

## THE SCIENCE OF CLEANING OF DAIRY FOULING LAYERS

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### ABSTRACT

The science underlying the removal of dairy fouling layers, and particularly the dissolution of proteinaceous deposits in alkaline solution, is relatively poorly understood even though this is a critical feature of many cleaning-in-place operations. We report key results from a series of investigations on heat-induced gels of  $\beta$ -lactoglobulin, the primary whey protein component in milk and whey foulant. These model systems were used to elucidate the reaction behaviour of gels and aggregates whereby the proteinaceous material is converted to a softer, swollen form that can then be removed by fluid shear or diffusion. We show that several features, such as the occurrence of an optimal pH for cleaning, can be related by analogy to the behaviour of synthetic polyelectrolyte polymers. The structure and history of the foulant, pH, ionic strength and salt concentration in the cleaning solution are all shown to be important factors in the chemistry of inter- and intra-molecular interactions explaining why it has been difficult to generalise about the mechanisms involved and to write simple models of their kinetics.

### INTRODUCTION

The processes of fouling (formation) and cleaning (removal) of deposits from process surfaces are intimately linked, as each sets the initial condition for the other (Wilson, 2005). Heat transfer equipment subject to fouling is frequently returned to service following cleaning. The requirements of both operating (*i.e.* fouling) and cleaning cycles have to be considered at the design stage, *e.g.* the material of construction has to be compatible with the process and cleaning streams. Furthermore, the rate of each process is determined by the history of the previous stage; an aged deposit usually proves harder to clean.

Regular fouling and cleaning is a longstanding and problematic feature of dairy heat exchangers such as sterilisers, as the operating conditions required by the process promote fouling (Changani *et al.*, 1997) and the scope for mitigation by chemical treatment and process modification are limited. At lower temperatures ( $< 110^\circ\text{C}$ ) milk and whey stream fouling is dominated by the formation of soft proteinaceous layers. At higher temperatures inverse solubility salts, particularly calcium phosphates, dominate (Burton, 1968), yielding harder deposits. Most process plants respond to exchanger fouling by regular cleaning-in-place (CIP) using alkali-based cleaning solutions for proteinaceous foulants, and acid solutions for deposits containing high levels of mineral scale. The scheduling of cleaning can be

determined by process inefficiency or microbiological criteria, depending on the application.

Physical cleaning methods could be used to remove these deposits but are not used regularly in the food sector as these usually require the equipment to be opened in order for the tool (*e.g.* a high pressure water lance) to access the fouled surface, with a high risk of microbiological contamination, compromising hygienic operation. Chemical cleaning methods are therefore favoured: the cleaning solution serves to convert the foulant to a softer, swollen form which can then be removed by fluid shear or simply by diffusion into the flowing liquid. The dominant cleaning agent in dairy applications is sodium hydroxide, with detergents, sequestering agents or anti-corrosion agents added to improve its efficacy. The action of NaOH is illustrated in Figure 1, which shows the change in thickness of a whey protein fouling layer on exposure to a 0.5 wt% solution in a laboratory apparatus simulating CIP.

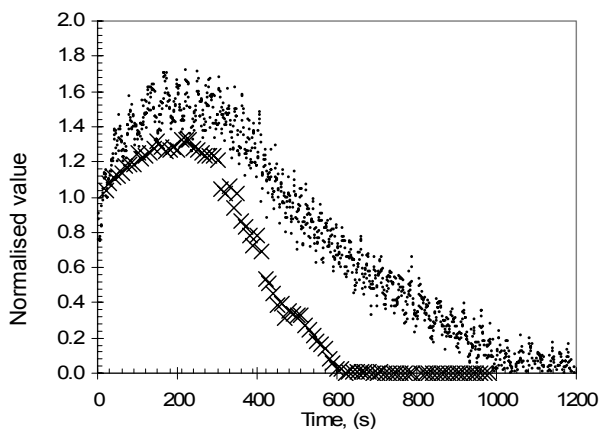


Figure 1. Thickness ( $\times$ ) and thermal resistance  $R_d$  (points) of whey protein fouling deposit exposed to 0.5 wt% NaOH at  $30^\circ\text{C}$  and  $Re \sim 3000$ . Data normalised against value at  $t = 0$  s. After Tuladhar *et al.* (2002)

Data obtained using two measurement techniques are presented: an external measure of thermal resistance,  $R_d$ , obtained using heat flux sensors; and a 'direct' measurement of the thickness of the soft layer obtained using fluid dynamic gauging (Tuladhar *et al.*, 2000): this technique was developed in order to allow such soft, liquid-saturated layers to be studied *in situ* and in real time. The layer thickness increases, *i.e.* swells, on exposure to the hydroxide and then decreases as material dissolves at an almost steady rate (which we will term  $R_0$ ),

before decreasing rapidly in a final stage where the swollen material is weak and its removal by chemical/diffusion processes is augmented by shear. The latter aspect is evident from the difference in profiles between the two measurements, where the stress imposed by the flow of the liquid during dynamic gauging erodes the film and causes it to be removed earlier. This critical stress can be quantified using computational fluid dynamics simulations (Chew *et al.*, 2004)

### MECHANISMS

CIP processes therefore employ both chemical and physical processes to remove dairy fouling deposits from the surface. A moving fluid imparts a shear force on a surface and an associated enhancement of heat and mass transfer by convection. We note that in the turbulent regime, turbulent ‘bursts’ of momentarily enhanced activity occur. These ‘physical’ mechanisms are responsible for the initial removal of loosely bound foulant; diffusion of dissolved material held within a foulant matrix; diffusion of soluble material away from the foulant-solution interface (at rate  $R_0$ ), and erosion of the weakened foulant obtained by extended contact with alkali. Cleaning models such as those reviewed by Leclercq-Perlat and Lalande (1991) and that of Xin *et al.* (2004) treat  $R_0$  as being controlled completely by diffusion and convection of disengaged proteins through the film at the foulant/solution interface.

Many CIP systems operate in the turbulent regime with high recirculation velocities, which will increase mass transfer through the liquid but will also promote physical erosion. At high enough velocities, the cleaning rate will become dominated by the rate of generation of weaker or soluble species. The reaction chemistry has been recognised as important for some time but has not been studied in depth: Bird and Fryer demonstrated the existence of an optimal concentration of NaOH (aq) for the cleaning of whey protein heat exchanger deposits, at 0.5 wt%, in 1991 but this has not been explained until relatively recently. Christian *et al.* (2006) studied the effect of applying cleaning chemicals in short campaigns rather than continuous application and demonstrated that the diffusion of hydroxide into the foulant and subsequent reaction played a critical role in determining the rate of cleaning. Moreover, studies using foulants generated under well-controlled conditions such as heat-induced gelation (*e.g.* Mercadé-Prieto and Chen, 2006) have demonstrated that the conditions under which whey proteins form a gel have a significant effect on the steady removal rate  $R_0$ . These observations all indicate that the structure and chemistry of proteinaceous fouling deposits play an important role in their cleaning behaviour, which is consistent with the fouling-cleaning symbiosis alluded to in the introduction.

Two areas of fundamental scientific knowledge are therefore required to elucidate the cleaning behaviour of milk-based deposits:

(i) the reaction behaviour of these fouling layers under alkaline conditions. The dissolution of mineral-based deposits is relatively well understood, and involves dissolution of crystalline material into solution and diffusion through the foulant matrix where the crystals are not at the foulant-solution interface. This topic has attracted attention due to its importance in the dissolution of detergents and controlled-release agents. The reaction behaviour of proteinaceous deposits is considered here.

(ii) the deformation behaviour (rheology) of fouling layers and the material generated on contact with cleaning solutions. Techniques for measuring the forces required to remove such layers, such as micromanipulation and fluid dynamic gauging (Hooper *et al.*, 2006a), have been developed and demonstrate the range of interactions in deposits from adhesive, where the foulant detaches from the surface as a result of weakening of interactions with the substrate, and cohesive, where the interactions within the foulant layer decrease so that material can erode.

Ultimately, one would aspire to link (i) and (ii) by predictive modelling based on knowledge of the microstructure of the deposit. Progress in (i) is hindered by the complexity of the material, and in the range of reactions that can arise between protein, fat, mineral and alkali at the temperatures of interest.

We have therefore chosen to study the behaviour of the whey protein  $\beta$ -lactoglobulin ( $\beta$ -lg) in isolation. This component forms a significant fraction of the protein content in milk and whey protein concentrates (WPCs), and is the major contributor to the formation of proteinaceous foulants in such liquids (Lalande *et al.*, 1985). Proteinaceous dairy fouling layers are complex heat-induced gels and fundamental understanding gained from studying  $\beta$ -lg behaviour in isolation will allow us to build up a framework for understanding the impact of other components such as fat, casein and calcium, and also allows us to compare its behaviour with synthetic polymers where the phenomenon of dissolution has received much more attention: the food sector has concentrated on the *formation* of gels rather than their breakdown.

Whey proteins can be considered to be polyelectrolyte biomacromolecules which differ from synthetic polymers in (i) the size distribution of aggregates (ii) the number and range of ionisable groups on the polymer backbone, (iii) their complex secondary and tertiary structure; and (iv) the variety of possible intermolecular interactions, including covalent disulfide bonds, inter-protein  $\beta$ -sheets and hydrophobic interactions.

This paper presents a summary of the work in this group on this topic. Experiments were performed on  $\beta$ -lg and WPC gels formed by heat-induced or caustic-induced

gelation in capsules rather than on heat transfer surfaces, as the formation conditions could thereby be closely controlled. This approach has been championed by Chen and co-workers (*e.g.* Xin *et al.*, 2004) and its validity for heat-exchanger deposits questioned by Hooper *et al.* (2006b), but its value in elucidating the fundamental behaviour of whey protein gels is unquestioned. The gels were dissolved in alkali under conditions where external mass transfer effects were not rate-limiting so that the rates are determined by internal diffusion and reaction. A detailed description of the experimental techniques and studies is given in Mercadé-Prieto *et al.* (2006).

## SWELLING

The phenomenon of swelling of a polymer layer in contact with a solvent has been studied at length for synthetic polymers, including polyelectrolyte gels (English *et al.*, 1997). Its importance in cleaning is demonstrated in Figure 2, which shows how heat-induced WPC gels swell on contact with NaOH but do not release significant amounts of protein until extensive swelling has occurred. Similar plots have been obtained for  $\beta$ -lg and other WPC gels. Swelling is therefore an important stage in the cleaning process, although its role has not been elucidated to date.

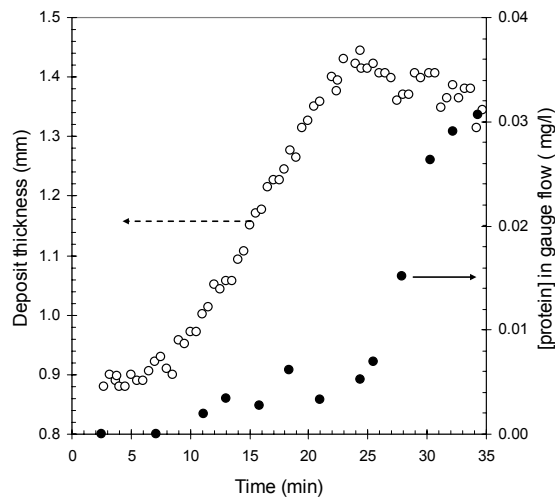


Figure 2. Protein concentration in solution and deposit thickness on contact with quasi-stagnant 0.25 wt% NaOH at 25°C obtained using FDG. Deposit: heat induced WPC80 gel. After Sahoo *et al.* (2007)

Polyelectrolyte polymers swell on contact with alkali because more charged groups on the polymer backbone are ionised at the higher pH, creating electrostatic repulsion. For  $\beta$ -lg, between pH 10-13 the number of (negatively) charged amino acids increases from 15 to 35 out of 162, giving a large fraction of charged residues. The corresponding electrostatic repulsion is, however, short range. Nevertheless, this large increase in charge also promotes the denaturation of the protein (Taulier and Chalikian, 2001) so that it loses its initial globular

(compact) structure, allowing the protein to swell/unfold at the molecular level (van der Leeden *et al.*, 2000). Furthermore, the net negative charge on the biopolymer causes anions from the solution to diffuse into the gel, either to compensate charges or due to a concentration gradient, and this creates an osmotic pressure that draws water into the biopolymer matrix and causes it to swell.

We have been able to model the swelling of  $\beta$ -lg gels quantitatively by adapting models developed for simple synthetic polymers and some of the results are shown in Figure 3. The equilibrium swelling degree,  $Q$ , is the ratio of the swollen volume to the initial volume, obtained gravimetrically. We study the equilibrium swelling degree because it is widely assumed in the dissolution of synthetic polymers that the layer next to the solution will be at equilibrium (Narashimham and Peppas, 1996).

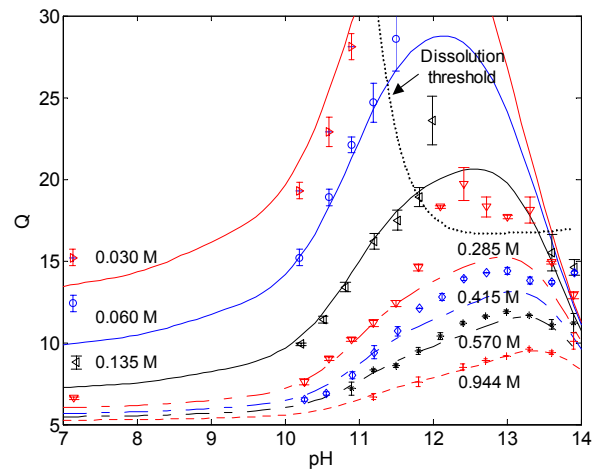


Figure 3. Effect of pH on the equilibrium swelling degree,  $Q$ , for different NaCl concentrations at 25°C. Heat induced  $\beta$ -lg gel. Dashed line shows the observed dissolution threshold: above and to the right of this locus stable equilibrium was not obtained. Solid lines show the predictions of the model reported by Mercadé-Prieto *et al.* (2007a) at different NaCl concentrations.

The figure shows that with little NaCl present the extent of swelling increases with pH noticeably above pH 10 and then decreases at high pH. Above 11.5 or so no data for the equilibrium degree of swelling were obtained because the gel started to dissolve before equilibrium could be reached.

At the higher pH values in Figure 3, however, stable  $Q$  values could be obtained, indicating that dissolution was being suppressed. The Figure also shows that swelling is suppressed by the addition of NaCl, which is due to the sodium ions screening the charges on the protein (English *et al.*, 1997). The addition of NaCl can even prevent dissolution, as demonstrated by the loci for high NaCl concentrations. Addition of salts will therefore

enhance this polyelectrolyte screening effect, reducing the extent of swelling with commensurate negative impact for dissolution and also for the formation of sparse (and weak) layers that can be removed by shear.

These results indicate that high salt levels should be avoided in cleaning solutions. The selection of additives should consider their impact on the ionic strength of the solution, as the reduction in swelling resulting from an increased ionic strength may counter the beneficial effect of the additive.

The effect of salts has been recognized in the literature via, for example, the knowledge of the existence of an optimal NaOH concentration for dairy cleaning applications. Bird and Fryer (1991) reported that the rate of cleaning WPC fouling layers decreased noticeably above 0.5 wt%. Industry would like to increase cleaning rates by using higher concentrations, since increasing the concentration of a reactant usually increases the rate but this is clearly not the case with proteinaceous deposits. Figure 3 gives some explanation: the equilibrium swelling degree is small above pH 13.5, while Figure 4 shows that the associated *swelling* kinetics are very slow. This change in rate is again caused by the polyelectrolyte screening effect of the base cations (English *et al.*, 1997).

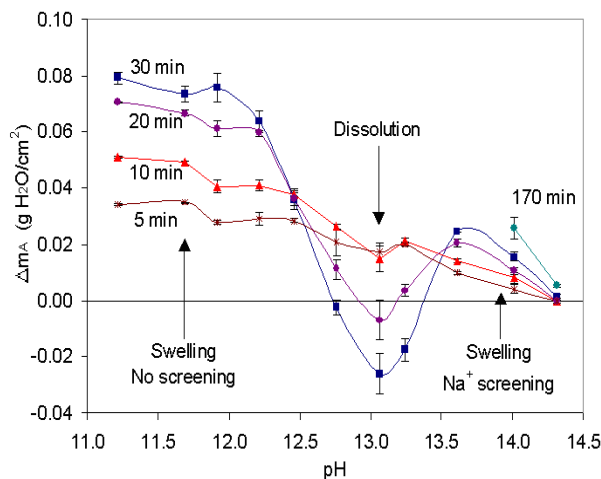


Figure 4. Water uptake per unit gel surface area,  $\Delta m_A$ , at different soaking times and pH at 19°C.  $\beta$ -Ig gels prepared at pH 11.2, 50°C for 20 min, no salt added. After Mercadé-Prieto *et al.* (2007c).

### SWELLING AND DISSOLUTION

The impact of salt addition on the dissolution rate is illustrated by Figure 5. As with swelling, increasing the salt concentration and ionic strength has a profound effect of the dissolution rate of gels. These rates were calculated from on-line measurements of the bulk solution in contact with a gel sample.

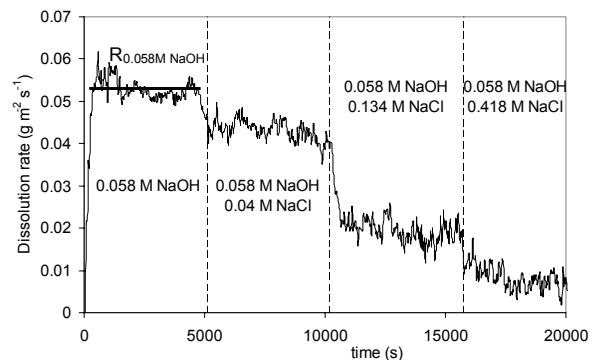


Figure 5. Effect of increasing NaCl concentration on gel dissolution rate at pH 12.76, 24°C. Dashed vertical lines indicate when NaCl was added. Gelation conditions: heat-induced gel (HIG)  $8 \times 10^{-3}$  M  $\beta$ -Ig, pH 7.45, 80°C for 20 min. After Mercadé-Prieto *et al.* (2007c).

Our experiments (Mercadé-Prieto *et al.*, 2007c) indicated that the dissolution rate decreased rapidly at NaOH concentrations above 0.4 M NaOH, and fell to similar values to those obtained by the addition of NaCl (Figure 6). At such high concentrations of base cations, the charges between proteins are completely screened and the protein then behaves as a neutral polymer; with little repulsion between chains the gel will collapse. Similar behavior has been reported for polyelectrolyte polymers at high pH (Mahdavinia *et al.*, 2004; Zhao *et al.*, 2005).

It should be noted that although the kinetics of swelling are faster at higher temperatures, the equilibrium swelling degree is relatively insensitive to temperature (Mercadé-Prieto *et al.*, 2007c) and any mechanism that is controlled by the extent of swelling will not be enhanced by operating at higher temperature. This needs to be considered when designing CIP protocols.

The NaCl concentrations reported above are very unlikely to be encountered in practice, but real foulants do contain significant levels of calcium as calcium phosphate: this divalent ion contributes strongly to ionic strength as  $I \propto z^2$ . Calcium also reduces swelling of  $\beta$ -Ig gels more efficiently than NaCl (Mercadé-Prieto *et al.*, 2007c) because it can bind specifically to the carboxylate groups on the protein, screening these negative charges more effectively (Simons *et al.* 2002).

Figure 6 shows that the strong effect of Ca on swelling is mirrored in the dissolution rate: the addition of 10 mM  $\text{CaCl}_2$  decreases the dissolution rate by 80%. This suggests that the dissolution rate of real whey protein and milk deposits could be significantly limited by the presence of calcium. We are currently investigating the effect of calcium added before gelation on subsequent cleaning.

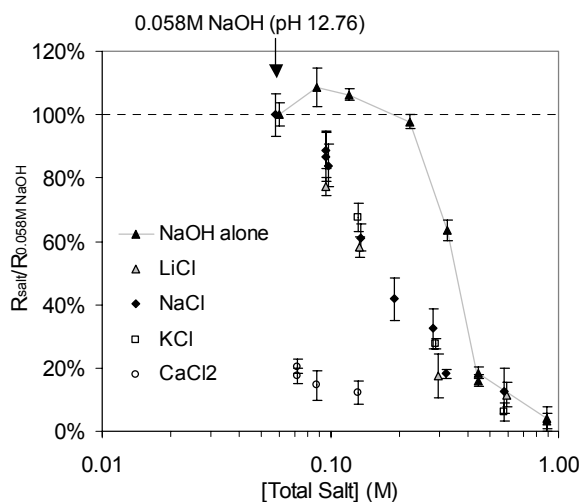


Figure 6. Effect of different salts on the constant dissolution rate at pH 12.76 and 24°C. Gelation conditions as in Fig. 5. Experiments where no salt was added but the NaOH concentration was changed are also shown for comparison, linked by the solid line. After Mercadé-Prieto *et al.* (2007c).

### DESTRUCTION OF PROTEIN GELS AND AGGREGATES IN ALKALI

Proteinaceous gels swell extensively under alkaline conditions but this is not a sufficient condition for chemical cleaning: it may suffice if removal is mainly controlled by erosion of the deposit. For example,  $\beta$ -lg gels exposed to alkali at NaCl concentrations  $< 0.06$  M in Fig. 3 can swell extensively, with  $Q > 20$ , but are stable at  $\text{pH} < 11$ : there is clearly more to cleaning than swelling. Many general statements have been made on why alkali conditions promote cleaning but a coherent explanation has not yet been accepted. We therefore performed a series of investigations to elucidate the basic question, of why alkali can dissolve gels promptly, so that it is a near-universal feature of cleaning formulations.

Consider first the nature of proteinaceous fouling deposits. When protein gels are formed of large protein aggregates, with a range of interactions, including disulfide crosslinks (Shimada and Cheftel, 1988; Verheul *et al.*, 1998), and non-covalent interactions (Bauer *et al.*, 2000; Havea *et al.*, 2004) such as intermolecular  $\beta$ -sheets (Lefevre and Subirade, 2000) and hydrophobic interactions (Shimada and Cheftel, 1988).

Deposits and gels are cleaned by breaking down these large protein aggregates, which in turn requires some or all of these interactions to be interrupted. To determine how alkali destroys protein aggregates, we studied the behaviour of aggregates formed at the same temperature and preparation time as the gels, but at lower protein concentration, so we start with a solution rather than a gel. The size of aggregates and breakdown

fragments released during the cleavage with alkali was monitored using size exclusion chromatography (SEC; Mercadé-Prieto *et al.*, 2007b)). An example of the results obtained is plotted in Figure 7, where the parameter  $f$ , defined thus

$$f = \frac{\text{Absorbance}_{\text{peak}}}{\int \text{Absorbance} \cdot d(\text{Elution volume})} \quad (1)$$

is used to monitor the evolution of the different SEC peaks and particularly that corresponding to the original aggregates ( $>600$  kDa). The data show that at room temperature the aggregates are destroyed very quickly at  $\text{pH} > 12$  but are stable at  $\text{pH} 11.1$ .

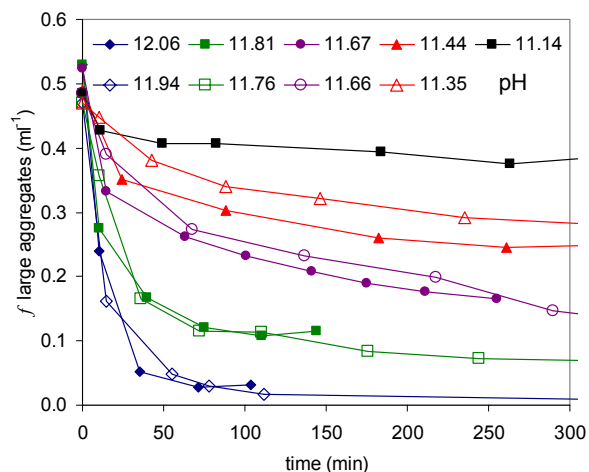


Figure 7. SEC of NaOH solution in contact with  $\beta$ -lg aggregates:  $f$ -values of largest components, calculated using eq. (1). Aggregates formed from 3 mM  $\beta$ -lg at 80°C for 20 min (A80/20m), after incubation in solutions at different pH (indicated on plot). Open symbols are for unbuffered NaOH solution; filled symbols are for 75 mM  $\text{Na}_2\text{HPO}_3$  buffer. After Mercadé-Prieto *et al.* (2007b).

The distribution of size fractions in Figure 8 shows that large aggregates, representative of the matrix in a protein gel, are destroyed only between  $\text{pH} 11.2$ - $12$ : this is accompanied by the formation of small oligomers (monomers, dimers and trimers). A pH threshold therefore exists for the breakdown of the gel.

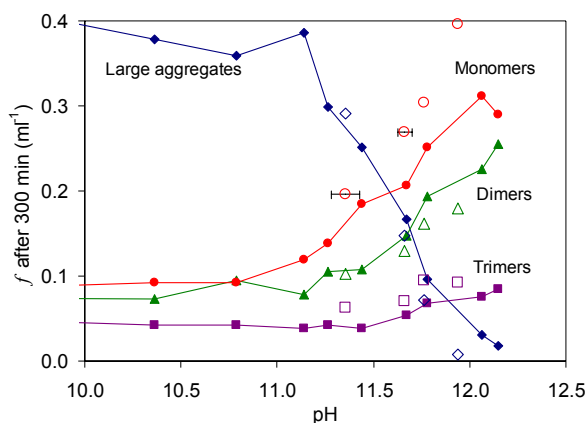


Figure 8.  $f$ -values of different SEC peaks for A80/20m gel incubated in alkaline solutions after 300 min (open symbols - unbuffered NaOH solution; filled symbols, with interpolating lines, -  $\text{Na}_2\text{HPO}_3$  buffers). After Mercadé-Prieto *et al.* (2007b).

The breakdown of aggregates between pH 11.2 and 12 occurs because of the destruction of non-covalent interactions between proteins. This was confirmed by using molecular-level techniques to study interactions between protein chains. Several spectroscopic techniques confirmed that the pH at which  $\beta$ -lg aggregates denatured was shifted about one pH unit above that of native  $\beta$ -lg due to the presence of the interprotein interactions in the aggregates. For example, tryptophan fluorescence spectra such as those in Figure 9 confirmed that the disruption of the major structure in the native  $\beta$ -lg protein, the  $\beta$ -barrel, occurs at a transition pK of  $\sim 10.8$ . A similar transition is observed in  $\beta$ -lg aggregates, starting from a higher wavelength as the protein is slightly unfolded during the heat treatment, but at higher pH (11.6-11.8).

Similarly, circular dichroism indicated that the secondary structure content in aggregates only starts to decrease above pH 11 (Figure 10), whereas for unheated  $\beta$ -lg this transition starts at pH  $\sim 10$ .

The alkaline denaturation of aggregates was found to be hindered by the interprotein interactions, but when these interactions are destroyed between pH 11.2 and 12, as shown by SEC (Fig. 8), the proteins can unfold. For aggregates formed above the denaturation temperature of  $\beta$ -lg (*i.e.* 80°C) and over a short time (*i.e.* 20 min), the interactions destroyed were found to be mainly non-covalent, *i.e.* either intermolecular  $\beta$ -sheets or hydrophobic interactions (our results cannot currently discriminate between the two). Conversely, for aggregates formed over a long period (*e.g.* 24 h), the break down of aggregates was also constrained by the destruction of intermolecular disulfide bonds, which stabilize the whole protein network in gels formed under those conditions.

For short heating times, disulfide bridges are present only between the small oligomers; the aggregates are linked by non-covalent interactions (Bauer *et al.*, 2000).

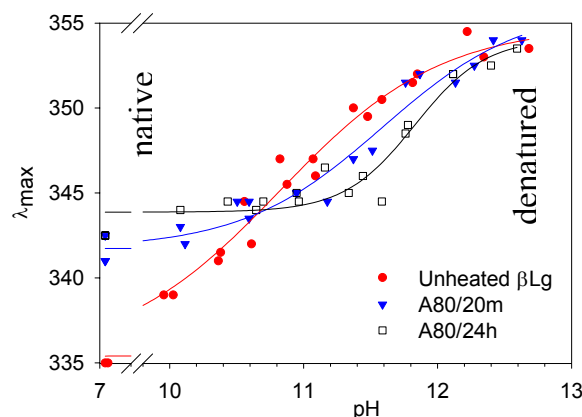


Figure 9. Wavelength (in nm) at maximum fluorescence intensity obtained using tryptophan fluorescence. Calculated transition pK:  $10.8 \pm 0.1$  - unheated  $\beta$ -lg;  $11.6 \pm 0.1$  - A80/20m aggregates; and  $11.8 \pm 0.15$  - A80/24h aggregates. After Mercadé-Prieto *et al.* (2007b).

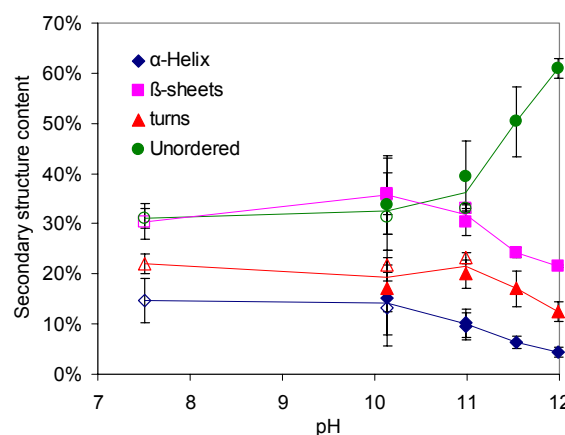


Figure 10. Calculated secondary structure content of A80/20m aggregates in solutions at different pH obtained from the circular dichroism spectra. After Mercadé-Prieto *et al.* (2007b).

The destruction of non-covalent interactions and the alkali-induced denaturation of aggregates is important for cleaning because these phenomena determine the lower pH threshold at which significant dissolution is observed. The constant dissolution rate,  $R_0$ , data in Figure 11 exhibit a noticeable increase or 'threshold' at a pH value of 11.6, which is in good agreement with the value indicated by the spectroscopic studies.

The comparison between native and aggregated  $\beta$ -lg indicates that the processing history of the  $\beta$ -lg (and, by extension, other proteins) will determine the exact location of the threshold and this value will shift slightly depending on the gelation conditions. Effective cleaning will need to be performed above this value, and it is recommended to use a pH above which the alkali

denaturation of aggregates is complete, found here at pH 12.

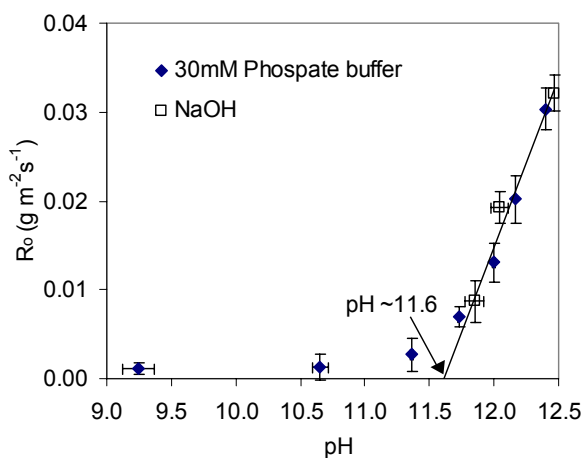


Figure 11. Effect of pH on steady dissolution rate,  $R_0$ , in NaOH and phosphate pH buffer for 15 wt%  $\beta$ -lg heat-induced gel formed at 80°C for 20 minutes. After Mercadé-Prieto *et al.* (2007b).

**DISSOLUTION RATES**

The rate of dissolution of a gel in alkali depends on many parameters. The effect of each parameter on the overall dissolution rate (which can be measured) can be analysed to indicate which mechanisms control the dissolution process. Figures 5 and 6 show that the addition of salts reduces the dissolution rate of gels, but it also reduces the maximum extent and the rate of swelling (Figs. 3, 4). Figure 12 shows a very strong correlation between the dissolution rate and swelling ratio, indicating that the phenomena are related: the decrease of swelling causes a decrease of the dissolution rate, irrespective of both the salt used and the gelation conditions.

However, the dissolution rate is not determined by the dissolution conditions alone, as the gelation conditions also have a profound effect via their influence on the gel microstructure. In the pH range 12-13 the dissolution rates for a wide range of conditions were found to follow a relationship of the form

$$R_0 = k'_g [\text{OH}^-] + R_w \tag{2}$$

where  $R_w$  is the dissolution rate in water at pH 7 and  $k'_g$  is the (lumped) dissolution rate constant. The  $k'_g$  values decrease with longer gelation times and higher gelation temperatures (Mercadé-Prieto *et al.*, 2006). Under the same conditions, solubility studies indicated that gels become increasingly insoluble in buffers lacking in dithiothreitol, a component that can cleave disulfide bonds.

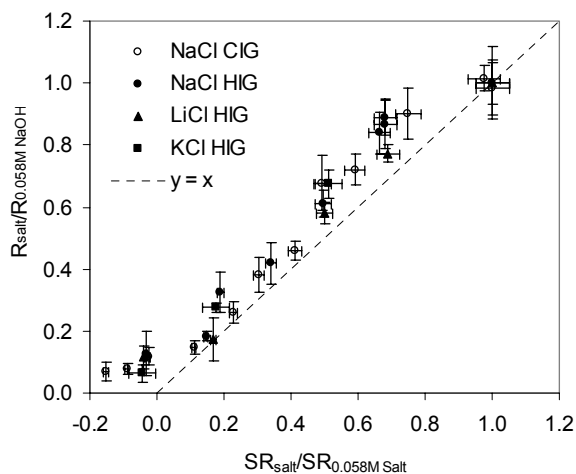


Figure 12. Correlation between the decrease in the constant dissolution rate and the swelling ratio due to the addition of alkali salts, for both heat-induced gels (HIG) and caustic-induced gels (CIG). HIG gelation conditions as in Fig. 5, CIG as in Fig. 4. Dashed line shows line of equality. After Mercadé-Prieto *et al.* (2007c).

Similarly, several of our investigations indicated that the amount of protein stabilized by covalent bonds, such as intermolecular disulfide bonds, correlate well with  $k'_g$  (Fig. 13).

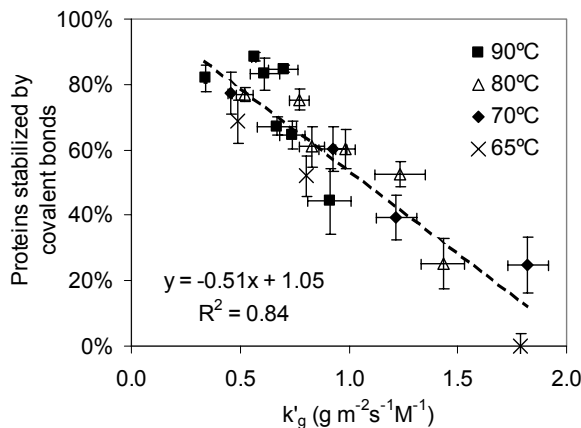


Figure 13. Correlation between the percentage of proteins stabilized by intermolecular covalent bonds, and the dissolution rate constant  $k'_g$  (eq. 2) for gels formed at different gelation times and temperatures. Dashed line shows the best fit through all data. After Mercadé-Prieto *et al.* (2006).

Weakly crosslinked gels dissolved very quickly, and *vice versa*. Furthermore, studies of the kinetics of the alkali cleavage reactions of disulfide bonds showed that these breakdown reactions could not be the limiting step (they are too slow under the conditions used during most of these tests). We suggest that the dissolution rate was

reduced when aggregate sizes were large, caused by the larger number of crosslinks.

The importance of the protein aggregate size during dissolution, together with the importance of gel swelling, suggest that one of the steps controlling dissolution involves the diffusion of proteins inside the swollen matrix before they reach the boundary layer. This intragel diffusion would be greatly influenced by the initial size of the aggregates (see Fig. 13), as well as by the breakdown reactions in the alkali to form small oligomers, and with the free volume in the swollen layer (Fig. 12).

### EXTENSION AND APPLICATION

These studies on model foulants, namely whey protein gels, have identified and quantified the importance of factors which play critical roles in the cleaning behaviour of proteinaceous dairy deposits: the formation conditions (and thereby the structure of the foulant); swelling, and the importance of free volume for protein disentanglement processes and the kinetics of dissolution; pH, ionic strength and the role of salts. Dissolution involves breakdown of interactions within and between proteins and the interactions being broken down vary with the nature of the gel and the pH. Figure 13 is direct macromolecular evidence of the importance of ageing on dissolution, while Figures 9 and 10 show how ageing changes the molecular-level structure from the native protein.

This work provides a fundamental understanding of the sub-processes involved in dissolution and identifies some of the parameters that should be monitored when considering the cleaning behaviour of mixtures of proteins, and particularly proteins and salts such as calcium. There is much work yet to be done to build up an understanding of the interactions in real milk deposits, but this is not within the scope of the present study.

The observed rate of cleaning depends on the balance between chemical reaction modifying the interactions in the foulant layer, and physical removal either by mass transfer or erosion. Areas requiring further study include (i) cleavage reactions, as they have received little attention to date: the dominant interest has been in the formation of bonds in order to promote gelation; and (ii) the interaction between reaction and rheology of the foulant layers. The results reported here have been performed under conditions approaching chemical reaction control, but diffusion of hydroxide into a swelling foulant layer and disentanglement and release of aggregates also present key steps in the kinetic mechanism. These are currently under investigation.

### CONCLUSIONS

It is no longer necessary to view the chemical reactions involved in the alkaline dissolution of whey protein gels as hidden within a 'black box'. Gels and aggregates formed by heat- or caustic-treatment of WPC,

and WPC-derived  $\beta$ -lactoglobulin, have been exposed to a range of temperatures, pHs and salt concentrations under conditions where interphase mass transfer does not control the kinetics. The chemistry has been sufficiently illuminated that theories originally developed for synthetic polymers have been adapted successfully to model the swelling and dissolution of these protein gels by aqueous NaOH, under the influence of polyelectrolyte screening effects. The practical implications include a better understanding of the optimal pH to be used for cleaning, and prediction of the effect of calcium ions in milk on cleaning of dairy plant. The scientific implications are perhaps particularly strikingly displayed in (a) the effect of the history of the foulant – revealed by the correlation between dissolution rate constant and the fraction of protein initially stabilised by intermolecular covalent bonds, and (b) the progress of alkaline attack on the gels – revealed by the correlation between dissolution rate and equilibrium swelling ratio.

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### NOMENCLATURE

$f$	fraction, absorbance spectrum, -
$k_g'$	constant dissolution rate, $\text{g m}^{-2} \text{s}^{-1} \text{M}^{-1}$
$\Delta m_A$	water uptake per unit surface area, $\text{g m}^{-2}$
$Q$	equilibrium swelling degree, -
$R_d$	thermal resistance, $\text{m}^2 \text{K W}^{-1}$
$R_o$	constant dissolution rate, $\text{g m}^{-2} \text{s}^{-1}$
$R_w$	constant dissolution rate in water, $\text{g m}^{-2} \text{s}^{-1}$
$Re$	Reynolds number, -

### REFERENCES

- Bauer, R., Carrotta, R., Rischel, C., Øgdenal, 2000, Characterization and isolation of intermediates in beta-lactoglobulin heat aggregation at high pH, *Biophysical J.*, 79, 1030-1038.
- Bird, M.R., Fryer, P.J., 1991, An experimental study of the cleaning of surfaces fouled by whey proteins. *Food Bioprod Proc.*, 69, 13-21.
- Burton, H., 1968, Deposit from whole milk in heat treatment plant - A review and discussion. *J Dairy Res.*, 35: 317-330.
- Changani, S.D., Belmar-Beiny, M.T., Fryer, P.J., 1997, Engineering and chemical factors associated with fouling and cleaning in milk processing, *Exptl. Thermal Fluid Sci.*, 14(4), 392-406.
- Chew, Y.M.J., Paterson, W.R., Wilson, D.I., 2004, Fluid dynamic gauging for measuring the strength of soft deposits, *J. Food Engng.*, 65, 175-187.

- Christian, G.K., Fryer, P.J., 2006, The effect of pulsing cleaning chemicals on the cleaning of whey protein deposits, *Food BioProd. Proc.*, 84C, 320-328
- English, A. E., Tanaka, T., Edelman, E.R., 1997, Equilibrium and non-equilibrium phase transitions in copolymer polyelectrolyte hydrogels, *J. Chem. Physics*, 107(5), 1645-1654.
- Havea, P. Carr, A. J. Creamer, L. K., 2004, The roles of disulphide and non-covalent bonding in the functional properties of heat-induced whey protein gels, *J. Dairy Res.* 71, 330-339.
- Hooper, R.J., Liu, W., Fryer, P.J., Paterson, W.R., Wilson, D.I., Zhang, Z., 2006a, Comparative studies of fluid dynamic gauging and a micromanipulation probe for strength measurements, *FoodBioprod.Proc.*, 84C, 353-358.
- Hooper, R.J., Paterson, W.R., Wilson, D.I., 2006b, Comparison of whey protein model foulants for studying cleaning of milk fouling deposits, *Food Bioprod. Proc.*, 84C, 329-337.
- Lalande, M., Tissier, J.P., & Corrieu, G., 1985, Fouling of heat transfer surfaces related to  $\beta$ -lg denaturation during heat processing of milk. *Biotechnology Processing*, 1(2): 131-139.
- Leclerq-Perlat, M.N., Lalande, M., 1991, A review of the modelling of the removal of porous contaminants deposited on heat transfer surfaces, *Intl. Chem. Eng.* 31(1), 74-93.
- Lefèvre, T., Subirade, M., 2000, Molecular differences in the formation and structure of fine-stranded and particulate  $\beta$ -lg gels, *Biopolymer*, 54, 578-586.
- Mahdavinia, G.R., Pourjavadi, A., Hosseinzadeh, H., Zohuriaan,M.J., 2004, Modified chitosan 4. Superabsorbent hydrogels from poly(acrylicacid-co-acrylamide) grafted chitosan with salt- and pH-responsiveness properties, *Eur. Poly. J.*, 40(7), 1399-1407.
- Mercadé-Prieto, R. and Chen, X.D., 2006, Dissolution of whey protein concentrate gels in alkali. *AIChE J.*, 52(2), 792-803.
- Mercadé-Prieto, R., Falconer, R.J., Paterson, W.R., Wilson, D.I., 2006, Effect of gel structure on the dissolution of heat-induced  $\beta$ -lg gels in alkali, *J. Agric. Food Chem.*, 54(15), 5437-5444.
- Mercadé-Prieto, R., Falconer, R.J., Paterson, W.R., Wilson, D.I., 2007a, Swelling and dissolution of  $\beta$ -lg gels in alkali, *Biomacromol.*, 8(2), 469-476.
- Mercadé-Prieto, R., Paterson, W.R., Wilson, D.I., 2007b, pH threshold in the dissolution of  $\beta$ -lg gels and aggregates in alkali, *Biomacromol.*, 8(4), 1162-1170.
- Mercadé-Prieto, R., Sahoo, P.K., Falconer, R.J., Paterson, W.R., Wilson, D.I., 2007c, Polyelectrolyte screening effects on the dissolution of whey gels at high pH conditions, *Food Hydrocolloids*, 21, 1275-1284.
- Narasimhan, B., Peppas, N. A., 1996, Disentanglement and reptation during dissolution of rubbery polymers, *J. Poly. Sci. B: Polymer Physics*, 34(5), 947-961.
- Sahoo, P.K., Mercadé-Prieto, M., Chew, Y.M., Wilson, D.I., 2007, Fluid dynamic gauging studies of swelling behaviour of whey protein gels in NaOH/NaCl solutions, submitted to *Intl. J. Food Sci. & Tech.*
- Shimada, K. Cheftel, J. C., 1988, Texture characteristics, protein solubility, and sulfhydryl-group disulfide bond contents of heat-induced gels of whey-protein isolate, *J. Agric. Food Chem.*, 36, 1018-1025.
- Simons, J. F. A., Kusters, H. A., Visschers, R. W., de Jongh, H. H. J., 2002, Role of calcium as trigger in thermal  $\beta$ -lactoglobulin aggregation, *Archives Biochem. Biophysics*, 406(2), 143-152.
- Taulier, N., Chalikian, T. V., 2001, Characterization of pH-induced transitions of  $\beta$ -lg: Ultrasonic, densimetric, and spectroscopic studies, *J. Mol. Biol.*, 314, 873-889.
- Tuladhar, T.R., Paterson, W.R., Macleod, N., Wilson, D.I., 2000, Development of a novel non-contact proximity gauge for thickness measurement of soft deposits and its application in fouling studies, *Can. J. Chem. Eng.*, 78, 935-947.
- Tuladhar, T.R., Paterson, W.R., Wilson, D.I., 2002, Investigation of alkaline cleaning-in-place of whey protein deposits using dynamic gauging, *Food Bioprod. Proc.*, 80C, 199-214.
- van der Leeden M.C., Rutten A.A.C.M., Frens G., 2000, How to develop globular proteins into adhesives, *J. Biotechnol.*, 79(3), 211-221.
- Verheul M, Roefs SPFM, de Kruif K.G., 1998, Kinetics of heat-induced aggregation of  $\beta$ -lg, *J. Agric. Food Chem.*, 46 (3): 896-903
- Visser, H., Jeurink, J.M., 1997, General aspects of fouling and cleaning, in fouling and cleaning of heat treatment equipment. In Visser H., editor, *Bulletin of the International Dairy Federation No. 328*, page 5. IDF, Brussels, Belgium.
- Wilson, D.I., 2005, Challenges in cleaning: Recent developments and future prospects, *Heat Transfer Eng.*, 26(1), 51-59.
- Xin, H., Chen, X.D., Özkan, N., 2004, Removal of a model protein foulant from metal surfaces, *AIChEJ*, 50(8) 1961-1973.
- Zhao, Y., Su, H., Fang, L., Tan, T., 2005, Superabsorbent hydrogels from poly(aspartic acid) with salt-, temperature- and pH-responsiveness properties, *Polymer*, 46(14), 5368-5376.