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THE POOR PERFORMANCE OF NaOH IN THE DISSOLUTION OF WHEY PROTEIN GELS AT VERY HIGH pH

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ABSTRACT

It is reported in the literature that when cleaning whey protein concentrate fouling layers or gels with highly concentrated NaOH solutions the cleaning rate is very low. This phenomenon is particularly evident at pH values above 13.5 and at temperatures below 50°C. Experiments have been performed on whey gels at the Universities of Auckland and Cambridge to elucidate the mechanisms involved in those conditions, as well as for lower pH values. The results suggest that at pH < 13, the dissolution rate is controlled by the β-elimination of the intermolecular disulfide bonds present in the WPC gels. At pH > 13, the NaOH in the gel is suggested to induce new intermolecular crosslinks that make the gels more alkali-resistant. Dissolution experiments with caustic-induced gels show that the presence of NaOH in whey gels can greatly enhance their resistance to alkali.

INTRODUCTION

The existence of an optimum NaOH concentration for the cleaning of milk and whey fouling has been reported several times (*e.g.* Bird and Fryer, 1991). Using high NaOH concentrations leads to surprisingly low cleaning rates (Jeurnink and Brinkman, 1994), especially at low temperatures (Xin *et al.*, 2002b). The reasons for the poor performance of NaOH at high concentrations are unclear, although some hypotheses have been presented. Tuladhar *et al*. (2002) suggested that it may be due to the less open micro-structure of the fouling deposit, while Morison and Thorpe (2002) attributed it to the viscosity increase in the boundary layer, and Xin *et al.* (2002b) proposed that new bonds could be formed to explain the increase of the hardness of gels attacked by highly concentrated caustic solutions.

The cleaning profile of protein foulants on surfaces typically consists of three stages: swelling, plateau, and decay (Xin *et al.*, 2002a). During the plateau stage, where the cleaning rate is at its maximum, most of the dissolution occurs. Therefore, it is of interest to study the plateau stage to elucidate the mechanisms involved at high caustic concentration. Experimentally, it is more convenient to use protein gels formed inside capsules than thermally-induced fouling deposits or gels on a surface, whether a plate or a tube. Very thick gels can be formed readily using this technique, allowing the dissolution to be studied accurately over long periods. This semi-infinite dissolution process allows one to focus only on the cleaning mechanisms inside the gel, where the gel-surface interactions are not relevant. Using gels generated inside capsules has also the benefit of eliminating flow effects (results not shown), so that the effect of chemical reactions can be identified more readily.

EXPERIMENTAL

Heat-induced gels (HIG) were formed inside small cylindrical glass capsules, internal diameter 5 mm and height 25 mm, using a well homogenized 16.7 wt% whey protein concentrate (WPC) solution (~80% protein WPC, New Zealand Milk Products). The sample was then sealed with foil and held in a water bath at the desired gelation temperature for 20 minutes. Most of the gels were made from 16.7 wt% WPC at 80ºC; these are termed standard HIG. Heat-induced gels were also prepared from a purer β-lactoglobulin source (92% β-Lg dry basis from Davisco Foods Intl. Inc, MN, USA) using 15.0 wt% β-Lg solutions at 80ºC for 20 min.

Caustic-induced gels (CIG) were prepared by mixing known amounts of a mother WPC solution, water and concentrated NaOH solution (~4 M) in a test tube. After homogenisation, the solution was introduced into capsules, sealed with foil and submerged in a water bath at 50ºC for 20 minutes. The final whey protein concentration was 16.9 wt%. The gelation pH was calculated using the hydrogen ion equilibrium method for WPC solutions reported by Mercadé-Prieto and Chen (2005).

Figure 1 shows the experimental configuration for dissolution experiments. 200 ml of caustic solution (analytical grade) were added to a 250 ml Erlenmeyer flask. The Erlenmeyer was insulated and the temperature held constant to ±2 ºC. The solution was homogenized using a magnetic stirrer at ~350 rpm. The pH was measured in each experiment by titration of an aliquot with 0.02 N HCl (standard analytical grade). Once the solution reached the desired temperature, a blank was scanned with a UV spectrophotometer (HP 8452A). More information on the continuous non-invasive UV measurement technique is given in Xin *et al.* (2002a). Finally, the capsule with the gel was suspended upright from the top of the Erlenmeyer by a wire and the spectrophotometer started to record. A small

part of the solution was pumped through the spectrophotometer and the UV absorption recorded at 30 s intervals.

The protein concentration in solution was determined using four wavelengths (224, 240, 250 and 280 nm). The dissolution rate was then calculated numerically using three-point differences of the concentration. The rates were subsequently smoothed to eliminate background noise.

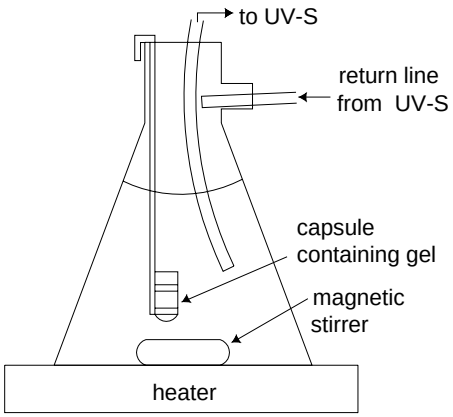


Fig 1. Schematic diagram of the gel dissolution experiments. UV-S: UV spectrophotometer.

RESULTS

Influence of Dissolution pH

The swelling and the plateau stage expected from the literature were observed when dissolving WPC gels at low NaOH concentrations (Fig. 2, pH 12.95). Where constant dissolution rates were observed, these were considered to represent the rate of a chemical reaction. The dissolution produced by the presence of caustic is termed $R_{\beta E}$ in connection with the β-elimination of intermolecular disulfide bonds, which is the most likely reaction involved (Whitaker and Feeney, 1983). Some dissolution is also observed in deionised water due to the partial solubility of the WPC gels, and this is termed R_w. The constant dissolution rate, R_o, can then be expressed with the following equation:

$$R_o = \frac{1}{A}\left(\frac{dn}{dt}\right)_{uniform} = R_{\beta E} + R_w = k_{\beta E}\left[OH^-\right]^{m_{\beta E}} + R_w \quad (1)$$

Values of the parameters obtained from regression of data from dissolution experiments over $0 < [OH^-] < 0.15$ M were found to be: $k_{\beta E} = 0.46 \pm 0.01$ g/m^2 s M; $m_{\beta E} = 1.02 \pm 0.03$; $R_w = (8 \pm 3) \times 10^{-4}$ g/m^2 s.

However, Figure 2 shows that the plateau stage behavior differs significantly when a NaOH concentration near and above the reported optimum (~0.5 wt% NaOH, pH 13.1) is used: the rate declines over time and the maximum rates do not follow Eq. (1), as shown in Figure 3.

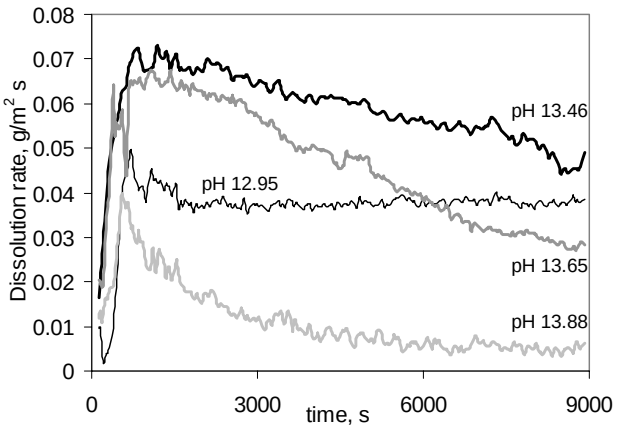


Fig. 2 Dissolution rate profiles at different pH values. Conditions: standard WPC HIG at 21ºC.

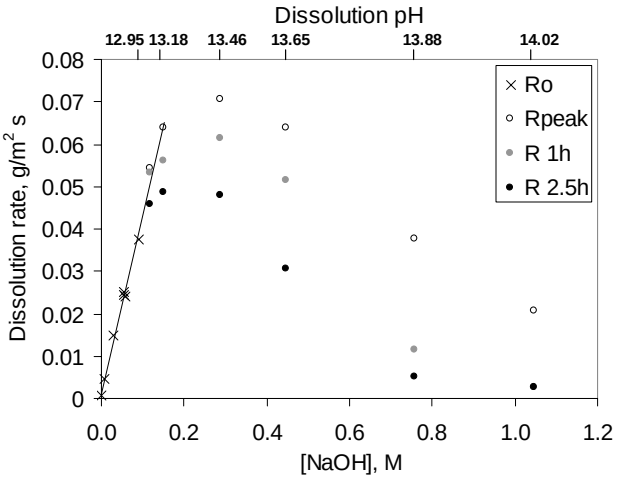
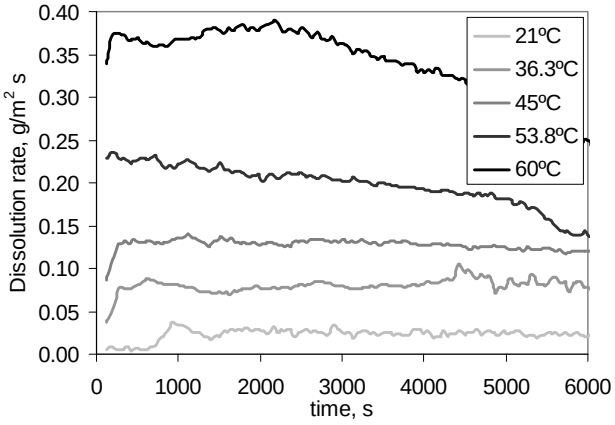


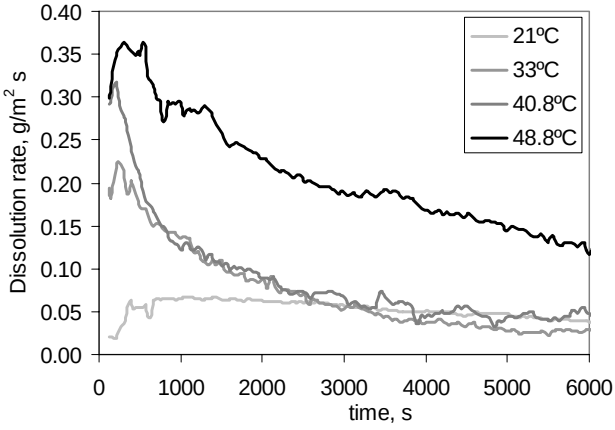
Fig. 3 Dissolution rate for standard WPC HIGs at different NaOH concentrations at 21ºC. R_o refers to the constant dissolution rate, R_{peak} is the maximum rate observed, and R_{1h} and $R_{2.5h}$ are the rates after 1 and 2.5 hours, respectively. The line shows Eq. (1).

Influence of Dissolution Temperature

Dissolution experiments performed below and above the optimum NaOH concentration at other temperatures showed similar behaviour to that at room temperature. At a low NaOH concentration, the dissolution rate increased with temperature, which could be described by an activation energy of 54.7 ± 1.4 kJ/mol (pH 12.10 and 12.72), and the rates were relatively constant over time (Fig. 4(a)).



(a) pH 12.72



(b) pH 13.65

Fig. 4 Effect of temperature on dissolution rate. Conditions: Standard WPC HIG.

Figure 4(b) shows the dissolution rate profiles for pH 13.65 (above the optimum). It should be noted that between room temperature and 41ºC, the dissolution rate decline becomes more severe with increasing temperature, even though the maximum initial rate also increases. This leads to the unexpected result that after one hour, the dissolution rate for all three temperatures is approximately equal. However, at 49ºC the decline in rate is less severe, and at 60ºC the dissolution is so fast that at the end of the experiment less than the 10% of the initial gel remained in the capsule (data not shown).

The region of the gel attacked by the caustic experiences some colour changes. At 21ºC and $pH_s \leq 13.5$ the caustic attacked phase is colourless whereas, at higher concentrations, a faint yellow colour is visible in the reaction zone; its brightness increases with pH_s and temperature (Fig. 5).

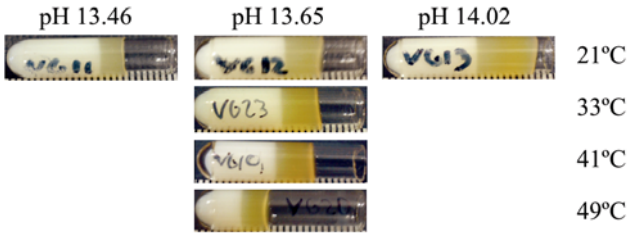


Fig. 5 Effect of pH on HIG appearance: standard WPC HIG after 2.5 h.

Influence of Gelation Temperature

The gelation temperature is one of the key parameters in heat-induced gelation and this was varied to assess how micro-structural changes in the gel affect the constant dissolution rate. Fig. 6 shows how increasing the gelation temperature results in a marked decrease in the constant dissolution rate at pH 12.72.

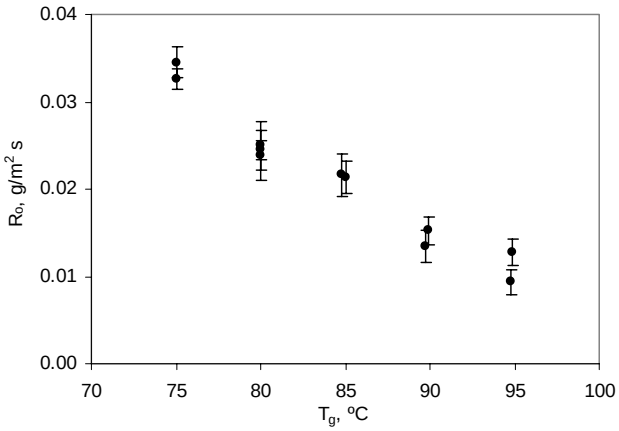


Fig. 6 Constant dissolution rate (R_o) for gels formed at different gelation temperatures. Dissolution conditions: standard WPC HIG at pH 12.72 and 21ºC.

Caustic-Induced Gels

It is known that WPC solutions can readily gel in the presence of NaOH, even at low temperatures (Mercadé-Prieto and Chen, 2005). The higher the NaOH concentration, the faster the gelation process. The structure and properties of caustic-induced gels (CIG) differ from those of the heat-induced analogues. Intermolecular disulfide bonds play a more important role due to the increased reactivity of cysteine in alkali, and hydrophobic interactions are less significant due to the high electronic repulsion (Monahan *et al*, 1995; Shimada and Cheftel, 1988). The dissolution of CIG is not reported in the literature, even though caustic may produce alterations in HIG in high pH environments.

Influence of Gelation pH in CIG. Fig. 7 confirms that the gelation pH strongly affects the resistance to alkali of these gels. Those produced at pH > 11.7 gave a yellow colour similar to that observed in the dissolution of HIGs at high pH (Fig. 5), with the yellow intensity again increasing with NaOH concentration.

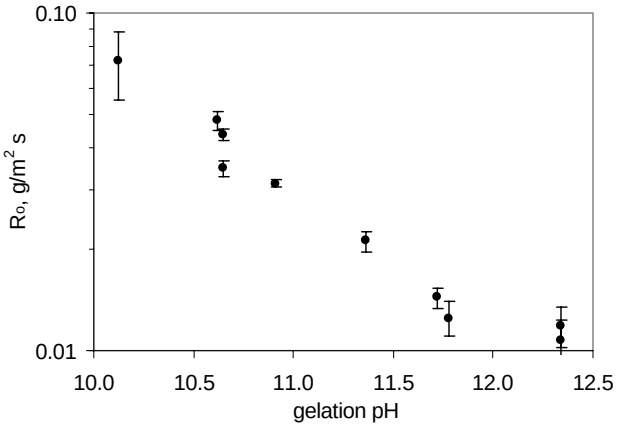


Fig. 7 Effect of gelation pH on R_o. Dissolution conditions: pH 12.72 and 21ºC. Note log scale on y-axis.

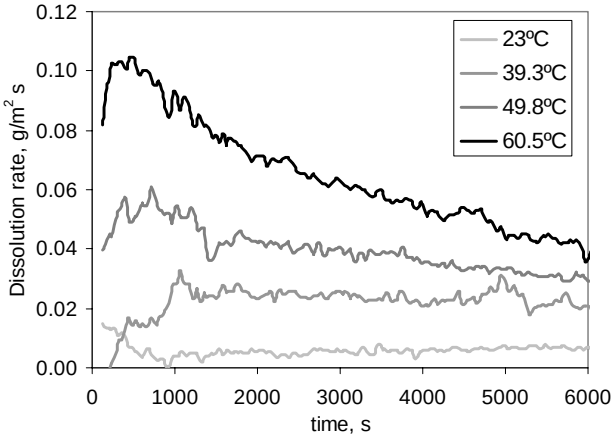


Fig. 8 Dissolution rate profiles for WPC CIG gels at different temperatures. Conditions: gelation pH 11.36 and dissolution pH 12.17.

Effect of temperature on CIGs. The dissolution rate profiles of CIGs at pH values below the NaOH concentration optimum at room temperature exhibited a plateau as observed in HIGs. However, at higher temperatures the rate declined over time, even when using relatively low pH values such as 12.17 (Fig. 8). At a pH of 13.06, the dissolution rate decreased with time at temperatures above 35ºC. As with HIGs, the intensity of the yellow colour observed in the reaction zone increased with temperature.

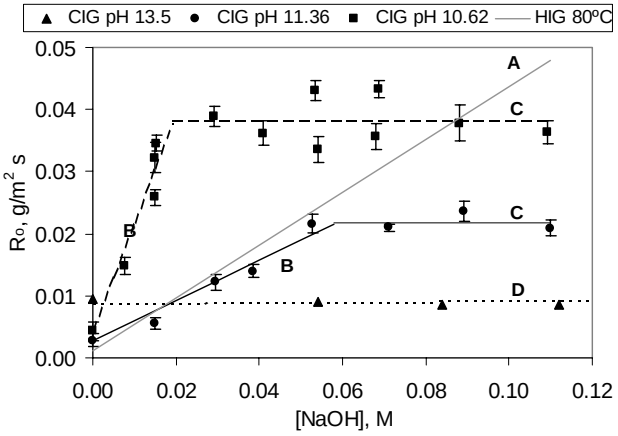


Fig. 9 Effect of dissolution NaOH concentration at 21ºC on R_o for CIG gels prepared at different gelation pH. A - dissolution by β-elimination of disulfide bonds for standard HIG, Eq. (1); B - dissolution by β-elimination of disulfide bonds for CIG; C - R_{limit} for different CIG; D – solubility (R_w) for CIG at pH_g 13.5.

Influence of Dissolution pH in CIGs. The dissolution rate of CIGs prepared at a gelation pH 13.5 remained constant over 7 < pH < 13.6, at $1 \pm 0.3\times10^{-2}$ g/m^2 s at 21ºC (Fig. 9, triangles). The rate only increased slightly, to ~1.5×10^{-2} g/m^2 s, at larger pH values, 13.88-14.02. For CIGs prepared at lower gelation pH, Figure 9 shows markedly different effects of dissolution pH. If the dissolution pH is low, from 7 to ~12.3 for a gelation pH of 10.6, or up to ~12.8 for a gelation pH 11.4, R_o increases linearly with hydroxide concentration. There is also a minimum value corresponding to the dissolution in deionised water, R_w. A first order dependency on hydroxide concentration and the presence of a solubility threshold value suggests that the dissolution observed at these pH values is equivalent to that seen for HIG. R_o above the dissolution pH thresholds is, within experimental accuracy, constant. These constant values, which are the maximum R_o observed at each gelation pH, have been labelled R_{limit}.

β-Lactoglobulin gels

Figure 10 shows that the dissolution profiles observed with HIGs prepared using relatively pure β-Lg were similar to the WPC gels. At high pH values, *e.g*. 13.95, the dissolution rate is similarly low: if that gel is then transferred to a dissolution experiment at a lower pH, say 12.77, the constant dissolution rate observed is significantly lower than in with a fresh gel.

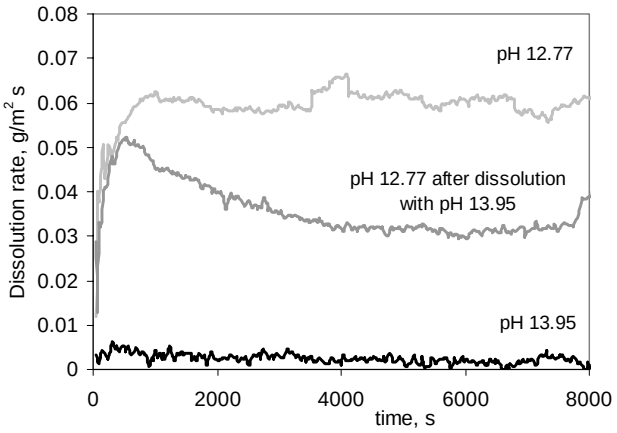


Fig. 10 Effect of pH on dissolution rate for β-lactoglobulin heat-induced gels at 24ºC.

DISCUSSION

Reactions involved in the dissolution process

The relevance of intermolecular disulfide bonds in the formation of aggregates and larger oligomers has been widely reviewed (Hoffmann and Van Mil, 1999; Surroca *et al*, 2002). Even though non-covalent bonds also play a role in the formation of HIG gels (Havea *et al.*, 2004), it has been recognized that for the kind of HIG generated here the main cohesive attractions are due to interprotein disulfide bonds (Shimada and Cheftel, 1989). Therefore, covalent bonds should be broken in order for the gel to dissolve, specifically the intermolecular disulfide bonds or the intramolecular peptide bonds. Chemical hydrolysis of the peptide bond requires harsh conditions, whereas under the pH and the temperature conditions employed here, little evidence was found for the hydrolysis of ribonuclease A and lysozyme (Hayashi and Kameda, 1980).

Three mechanisms are recognized in the destruction of disulfide bonds: α-elimination, hydrolysis and β-elimination. The first two mechanisms are known to be not significant for proteins (Schneider and Westley, 1969; Stapleton and Swan, 1960), whereas the β-elimination mechanism has been widely verified (Kim and Kim, 2001; Whitaker and Feeney, 1983), even for β-lactoglobulin (Noetzold *et al*, 1972). It can explain for example the release of H_2S seen in the alkali treatment of proteins, reported many times (in whey proteins (Noetzold *et al*., 1977)) and determined in large quantities in ad hoc dissolution experiments (results not shown). The mechanism is first order with respect to the hydroxide concentration (Florence, 1979), in good agreement with the value found for $m_{\beta E}$ (Eq. 1). However, the activation energy found for the dissolution of HIG and CIG at low alkaline pH, ~55 kJ/mol, is smaller than that reported for the β-elimination of intramolecular cystine residues in different proteins (60-100 kJ/mol) (Feeney et al, 1977). Similar dissolution behaviour to HIG is also seen for CIG (gelation pH 10.62 and 11.36) at low dissolution pH (Fig. 9). The present study, therefore, agrees with those cleaning mechanisms based on a first order reaction with the hydroxide concentration, *e.g*. Jennings (1965).

The yellow colour observed in Figure 5 may be due to Maillard reactions, even though the temperatures used are low and the lactose content very small (the β-Lg used had only 0.1 wt% lactose, but intense yellowness was also seen at 24ºC and pH 13.95). On the other hand, the colour change seen may be due to the formation of polysulfides after the β-elimination of disulfide bonds (Stapleton and Swan, 1960). Sodium disulfide (Na_2S_2) may be involved, as when dissolved the solution colour is described as deep yellow (Karchmer, 1970). Polysulfides are known to be unstable in acidic conditions, as they break up to form elemental sulfur and hydrogen sulfide, and hence the bright colour disappears. This was tested and observed in all the yellow solutions and gels in the present study. In addition, the smell of H_2S was noticeable after acidification.

Non-constant dissolution rates

Nevertheless, the linear relationship of the dissolution rate with the NaOH concentration, which has been assigned to the β-elimination mechanism, has a limited applicability both for HIG and CIG. In HIG the dissolution rate decreases with the NaOH concentration (Figure 3), while in CIG the rate remains constant (Figure 9).

The significant role of gelation conditions in the dissolution process shown in Fig. 6 and 7. Other gelation variables, like the protein concentration (Xin, 2003), are also known to influence the posterior dissolution rate in alkali. This behaviour is particularly significant as the dissolution conditions, NaOH concentration and temperature, are kept constant, and mechanical action inside the capsules is negligible. This suggests that characteristics of the gel, such as the interprotein crosslink density, determine the dissolution rate observed under the same dissolution conditions.

Caustic-induced gels show some similarities with HIG attacked at high pH, such as the presence of an intense yellow colour, very low dissolution rates or the sharp rate decline with increasing temperature. It is plausible to suggest that the NaOH inside the gel can induce structural changes that may change a HIG into a form similar to a CIG: fewer hydrophobic and hydrogen bonds interactions, and more intermolecular disulfide bonds (Monahan *et al*., 1995).

Experiments with β-Lg gels showed similar profiles to WPC gels (Figure 10), suggesting that β-Lg, which is the main protein in WPC, can be used as a model system.

Preliminary results show that structural modifications after treatment at high NaOH concentrations are likely to occur, as the dissolution rate at pH 12.77 is only half that when a β-Lg HIG is previously dissolved with pH 13.95. Therefore, structural changes at high pH will be a key component of this complex behaviour, but others mechanisms may also play a role. Further study is still needed, especially on the structural modifications beyond the NaOH concentration optimum.

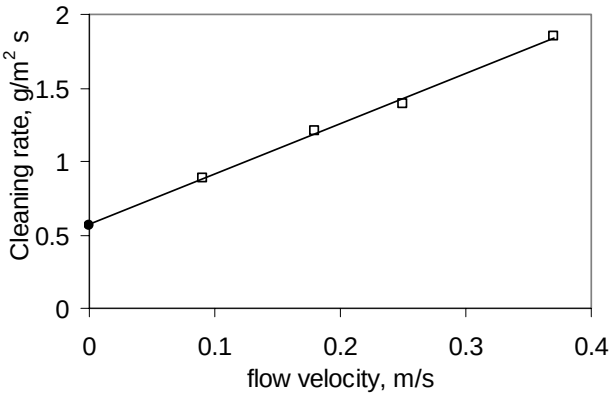


Fig. 11 Influence of velocity on the cleaning rate in pipes fouled with WPC gels. The empty square points and line are taken from the study by Xin *et al.* (2004) using 0.5 wt% NaOH at 65ºC. The solid square is R_o estimated for a standard HIG dissolved at the same conditions, assuming no velocity effects.

Influence of the flow velocity in cleaning

Even though the influence of flow velocity has not been studied explicitly in this work, it is of interest to compare the values observed here with those reported in the literature. The cleaning rate in pipes at different velocities reported by Xin *et al.* (2004) is shown in Figure 11. A linear extrapolation of the data predicts a non-zero cleaning rate at zero flow velocity. Other studies show the same trend (Bird and Fryer, 1991; Gillham *et al.*, 1999; Tuladhar *et al.*, 2002). The HIG used in these experiments can be compared directly with the fouled pipes used by Xin *et al.* (2004) as the same WPC material was used and the gelation conditions were similar. The calculated R_o for their experimental conditions of pH 13.10 and 65ºC is 0.56 g/m^2 s, which it is shown in Figure 11 as a solid square, assuming zero flow velocity. The formation of loose proteins was not enhanced by the stirring speed used (data not reported). The extrapolation of Xin *et al.*'s data in pipes to zero velocity is in good agreement with the value found in the present study using capsules. The present study also supports the experimental evidence that at the limit of zero fluid velocity, the cleaning rate is not zero. Flow velocity or shear stress should not affect the chemical reactions that produce loose proteins. They will affect the transport (*i.e.* removal) of loose proteins from the deposit to the bulk stream and the supply of hydroxide ions.

CONCLUSIONS

1. Dissolution of whey gels in capsules allows these materials to be studied over long times while minimizing flow effects. At high pH the dissolution rate decreases markedly over time.
2. Structural differences due to different gelation conditions (gelation temperature and pH) are important factors determining the different dissolution rates observed.
3. Intermolecular disulfide bonds are described in the literature as the key interactions in HIG, while the β-elimination of these bonds is the most accepted mechanism in proteins under an alkali treatment. Agreement is found between the first order dependence on NaOH concentration reported in the literature for a β-elimination mechanism, and the parameter $m_{\beta E}$ from Eq. (1) for the dissolution process. However, the activation energy found for the dissolution process is smaller than those reported for the β-elimination of intraprotein cystines.
4. Structural modifications in gels dissolved at high pH, such as an increased formation of inter-protein crosslinks, is likely to cause a reduction in the dissolution rate. However, other mechanisms are needed to fully explain the low dissolution rates seen when using high NaOH concentrations.

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