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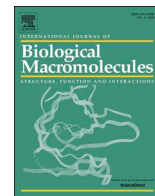
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## Exploring the formation of surficial whey protein deposits under shear stress by rheofluidic approach

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### ABSTRACT

Understanding how shear affects whey protein stability is crucial to deal with typical industrial issues occurring at the bulk solution/surface interface, such as fouling during heat treatments. However, at the state of the art, this effect remains unclear, contrary to that of temperature. This article presents a novel strategy to study the impact of shear rate and concentration on the accumulation of whey protein surficial deposits. It consists in applying a range of shear rates (0–200 s<sup>-1</sup>) at controlled temperature (65 °C) on whey protein solutions (5–10 wt %) by a parallel plate rheometer equipped with a glass disc, thus allowing the off-line characterization of the deposits by microscopy. Our results highlight an unequivocal effect of increasing shear stress. At 5 wt%, it fosters the formation of primary deposits ( $\approx 10 \mu\text{m}$ ), whereas at 10 wt% it results in the development of complex branched structures ( $\approx 50 \mu\text{m}$ ) especially for shear rates ranging from 140 s<sup>-1</sup> to 200 s<sup>-1</sup>. Based on the classification by size of the observed populations, we discuss possible hypotheses for the deposit growth kinetics, involving the interplay of different physico-chemical protein-surface interactions and paving the way to future further investigations.

### 1. Introduction

The stability of globular proteins can be challenged by various environmental factors, which result in their unfolding and aggregation. For example, temperature-induced unfolding is a well-known phenomenon, whose kinetics have been modeled by many studies available in the literature [26,33]. In contrast, the sensitivity of globular proteins to mechanic solicitation is still a matter of debate. [39] have reviewed the effects of shear on proteins in solution and concluded that shear itself rarely causes the instability of proteins but interfacial effects are to be considered. Jaspé & Hagen [23] have come to stronger conclusions, pointing out that even extremely high shear rates ( $\approx 10^7 \text{ s}^{-1}$ ) do not significantly destabilize the structure of globular proteins flowing through narrow channels. However, numerous papers [5,14,21,28] have shown that shear and extensional forces enhance protein denaturation and aggregation, leading for example to the formation of fibrils (e.g., amyloids) in solution. These works stressed out the crucial role of the exposure time, in addition to the shear intensity, on reversible or

permanent configurational changes resulting in protein unfolding [4,6].

Thermal sensitivity makes no exception for whey proteins (WPs), globular proteins issued from the whey, i.e., the by-product of cheese or rennet/acid casein manufacture. WPs represent 20 % of cow milk proteins and  $\beta$ -lactoglobulin ( $\beta$ -Lg) and  $\alpha$ -lactalbumin are quantitatively the main WPs.  $\beta$ -Lg are highly sensitive to their environment, including pH and ionic forces [11,30]. At neutral pH, these proteins have a denaturation temperature ( $T_d$ ) of 70 °C [12,35]. The denaturation is first initiated by the native proteins unfolding during heating, then followed by the aggregation of the unfolded proteins via intermolecular disulphide bonds and hydrophobic interactions [20,22,42].

Although the heating effect on WPs has been extensively studied in the literature, the impact of the shear on WP denaturation is far from being fully elucidated. Little of the literature focuses on the “bulk” solution, with an exception [2] investigating the events occurring at the solid-liquid interface. Controlled shear stresses were generally applied using rheometers equipped with Couette geometry [8,9,15,16,29,31] and microfluidics [40] and the characterization of the samples consisted

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in either the determination of degree of denaturation by chromatography and the size measurements of aggregates by dynamic light scattering. Available papers have studied WP aggregation at temperatures higher than  $T_d$ . Under such experimental conditions, the thermal unfolding takes over, making it difficult to discriminate the shear influence [9,25,34,41]. As a result, the conclusions of these works are not consensual. On one hand, some reported no effect of shear [40]. On the other hand, others have shown an impact of the shear rate on aggregate size and structure, underlining its strong dependence on WP concentration [3,15,36,37,43].

Therefore, unravelling the effect of shear on WP denaturation, alone or combined with other parameters such as temperature and protein concentration, remains an open question. In particular, understanding the mechanisms of interaction and deposit at the surface is necessary for controlling fouling phenomena at larger scales. The progressive growth of WP layers is massively observed in heat exchangers or falling film evaporators in the dairy industry and it has been usually linked to temperature-induced protein aggregation [7,12,19,44-46]. However, in the latter, where the gradual concentration of dairy solutions is obtained by different stages of evaporation under vacuum at fixed temperature, the fouling occurs even at temperatures lower than that of denaturation of WPs ( $\approx 40$ – $50$  °C) ([10]; [18]; [32]). Therefore, it is likely that factors other than temperature, such as shear stress and protein content in the solutions, influence the formation of WP deposits.

The aim of this study is thus to explore this hypothesis through a novel experimental approach conceived to analyze protein deposit accumulation on a surface under shear stress. Unlike previous works, here i) a range of shear stresses is simultaneously applied on WP solutions with different concentration using a rheometer equipped with a parallel plates geometry and ii) the temperature of the system is kept lower than  $T_d$ . The impact of increasing shear rates is then evaluated by characterizing the number, the shape and the size of the surficial deposits, developed on the same rheometer plate by microscopy. The suitability of this approach to investigate the effect of both the shear rate and the protein concentration on the mechanisms of surficial deposit formation is discussed through a case study, *i.e.*, the fouling of whey proteins during concentration by vacuum evaporation, with parameters, *i.e.*, temperature (65 °C), range of shear rate (0–200  $s^{-1}$ ) and concentrations (5–10 wt%), typically observed in falling film evaporators.

## 2. Materials and methods

### 2.1. Preparation of whey protein solutions

Suspensions at 5 wt% and 10 wt% total WPs concentrations were

prepared by dissolving commercial WP isolate (WPI) powders (Pronatix 95, Lactalis, France) in deionized water containing 0.02 wt% of sodium azide ( $NaN_3$ ) as a bacteriostatic agent. The pH of these solutions was measured by a pH-meter (Metrohm 744, Metrohm, Switzerland) and was  $6.56 \pm 0.02$  ( $n = 3$ ). WPI powders exhibited 89 % protein content. The solutions were stirred at 20 °C for 12 h and then stored at 4 °C until use within 7 days.

### 2.2. Shear treatment by rheometry

The impact of the shear on the formation of WP deposits was investigated using a rheometer (MCR 301, Anton Paar, Austria) equipped with a parallel plate geometry (diameter,  $D = 50$  mm, gap size,  $e = 1$  mm) and a Peltier temperature controller. A transparent glass disc was used as bottom plate to facilitate the microscopic observations offline (see the schematic representation in Fig. 1). The experiments consisted in shearing the WPI suspensions for 10 min at 65 °C. The parallel plate configuration enabled the application of a shear gradient on the samples, ranging from 0 to 200  $s^{-1}$  as a direct function of the distance from the center of the glass slide ( $r$ ). Both the intensity and the duration of the shearing treatment were chosen to mimic the flow conditions typical of falling film evaporators [1,38]. Three tests were performed for each concentration. At the end of the shear treatment, the glass slides were rinsed with wash bottle of deionized water for about 30 s to remove the supernatant. The glass slides were then dried in an oven at 40 °C before further analysis.

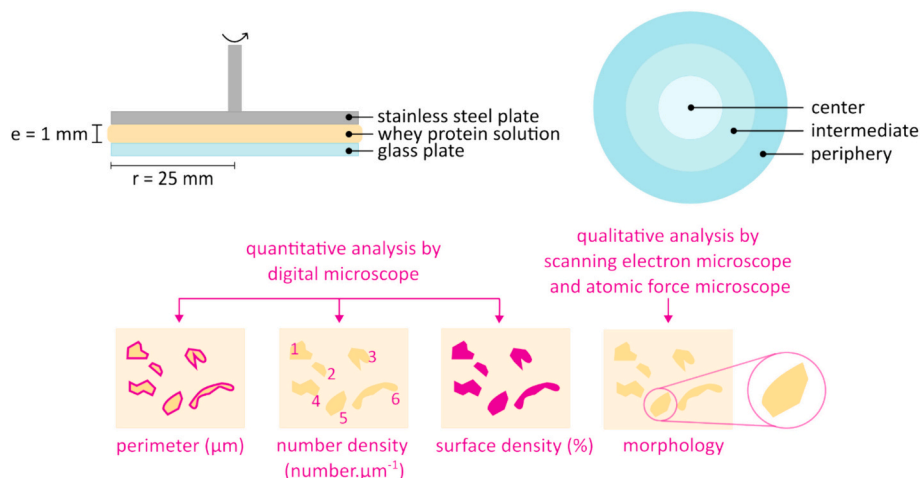
### 2.3. Microscopic analysis of the samples

#### 2.3.1. Quantitative analysis of WP deposit density and structure by digital microscopy

The surface of the glass plates was scanned using a Keyence digital microscope (VHX 7000 N, Keyence, Japan) and the deposit number and shape were characterized as a function of the shear intensity. The surface of the disc was divided into three zones, *i.e.*, center, intermediary

**Table 1**  
Definition of three zones of a 25 mm radius glass plate according to the different applied shear ranges in a rheometer equipped with parallel plate geometry.

	R (mm)	$\dot{\gamma}$ ( $s^{-1}$ )
Center	0–10	0–80
Intermediary	10–17.5	80–140
Periphery	17.5–25	140–200



**Fig. 1.** Schematic representation of the experimental strategy adopted to investigate the impact of shear on the accumulation of whey protein deposits on a glass surface by rheometry.

and periphery, corresponding to three shear ranges (Table 1, Fig. 1). Ten images were acquired per zone at a magnification of 500 $\times$ , and successively analyzed by a custom image analysis software (Serie CV-x software). Three indicators were chosen to quantitatively characterize the WP deposits on the surfaces: i) the number density (Eq. 1) expressed in number. $10^{-3} \mu\text{m}^{-2}$ , to evaluate the frequency of structure formation normalized by the glass surface, ii) the perimeter, expressed in  $\mu\text{m}$ , to estimate the mean size, and iii) the surface density (Eq. 2, dimensionless), defined as the ratio between the area occupied by deposits and the picture area.

$$\text{Number density} = \frac{\sum \text{Deposit number}}{\text{Picture area}} \quad (1)$$

$$\text{Surface density} = \frac{\sum \text{Deposit area}}{\text{Picture area}} \quad (2)$$

### 2.3.2. Observation and analysis of morphology of aggregates by scanning electron microscopy

Scanning electron microscopy (SEM) was used to provide more details on the evolution of the WP deposit structure with the applied shear rate. The glass plates first underwent a gold-palladium coating (thickness  $\approx 3 \text{ nm}$ ) using a Leica EM ACE200 sputtering system for  $\approx 30 \text{ min}$  in a vacuum-prepared chamber. The samples were then observed by detecting the secondary electrons with a field emission SEM (JEOL, JSM 7100F, Japan) at an accelerating voltage of 10 KV, under high vacuum and working distance of 10 mm.

The aggregates were classified depending on their size for both 5 wt% and 10 wt%. The main goal was to obtain a more complete overview of the deposits, from global morphology to structural details at the microscale.

### 2.3.3. Observation of aggregates by atomic force microscopy

WP deposits from 10 wt% samples were characterized by atomic force microscope (AFM) in resonant intermittent contact-tapping mode with a D3100 microscope (Bruker, USA). After shear treatment, the glass plate was fixed on a sample chuck and then explored with a probe (NCHV-A, Bruker) tuned at a frequency of 320 kHz. A laser pointed at the probe was reflected towards sensors favoring the translation of the changes in laser wavelength into an image. Images were processed with Gwyddion software. The AFM investigation allowed us to obtain information on the 3D characteristics of the deposits.

### 2.3.4. Statistical analysis

The dataset was composed of 3 quantitative variables (number density, perimeter, surface density) and 180 individuals (10 pictures for each zone on each sample, conducted in triplicate). The ‘‘Linear model’’ function was used to fit a linear model [27]. A one-way analysis of variance (ANOVA) was carried out using R (The R Foundation, 2014) and a Fisher’s exact test was performed with a significance level set at  $p < 0.05$ . Then, meansComp test was carried out.

## 3. Results

### 3.1. Impact of shear rate on the number and size of whey protein deposits at the surface

Representative images of the scanned surfaces are displayed in Fig. 2 for each shear range and WPI concentration to evaluate the impact of the shear intensity on the development of surficial deposits. At low concentration (5 wt%), increasing  $\dot{\gamma}$  resulted in a higher number of deposits, whose size (mainly  $\leq 10 \mu\text{m}$ ) and structure remained, however, comparable, net of the technical limitations of digital microscopy at this magnification. At higher WP content (10 wt%), the effect of the shearing treatment rather resulted in the gradual formation of larger structures (up to 200–300  $\mu\text{m}$ ), exhibiting a branched complex shape, especially in the intermediary and the periphery zones of the discs.

To corroborate this qualitative overview, three quantitative indicators (2.3.1) were systematically determined for each shear zone (Fig. 3). The results of ANOVA and Fisher’s test confirmed a significant effect of shear rate on number density, perimeter and surface density ( $p = 3.45 \cdot 10^{-7}$ ,  $2.47 \cdot 10^{-3}$ , and  $2.37 \cdot 10^{-8}$ , respectively) and that of concentration ( $p = 1.52 \cdot 10^{-2}$ ,  $1.33 \cdot 10^{-12}$  and  $6.32 \cdot 10^{-9}$ , respectively), without any effect of their interaction. The number density (Fig. 3A) allowed us to confirm that, for 5 wt%, increasing shear intensity fostered the development of a higher number of deposits on the glass surfaces ( $\approx 5$  times higher in average for 140–200  $\text{s}^{-1}$  compared to the 0–80  $\text{s}^{-1}$ ). It should be noted that the average values in such dilute conditions were characterized by large standard deviations, especially at higher  $\dot{\gamma}$ . This finds an explanation in the non-uniform distribution of WPI structures on the plates, which exhibited regions with sparse deposits and others with significant accumulation (Fig. S1). This increasing trend in the number of deposits with increasing shear rate observed at 5 wt% was nuanced at 10 wt% (Fig. 3A). Indeed, stronger shear rates rather resulted in the simultaneous increase in the size and the morphological complexification of deposits, as highlighted by the increase in their perimeter (Fig. 3B). The wide standard deviation registered for  $\dot{\gamma} = 140\text{--}200$

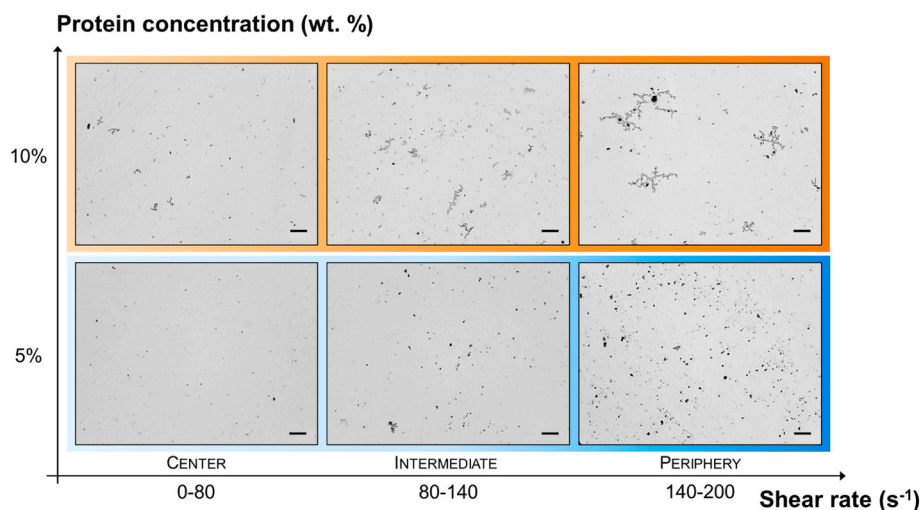


Fig. 2. Qualitative observation by digital microscopy of the impact of shear rate on the number and the morphology of whey protein surficial deposits (scale bars = 50  $\mu\text{m}$ ).

$s^{-1}$  at 10 wt% was due to the simultaneous presence of two main populations differing by size, *i.e.*, small compact structures ( $\leq 10 \mu\text{m}$ ) and growing branched ones ( $\geq 50 \mu\text{m}$ ). This is visually noticeable in Fig. 2 and it is also quantitatively represented by the deposit size distribution in Fig. 4. For 5 wt%, the deposits exhibited comparable size regardless of the applied shear rate, thus suggesting a weak impact on WP aggregation and anchoring to the surface in dilute conditions.

Lastly, the evaluation of the surface density (Fig. 3C) provided a more global picture since this parameter considers not only the appearance of WP structures on the glass surfaces but also the evolution of their size under increasing shearing conditions. All in all, the portion of plate surface occupied by the WP deposits grew as a function of the shear strength for both concentrations. The comparison test showed that for each concentration, the response belongs to a significantly different group as the shear range is increased. The surface density takes into account both the deposit number and size. Consequently, standard deviations of the surface density are amplified because they are the results of those of the deposit number and size. It is worth pointing out that the outcomes summarized here refer to ranges of shear solicitations rather than a fixed one, *i.e.*, different dynamics of protein unfolding and aggregation within the same regions.

### 3.2. Impact of shear rate on the morphology of whey protein surface deposits

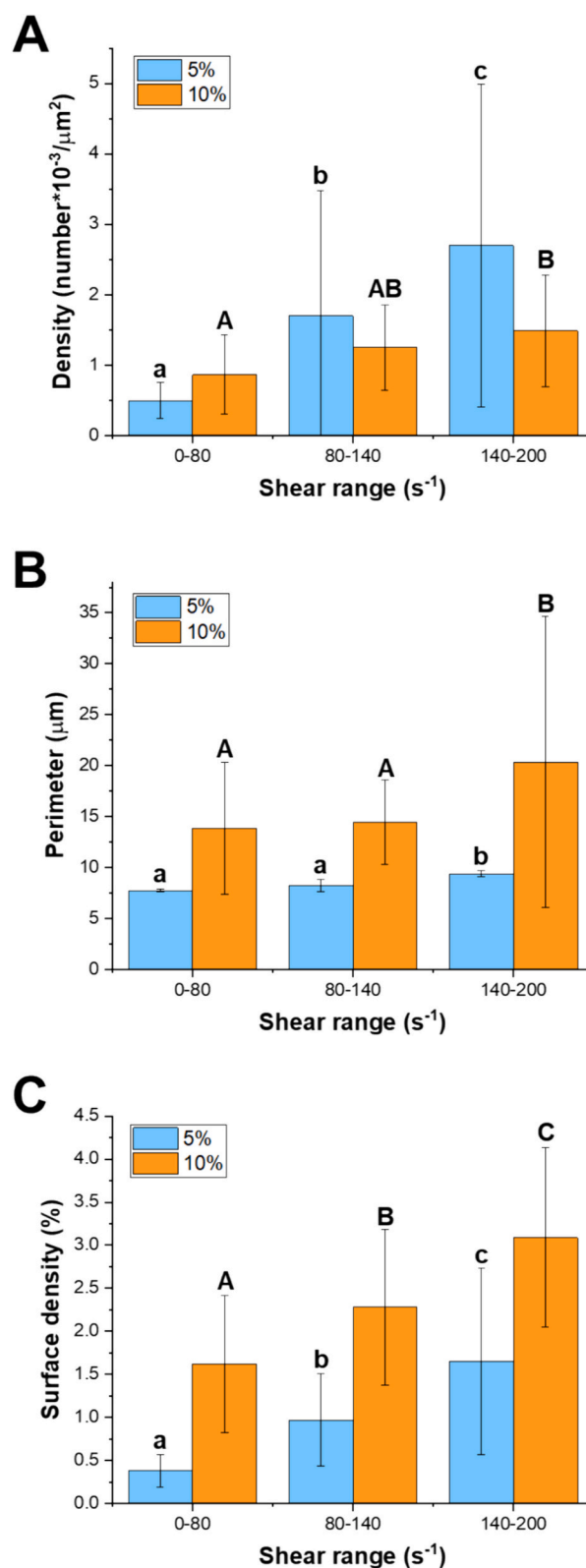
The investigation by digital microscopy favored the systematic analysis of WPI surface deposit number and size under the effect of shear. However, this experimental approach did not make it possible to provide a proper morphological characterization due to the small size of the aggregates, nor to detect the eventual presence of submicronic WP structures. For this reason, the glass plates were also explored by SEM after the shear treatment. Using the average size as main distinctive parameter and for all the shear ranges investigated, three populations of deposits were observed, to different extent, and classified as follows:

- I. Compact aggregates measuring a few hundred nanometers (Fig. 5A) composed of multiple granular subunits;
- II. Deposits (Fig. 5B) of approximately  $10 \mu\text{m}$  that do not exhibit a compact shape but rather a reticulate organization, contrary to what was hypothesized after the digital microscopy investigation. Granular subunits were still distinguishable, thus suggesting a possible building block development of the WP structures;
- III. Branched structures (Fig. 5C) larger than  $50 \mu\text{m}$ , in agreement with what was illustrated in Fig. 2.

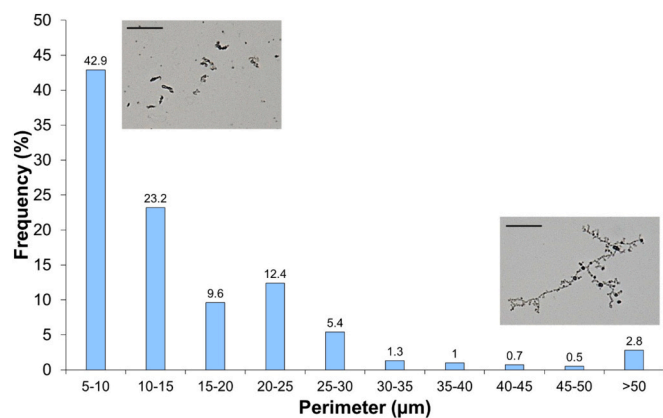
The eventual changes in the morphological characteristics of the deposits as a function of  $\dot{\gamma}$  were investigated in the three defined plate regions. The globular shape of the small WP aggregates ( $\leq 1 \mu\text{m}$ ) was not significantly affected by the shear strength (Fig. 5A). Similar results were obtained for the deposits of intermediate size ( $\approx 10 \mu\text{m}$ ) (Fig. 5B), whose rich variety of irregular morphologies complicated the determination of a consistent trend according to  $\dot{\gamma}$ . The only obvious shape modification induced by the increasing shear solicitation was registered for the large, branched structures ( $\geq 50 \mu\text{m}$ ). As shown in Fig. 5C, a squat appearance with short branches was observed for  $\dot{\gamma} = 0\text{--}80 s^{-1}$ , whereas the WP deposits displayed a thinner morphology characterized by more and complex ramifications when moving towards the periphery of the glass plates. Moreover, the observation by SEM highlighted that the structures at higher shear ( $\dot{\gamma} = 140\text{--}200 s^{-1}$ ) were often fractured in certain areas, a detail not visible by digital microscopy.

## 4. Discussion

The observation of deposits with significantly different size and morphology in the same shear range reveals that, when the temperature of the system is lower than  $T_d$ , the WP surficial accumulation depends on



**Fig. 3.** Characterization of whey protein deposits at 5 wt% (blue) and 10 wt% (orange) at increasing shear rates by three quantitative indicators: (A) the number density to evaluate the frequency of structure formation normalized by the explored surface; (B) the perimeter to estimate the mean size and (C) the surface density defined as the ratio between the area occupied by deposits and the picture area. Same letters within a concentration indicate no significant difference ( $p < 0.05$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** Size distribution of surficial whey protein deposits of 10 wt% solutions for a shear range of 140–200 s<sup>-1</sup>. In the insets, pictures of the two observed morphological categories are displayed: i) primary deposits ( $\leq 15 \mu\text{m}$ ) and ii) branched structures ( $\geq 50 \mu\text{m}$ ). Scale bar equal to 50  $\mu\text{m}$ .

the complex interplay of various factors rather than on a single predominant one. The impact of the shear stress itself, which is the main parameter investigated in this article, is strongly affected by the protein content in the solutions. Consequently, predicting the growth kinetics of WP deposits under shear remains a challenging open question. In this regard, deciphering the nature (*e.g.*, physical, chemical) of the protein-protein and protein-surface interactions characterizing their occurrence and propagation would represent a crucial step. To this end, the evolution of the three different categories of deposit morphologies observed under shear allows us to discuss on plausible hypotheses in the light of the current literature.

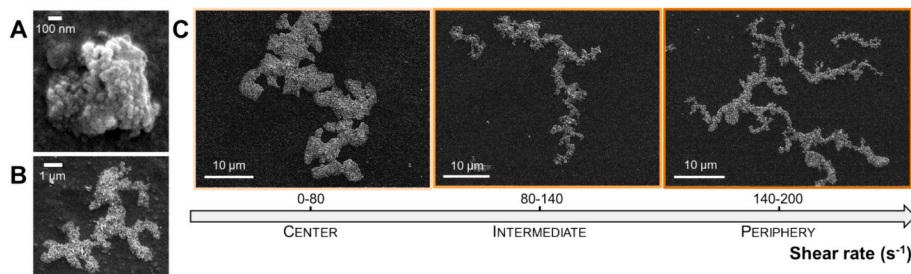
The presence of compact WP aggregates of few hundred nanometers (Fig. 5A) dispersed all over the explored surface has been already reported in a recent work on the study of milk fouling on stainless steel surfaces [2]. The thermally induced structural modifications of WP molecules favored their aggregation and possibly their adsorption onto the surface within few minutes. The authors did not consider the shear stress as a possible parameter influencing WP activation and adsorption, although the experiments were carried out under flow. Nevertheless, according to the work of Dunstan et al. [14] on protein unfolding under shear,  $\dot{\gamma}$  and shearing time strongly influence the development of stable submicronic fibrils in  $\beta$ -lg solutions during the process of amyloid formation, at pH = 2 and  $T = 80^\circ\text{C}$ . In our study, the bunch-like shape of the submicronic aggregates observed by SEM (Fig. 5A-6A) corroborates the hypothesis of active WP aggregates chemically bonded to the surface, although it does not clarify the eventual impact of the shear strength during this early stage of deposit accumulation on the surface. These clusters make potential initiation points for the growth of protein deposits. Their development would be fostered by the adsorption of further unfolded molecules approaching the surface and leading to the

formation of primary deposits of few microns ( $\leq 10 \mu\text{m}$ ). Since submicronic WP aggregates are still detectable after 10 min of shear treatment, it is evident there are limiting factors inhibiting reaction kinetics. Indeed, WP aggregates keep being adsorbed at the surface until the late stage of the shear process, resulting in a different reaction time and the heterogeneity of the deposits. On the other hand, protein-protein and protein-surface interactions are likely influenced by spatial reasons, *i.e.*, the initiation points can merge into primary deposits only if their distance ( $\delta$ ) is below a critical one ( $\delta < \delta_i$ ) (Fig. 6A). However, our experimental approach does not allow discrimination of such time and spatial effects, which would be better highlighted by the direct observation of protein accumulation.

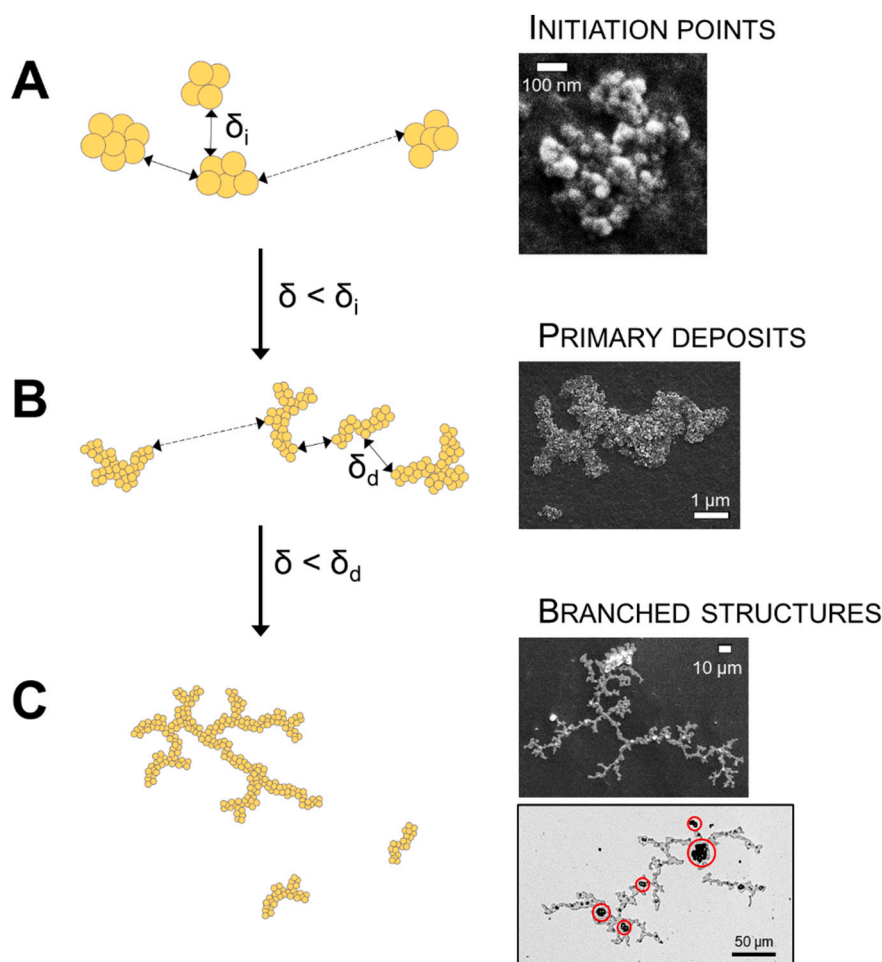
Regarding the role of  $\dot{\gamma}$  on the further stage of deposit development, a different impact was detected for the two concentrations. As underlined in Fig. 3A and B, at 5 wt%, the number of primary deposits increased without significant evolution of their size and morphology. In other words, high shear rates would enhance the molecular diffusion and, thus, the collisions with the surface. At 10 wt%, the higher number of macromolecules in the solution increased the probability of collisions between active aggregates and the glass plate. This did not lead merely to the expected increase in the primary deposit density at the solid-liquid interface, but rather for same shearing conditions to their interconnection and the development of branched structures depending on their interdistance ( $\delta < \delta_d$ ) (Fig. 6B). To corroborate this hypothesis, the investigation by digital microscope revealed that such morphologically complex deposits exhibit regions with heterogeneous thickness, as underlined by the different shades of gray in Fig. 6C (black frame). The primary deposits, *i.e.*, dark gray objects in the red circles, are here easily distinguishable and connected by light gray arborescent branches.

The morphology (Fig. 7A) and thickness (Fig. 7B) of these WP branched structures were investigated by AFM. Fig. 7A underlines the complex 3D shape of the deposits and their granularity, particularly in the more densely packed regions. Mostly, Fig. 7B provides a quantification of the variation in deposit height from bright clusters with micronic thickness to thinner darker branches ( $\approx$  few hundred nanometers), consistent with the hypothesis of a network of interconnected primary deposits. As already underlined for the initiation points, the quantification of a critical distance ( $\delta_d$ ) influencing deposit connection and complexification, although strongly suggested by the observation of scattered primary deposits far from the branched structures, requires an online approach (*e.g.*, microfluidics or rheoscopy). Nota bene, a different subscript has been adopted for the critical distances related to initiation points ( $\delta_i$ ) and primary deposits ( $\delta_d$ ), since they refer to objects with significantly different size.

The outcomes from the microscopic observations suggest that the deposit propagation into complex reticulates could involve interactions other than chemical ones. On the one hand, the shearing time and intensity would maintain the WP surficial deposits chemically active and favor their ramification, by analogy with amyloid formation [6]. On the other hand, the formation of networks of primary deposits and thin branches are clues of the development of shear-dependent short-range



**Fig. 5.** Qualitative classification by size of whey protein deposits after observation by scanning electron microscopy: (A) compact aggregates with granular structure ( $< 1 \mu\text{m}$ ); (B) primary deposits ( $\approx 10 \mu\text{m}$ ); (C) branched structures ( $\geq 50 \mu\text{m}$ ). The latter, whose morphology evolved with increasing shear rate, were observed only after shear treatment of 10 wt% whey protein isolate suspensions.



**Fig. 6.** Schematic representation of the three stages proposed for the whey protein deposit growth reaction. For each stage, representative images of the corresponding deposit morphology, acquired from the observation of 10 wt% samples by scanning electron microscopy and digital microscopy, are shown.

physical interactions, similar to what observed in colloidal gels [17]. This would explain, for example, the morphological evolution of the branched structures showed in Fig. 5C. These considerations pave the way to next studies aiming at unravelling the mechanisms of protein-protein and protein-surface interactions leading to WP surficial accumulation. In this light, Raman [24] or vibrational spectroscopy [13] could represent a powerful tool for a quantitative characterization.

## 5. Conclusions

This paper presents a novel methodology to study the influence of shear on the accumulation of protein deposits on a surface. Its originality lies in three aspects: i) possibility of exploring a wide range of shear rates in a single experiment; ii) reproducible control of operating conditions such as shear and time and iii) facilitated systematic observation of deposits using a glass disc.

The feasibility and utility of this methodology were demonstrated on soluble WPs at two different concentrations (5 and 10 wt%) across a shear range of 0–200  $s^{-1}$ . The quantitative analysis of deposits observed by digital microscopy and their qualitative analysis by SEM and AFM made it possible to better investigate the deposition and the growth of proteins on the surface. At 5 wt%, increasing shear rate resulted in a higher number of deposits without significant change in their size. At 10 wt%, increasing shear led to different types and sizes of deposits, including the formation of finer and more complex branched structures. According to these observations, we suggest three stages of deposit development, involving the formation of: (1) initiation points (few

hundred nm) chemically bonded to the surface, (2) primary deposits ( $\approx 10 \mu m$ ) formed from the bonding of these initiation points, and (3) thicker branched structures ( $\approx 50 \mu m$ ) resulting from interconnection of primary deposits. These latter seem to involve other interactions than chemical ones, much influenced by increasing shear. This work lays foundation for future investigations to elucidate the kinetics of deposit development by comparing WP stability in the bulk solution and on the surface, exploring higher concentrations and temperatures and varying other factors such as shear exposure time.

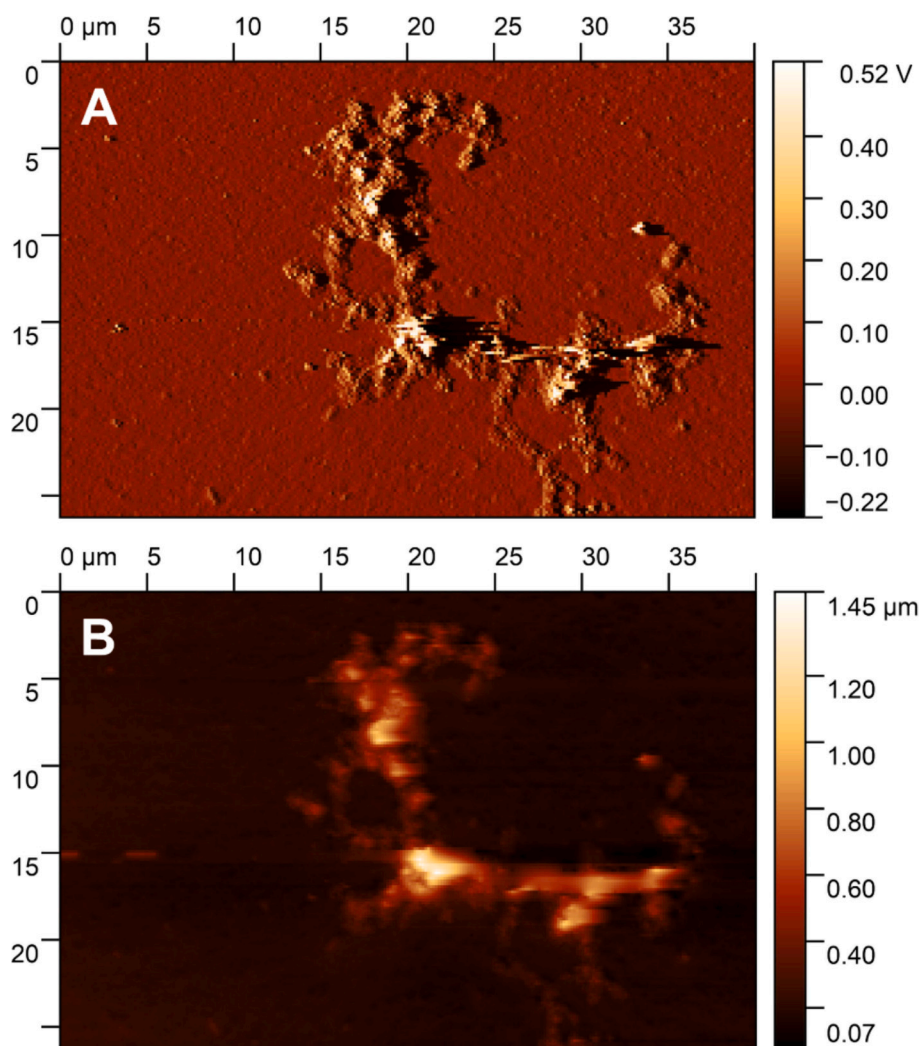
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## CRediT authorship contribution statement

**Margot Grostete:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Jeehyun Lee:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Zanele Msibi:** Investigation. **Françoise Boissel:** Investigation. **Maude Jimenez:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Romain Jeantet:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition,



**Fig. 7.** Observation by atomic force microscopy of a typical branched structure of whey proteins formed under intermediate shear rates (80–140 s<sup>-1</sup>). (A) Amplitude picture (B) Topography picture.

**Conceptualization.** Luca Lanotte: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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