ultrafiltration at  $T = 25^\circ\text{C}$ ,  $v = 0.03\text{m}\cdot\text{s}^{-1}$  and  $\text{TMP} = 110 \text{ kPa}$ .  $z = 30, 70, 50, 100, 150$  and  $200 \mu\text{m}$  from the top to the bottom.

### Concentrated layer formation and reversibility

Filtration run was carried out at  $v = 0.03 \text{ m}\cdot\text{s}^{-1}$  and  $\text{TMP} = 110 \text{ kPa}$  (step F in Fig. 3. a.). Then a second step of relaxation was applied by reducing the TMP from 110 kPa until 10 kPa. Finally erosion steps ( $E_1$  and  $E_2$  4 in Fig 4. a.) were applied to try to eliminate the accumulated layer. The aim of this experiment was to form a fouling layer and then applied different filtration conditions (release of the pressure, subsequent cross flow) to test its reversibility. Such conditions allow building a thick fouling layer of  $\sim 150 \mu\text{m}$  (Fig. 4. b.) with a maximal concentration of  $7.5 C_0$  (around  $195 \text{ g}\cdot\text{L}^{-1}$ ) close to the membrane ( $z = 30 \mu\text{m}$ ) above the sol- gel transition ( $180 \text{ g/L}$ ) according to Bouchoux *et al.* (2009) and Jin *et al.* (2014).

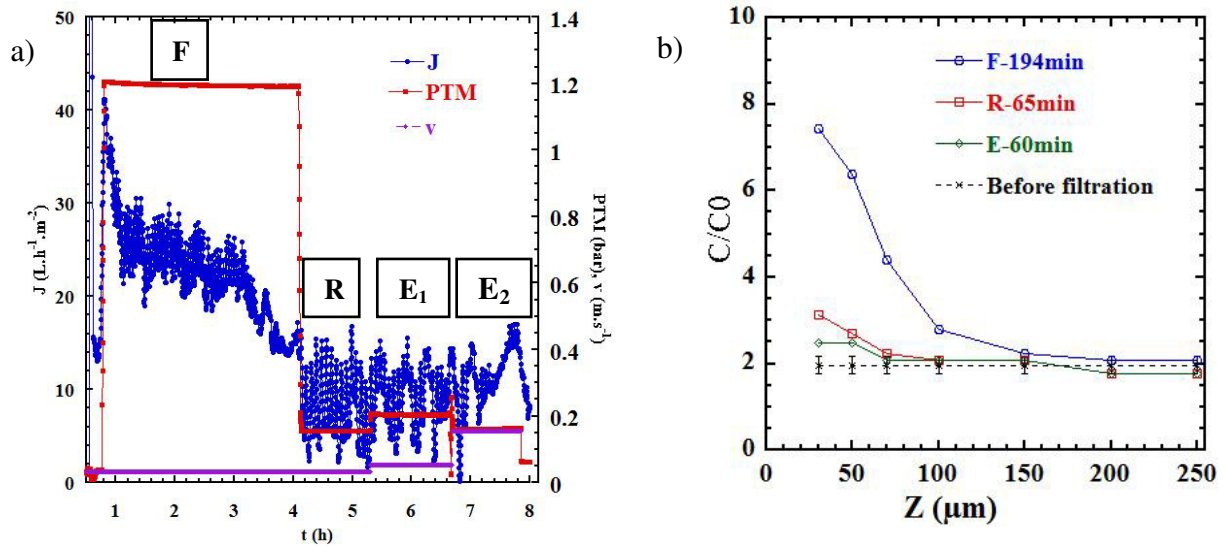


Figure 4. Macro-scale results and nano-scale detections during casein micelle cross-flow ultrafiltration ( $C = 2C_0$ ,  $\text{pH} = 6.7$ ,  $T = 25^\circ\text{C}$ ) (a) Cross-flow filtration curve: Evolution of  $J$ , TMP and  $v$  over time. (b) Related concentration profiles at each step from the membrane surface to the bulk, deduced from *in-situ* measurements. F: Filtration step, R: Relaxation step, E: Erosion from step  $E_2$ .

The concentrated layer reached a steady state after 130 minutes of filtration (Fig 5. a.) and after a pressure relaxation (Fig 5. b.), a majority of reversible concentrated layer disappears since it lasts a 50-70 $\mu\text{m}$  of casein micelle layer reaching  $3.5 C_0$  (around 91  $\text{g.L}^{-1}$ ) close to the membrane surface.

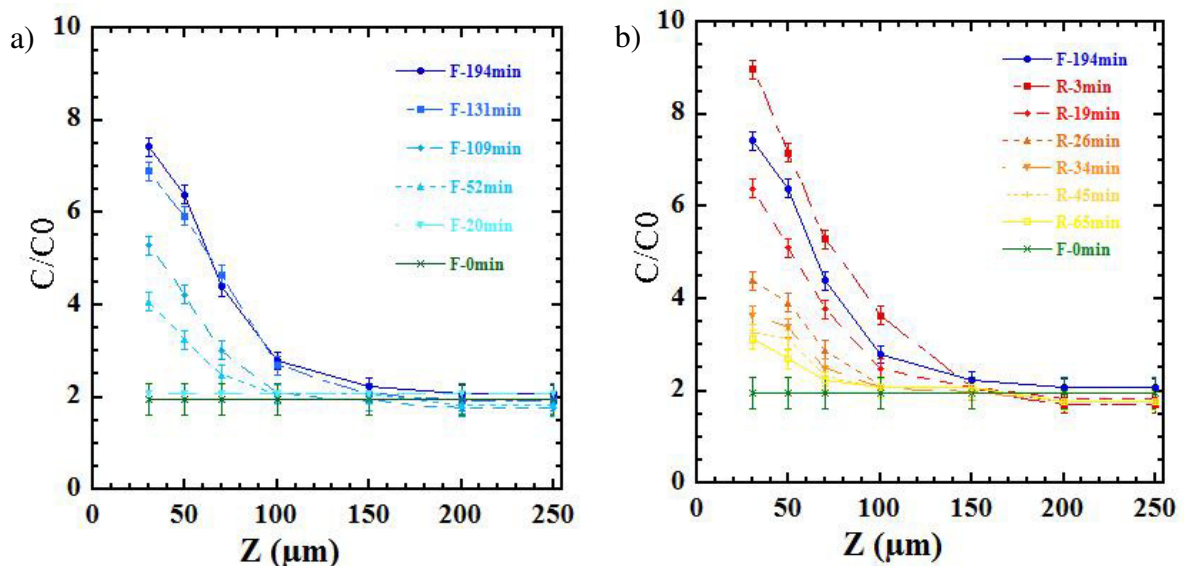


Figure 5. Evolution of concentration profiles within the accumulated layer over time during casein micelle cross-flow ultrafiltration ( $C = 2C_0$ ,  $\text{pH} = 6.7$ ,  $T = 25^\circ\text{C}$ ). 0 min represents the initial state before starting TMP (a) Filtration step. (b) Relaxation step. F: Filtration step, R: Relaxation step.

Some experiments in progress will determine further the irreversibility and reversibility parts after filtration and will furnish better permeate flux than ones presented in Fig 4.a.

### Effect of temperature

The effect of temperature on the concentration profile and its reversibility has been studied for three independent filtrations (Table 2). Concentrated layer thickness increased with temperature (Fig. 6.): from  $\sim 100 \mu\text{m}$  at  $12^\circ\text{C}$  to  $150 \mu\text{m}$  at  $25^\circ\text{C}$  and  $200 \mu\text{m}$  at  $44^\circ\text{C}$  (after  $183 \pm 9$  min of filtration) and decreased with shear. However, the concentration distribution profile did not evolve with layer thickness. A maximal concentration is observed for filtration performed at  $25^\circ\text{C}$ . Irreversibility is similar for all the temperatures since a  $3.5 C_0$  (around  $91 \text{ g.L}^{-1}$ ) concentrated layer close to the membrane is lasting after pressure relaxation.

Table 2. Evolution of concentration at  $z = 30\mu\text{m}$  from the membrane and thickness of total layer ( $e$ ) after filtration for different temperatures.

T ( $^\circ\text{C}$ )	$t_{\text{filtration}}$ (min)	$C/C_0$ (at $z=30\mu\text{m}$ )	$e$ ( $\mu\text{m}$ )
12	180	$3.5 \pm 0,2$	50-100
25	194	$7.4 \pm 0,2$	100-150
44	175	$3.5 \pm 0,2$	150-200

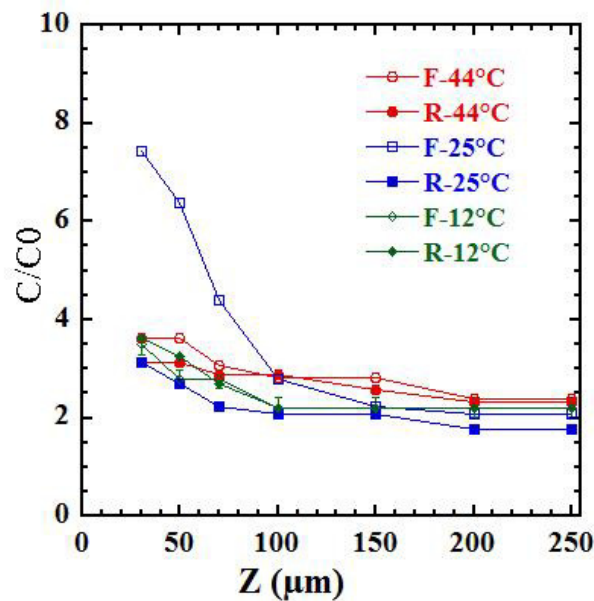


Figure 6. Evolution of concentration profiles within the accumulated layer over temperature during casein micelle cross-flow ultrafiltration ( $C = 2C_0$ ,  $\text{pH} = 6.7$ ). F: Filtration step, R: Relaxation step.

### CONCLUSIONS

*In-situ* SAXS measurements during cross-flow ultrafiltration of casein micelles allowed to follow the formation and reversibility of concentration polarization layers as a function of time, TMP, cross-flow and temperature conditions. It allows to follow sol-gel transition of casein micelles near the membrane surface and to distinguish which part was reversible and eliminated by pressure relaxation. Those results are important for understanding the mechanisms of concentration during cross-flow filtration and they could be used to adjust the filtration parameters in order to avoid the formation of an irreversible concentrated fouling layer at the membrane surface.

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