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Tuning heat-induced colloidal aggregation of whey proteins, sodium caseinate and gum arabic: effect of protein composition, preheating and gum arabic level

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Abstract

Heating can drive the colloidal complexation of negatively-charged proteins and polysaccharides by strengthening hydrophobic interactions and denaturing proteins, thereby exposing reactive sites for covalent and noncovalent bonding. We have previously shown that stable colloidal aggregates comprising whey protein, sodium caseinate and gum arabic can be produced by careful selection of heat treatment, pH and protein type. Here we tested how the size, composition, charge and morphology of colloidal aggregates are affected by the amounts of whey protein, sodium caseinate and gum arabic, as well as the thermal history of the proteins. Increasing amounts of whey protein resulted in larger particles, which were more prone to precipitate. Preheating whey protein slightly enhanced aggregation, and this effect was mitigated when sodium caseinate was present during preheating (chaperone effect). Increasing amounts of gum arabic produced larger particles with less charge, but the gum arabic effect was statistically confounded with ionic strength. We believe that both covalent (disulphide) and noncovalent interactions among protein molecules are required to overcome electrostatic repulsion at pH 7 and form stable aggregates.

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1 Introduction

Complexation between polysaccharides and proteins is a well-known phenomenon that finds application in protein purification (Wang, Gao, & Dubin, 1996), nutrient delivery (Zimet & Livney, 2009), and food structure design (Dickinson, 2012; Paquin, 1999). Both proteins and polysaccharides are polyelectrolytes whose charge depends on pH, and attractive or repulsive electrostatic interactions have a strong influence on phase behaviours in mixed aqueous systems. Most food polysaccharides have very low isoelectric pH (pI), so they are negatively charged in conditions relevant to food systems. By contrast, food proteins often have isoelectric points (pI) within the operating pH range of food manufacturing processes, so they may have either a net negative charge (pH > pI) or a net positive charge (pH < pI) depending on the pH.

Complexation often occurs in the pH range where protein and polysaccharide are oppositely charged, and may result in colloidal aggregates or a precipitate. The nature of aggregates depends on biopolymer type and concentration, ionic strength, pH, and temperature. Aggregates that are colloidally stable may be useful in foods as Pickering emulsifiers (Dickinson, 2012), fat mimetics (Paquin, 1999) or nutrient delivery vehicles (Zimet & Livney, 2009).

Earlier work has shown that heating at neutral pH drives reversible hydrophobic association between sodium caseinate (SC) and gum arabic (GA) (Ye, Edwards, Gilliland, Jameson, & Singh, 2012). We have shown that adding whey protein isolate (WPI) to mixtures of SC and GA promotes covalent aggregation, particularly at pH 7 but also to a limited extent at pH 3.5 (Loveday, Ye, Anema, & Singh, 2013). Here we extend this work to examine how heat-induced denaturation of milk proteins prior to mixing with GA affects the nature of aggregation.

Heating whey proteins at 80°C and neutral pH leads to unfolding of globular proteins and exposure of the reactive free thiol groups of β-lactoglobulin and bovine serum albumin (BSA), leading to complete denaturation within 12.5 min for β-lactoglobulin (Manderson, Creamer, & Hardman, 1999) or even faster for BSA (Baier, Decker, & McClements, 2004; Boye, Alli, & Ismail, 1996). Subsequent intermolecular disulphide bonding among whey proteins of the same or different type drives the formation of oligomers and polymers, whose size depends strongly on protein concentration, the proportions of different whey proteins, and ionic strength (Anema, 2009b; Livney, Corredig, & Dalgleish, 2003). α-Lactalbumin contains four disulphide bonds and no free thiol groups, but it nevertheless polymerises *via* disulphide bonds in the presence of other proteins with free thiol groups, which appear to catalyse sulphydryl-disulphide interchange in α-lactalbumin (De la Fuente, Singh, & Hemar, 2002).

The casein fraction of milk protein comprises four gene products, namely α_{s1}-, α_{s2}-, β-, and κ-caseins. Caseins are phosphoproteins in which the clustering of hydrophilic residues (particularly phosphoserine) and hydrophobic residues imparts a strongly amphiphilic character (Horne, 2009). This gives them a tendency to form micelles by hydrophobic association, and this tendency is enhanced with increasing temperature due to a strengthening of thermodynamic driving forces for hydrophobic association, relative to other effects opposing association (Schellman, 1997). α_{s1}- and β-casein contain no cysteine residues, whereas α_{s2}-casein contains two, which may form an intramolecular disulphide bond or may bond with Cys residues on another α_{s2}-casein molecule, forming a dimer (Zhang & Vardhanabhuti, 2014). The two Cys residues of κ-casein make it highly susceptible to polymerisation (Farrell Jr. et al., 2004; Hamilton-Brown, Bekard, Ducker, & Dunstan, 2008). The caseins are classed as 'intrinsically unstructured' due to their low level of secondary structure and lack of well-defined tertiary structure (Farrell, 2011).

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In previous work we fixed the ratio of SC to WPI at 1:1 and fixed the mass ratio of protein to polysaccharide (Pr:Ps) at 1:5. Here we tested SC to WPI ratios from 1:4 up to 4:1, as well as Pr:Ps from 0.2 to 1.0. The results show how colloidal protein-polysaccharide aggregation can be sensitively modulated at neutral pH by varying the protein type and thermal history, as well Pr:Ps.

2 Materials and Methods

2.1 Chemicals

Fonterra Cooperative Ltd. (Auckland, NZ) supplied WPI and SC, and Bronson and Jacobs Ltd. (Auckland, NZ) supplied GA. Sigma-Aldrich (St. Louis., MO, USA) supplied all other chemicals, which were of analytical grade.

2.2 Preparation of solutions

SC, WPI, and GA were dissolved in Milli-Q® water and neutralized to pH 7.0, as previously reported (Loveday et al., 2013). Where required, solutions of 1% w/w protein in 50 mL plastic tubes were preheated in a water bath (Lab Companion BS-11, Jeio Tech, Seoul, Korea) at 80 ± 0.2 °C for 30 min and then cooled to room temperature. After mixing, solutions of protein(s) and GA in small screw-top plastic bottles were heated in a water bath at 80 ± 0.2°C for 30 min and then cooled to room temperature. The experimental protocol is depicted schematically in **Figure 1**, which shows that protein solutions were either mixed with GA then heated or preheated alone, mixed with GA then heated again.

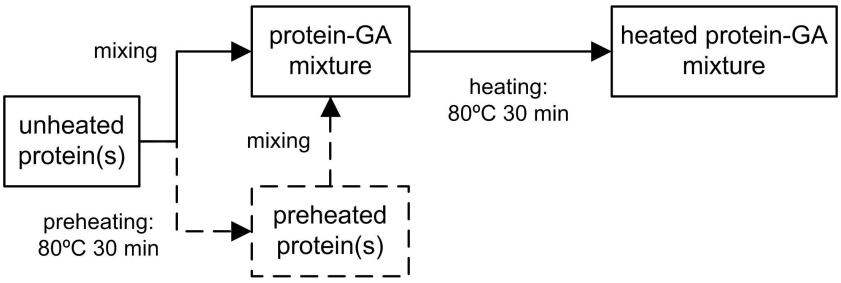


Figure 1. Schematic of the experimental protocol for sample preparation.

Where specified, heated mixtures were centrifuged at 4000 x g for 15 min at room temperature to sediment aggregates. All percentages given in the text are % w/v unless otherwise specified, and the '%' sign is omitted when referring to protein-polysaccharide mixtures, e.g. 0.3WPI|0.2SC|2.5GA refers to a mixture containing 0.3% w/v WPI, 0.2% w/v SC and 2.5% w/v GA.

2.3 Particle size and ζ-potential analysis

A Malvern Zetasizer Nano ZS (Worcestershire, England) was used to measure the size and ζ-potential of aggregates using dynamic light scattering (DLS) and cumulants analysis, as described previously (Loveday et al., 2013). The measurement temperature was 20 °C in all cases, and samples were not diluted before measurement.

2.4 Transmission electron microscopy (TEM)

Negative staining and microscopy followed published methods (Loveday, Wang, Rao, Anema, & Singh, 2012). Briefly, a drop of sample was placed on a TEM grid for 5 min then drained

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away from the edge of the grid with filter paper. A drop of uranyl acetate stain was applied for 5 min then drained with filter paper, and finally the grid was examined in the microscope.

2.5 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was carried out using a Mini Protean® system (Bio-Rad, Hercules, CA, USA), using gels prepared following the method of Manderson, Hardman and Creamer (1998). Electrode buffer consisted of 1g SDS, 3g Tris, and 14.4 g glycine, dissolved in 1 L Milli-Q® water and adjusted to pH 8.3. Each 100 mL of sample buffer contained 12.5 mL 0.5 M Tris (pH 6.8), 25 mL glycerol, 2 g SDS, and 10 mg Coomassie brilliant blue G250. For reduced SDS-PAGE, sample buffer also contained 5% v/v of β-mercaptoethanol. Staining solution contained 10% v/v acetic acid, 20% v/v isopropanol and 0.3% w/v Coomassie brilliant blue R. Destaining solution was 10% v/v acetic acid and 10% v/v isopropanol. For centrifuged samples, the pellet was dispersed in 1 mL of sample buffer and diluted 10-fold. PAGE bands were identified as particular proteins on the basis of published work (Anema, 2009a; Chevalier, 2011; Dupont, Grappin, Pochet, & Lefier, 2011).

3 Results

3.1 Effect of protein preheating in protein-only controls

Preheating proteins alone or in combination led to apparent changes in z-average particle diameter (**Table 1**), but analysis of the derived count rate (DCR) revealed some unexpected subtleties. Heating 1% WPI alone decreased z-average diameter from 150 nm to 116 nm, but increased the DCR 7-fold, which is not consistent with a simple decrease in particle size.

Table 1. Effect of preheating (30 min at 80°C) on the size of aggregates and derived count rate in 1% WPI, 1% SC or a 0.4% WPI + 0.6% SC mixture. Figures are the mean from 2-4 replicates, where variation among replicates was on the order of 1% of the mean.

WPI (% w/v)	SC (% w/v)	Z-average diameter (nm)		derived count rate (kilo counts per second)	
		unheated	heated	unheated	heated
1	0	150 (0.40)[a]	116 (0.39)	18,307	129,848
0	1	182 (0.22)	199 (0.21)	25,140	53,256
0.4	0.6	-[b]	192 (0.26)	-	59,992

a: figures in brackets are polydispersity indices.

b: not tested

The most likely explanation is that a small number of aggregates with diameter 100-200 nm were present in the unheated sample, as well as a large number of much smaller molecules and aggregates. The intensity of light scattered by small particles is proportional to the sixth power of diameter, so the contribution of intensities from these small particles/molecules to the overall scattering would be insignificant. The increase in DCR indicates that heating caused an increase in size and/or quantity of aggregates. We believe that the molecules and very small particles aggregated into particles around 100 nm in diameter, balancing the influence from larger aggregates and decreasing the z-average size.

Preheating 1% SC alone increased the z-average diameter from 182 nm to 199 nm, but the 112% increase in DCR in the face of only 9.3% change in z-average diameter suggests that

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particles much smaller than 182 nm had aggregated during heating, as with WPI. This phenomenon, which we have observed before (Loveday et al., 2013), highlights the fact that DLS estimates of particle size are influenced by both the size of particles and the number of particles, and that caution must be exercised when two or more particle populations with very different sizes may be present.

3.2 Effect of protein preheating in mixtures of protein and GA

It was evident from the visual appearance of samples (**Figure 2**) that both the preheat treatment and the proportions of WPI and SC affected particle size and phase behaviour when GA was present. Increasing the proportion of WPI made samples more turbid, and preheating WPI in the absence of SC led to precipitation when 0.3% or 0.4% WPI was present. Heating GA with 0.3% WPI and 0.2% SC that were previously preheated together did not result in a precipitate, suggesting that SC reduced aggregation of whey proteins. Particle size data (**Figure 3**) showed that increasing particle sizes were responsible for the increasing turbidity. Preheating SC alone had almost no effect on either turbidity or particle size.

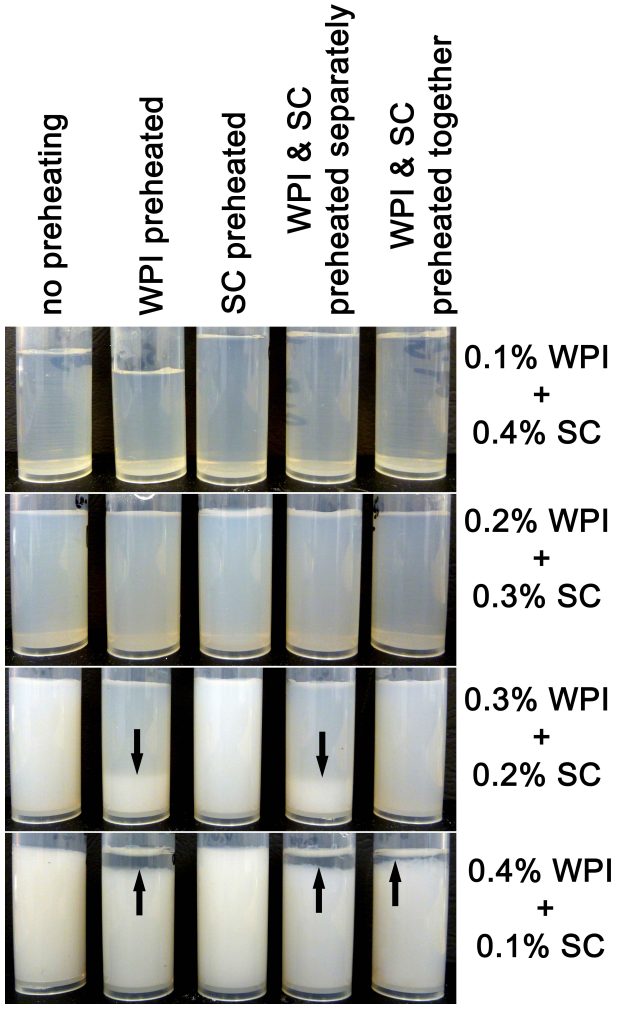


Figure 2. Appearance of heated mixtures of WPI, SC and GA, showing the effect of preheating WPI and/or SC and the effect of the WPI:SC ratio. All samples contained 2.5% GA. Arrows indicate precipitation.

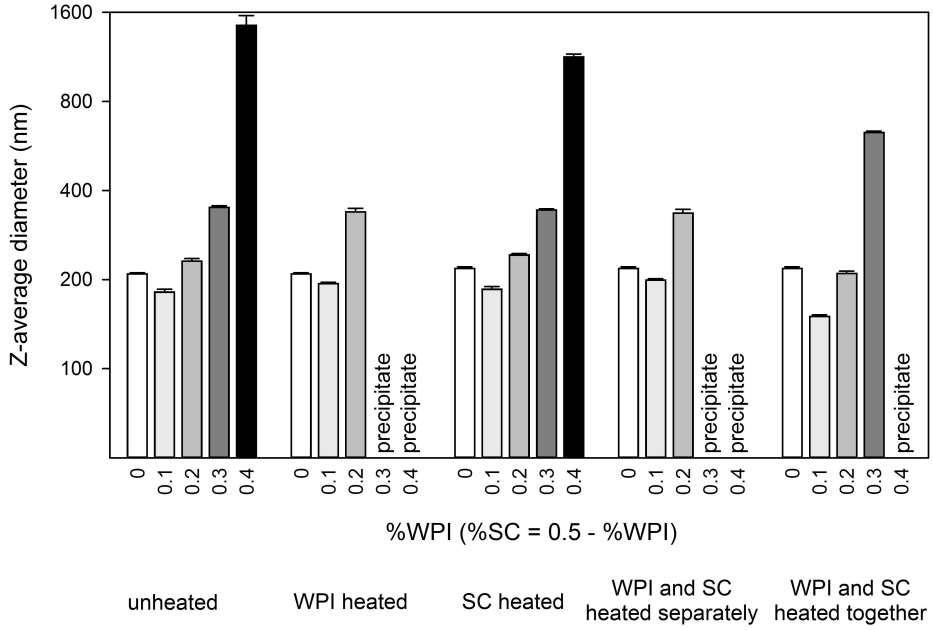


Figure 3. Size of colloidal particles formed by heating mixtures of WPI, SC and GA at 80°C for 30 min. The amount of WPI was varied between 0% and 0.4%, with SC making up the balance to 0.5%, and GA was 2.5% in all cases. Heat treatments of 30 min at 80°C were applied to WPI and/or SC in some cases, as shown by labels below the x-axis. Vertical bars are standard errors of 4 to 8 measurements.

3.3 Statistical testing of reproducibility and time effects

To check the reproducibility of results, the experiment measuring the effect of preheat treatment on particle size distribution was replicated for 0.2WPI|0.3SC|2.5GA and 0.1WPI|0.3SC|2.5GA. ANOVA showed some significant differences in z-average particle diameter (**Table 2**), but mean differences were only 10-20 nm, or ≤5%, and were not consistently positive or negative. Tukey's pairwise comparisons of treatment means showed no significant replicate effects. The effect of storing samples at 4°C for 6 days was tested with a similar analysis. Storage significantly increased the particle size for 0.1WPI|0.4SC|2.5GA ($p = 0.029$), but the difference was <2% on average, and pairwise comparisons of treatment means again showed no significant effects.

Table 2. Statistical analysis of differences in particle size and polydispersity due to independent replication or storage. Replication effects were tested by conducting 2 complete experiments in subsequent weeks, and storage was at 4°C for 6 days, i.e. a set of samples was analysed before and after storage. Each data set comprised 4 measurements for each of 5 preheat treatments, and data were analysed by two-way ANOVA. Preheat treatment effects were highly significant ($p < 0.0005$) in all cases.

		Z-average diameter (nm)	polydispersity index
effect	mixture	ANOVA p-values	
replication	0.1WPI|0.4SC|2.5GA	<0.0005	0.369
	0.2WPI|0.3SC|2.5GA	0.006	0.667
storage	0.1WPI|0.4SC|2.5GA	0.029	0.911
	0.2WPI|0.3SC|2.5GA	0.630	0.455

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3.4 Morphology of protein-GA aggregates

TEM on 0.2WPI|0.3SC|2.5GA with different preheat treatments revealed subtle differences in particle morphology and clustering behaviour (**Error! Reference source not found.**), but relatively consistent particle size, in agreement with DLS results (**Figure 3**). Two main morphology types were seen: almost round/spherical particles 50-200 nm in diameter with a smooth border (designated 'type A') and elongated particles that varied in length from a few tens of nm to approximately 100 nm and had an irregular border ('type B'). Type A particles were very similar to pH-induced SC-GA aggregates (Ye, Flanagan, & Singh, 2006). Type B particles were particularly prevalent when WPI alone was preheated (**Error! Reference source not found.**C) or WPI and SC were preheated separately (**Error! Reference source not found.**E), whereas type A particles predominated for other preheating treatments.

Particles sometimes clustered together, and the size and 'tightness' of clusters varied. Little clustering was seen when neither WPI nor SC were preheated (**Error! Reference source not found.**A and B), or when SC alone was preheated (**Error! Reference source not found.**D), whereas clusters were larger when WPI was preheated. This was reflected in slightly larger z-average particle size (**Figure 3**) and higher polydispersity (data not shown) for these samples.

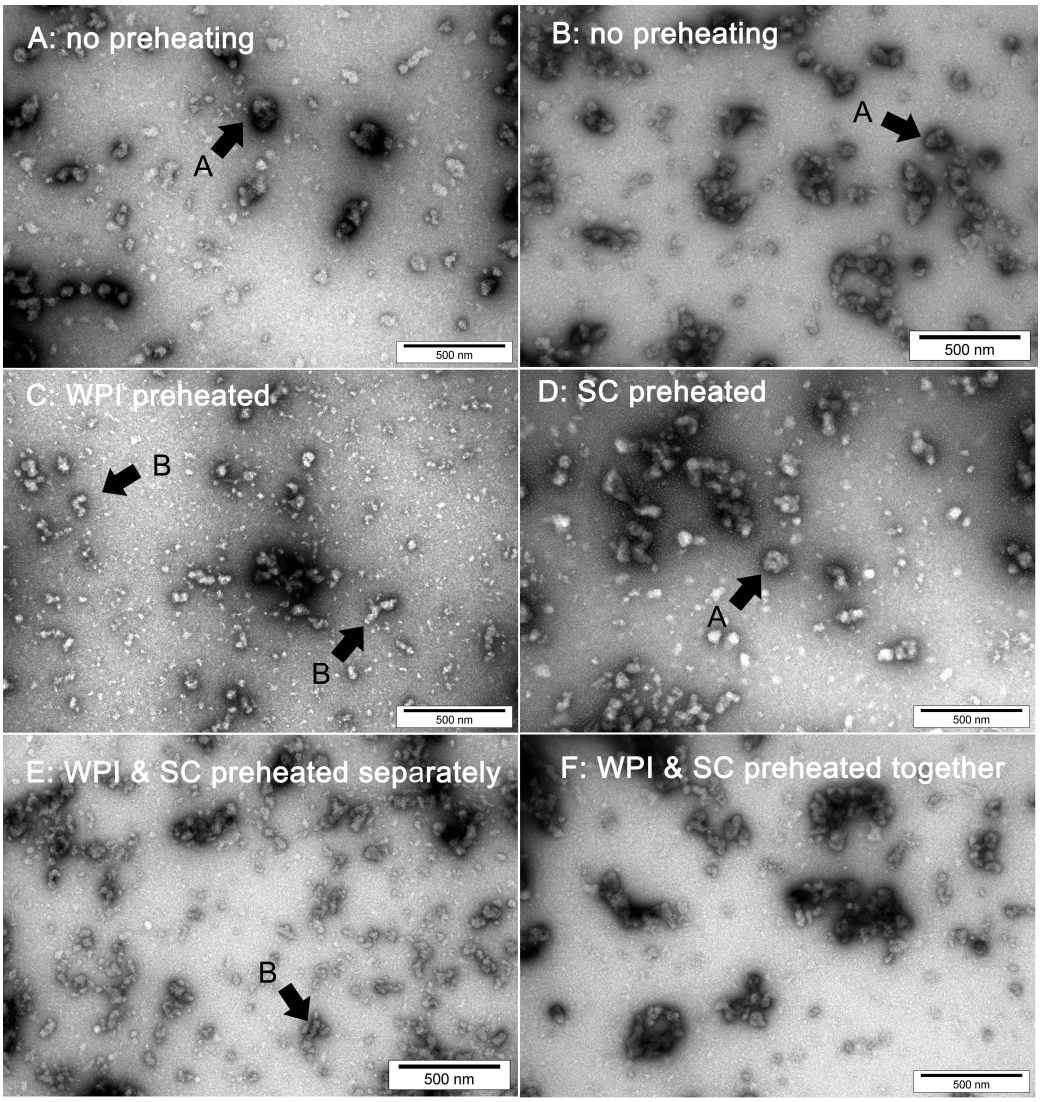


Figure 4. TEM images of particles formed by heating 0.2% WPI, 0.3% SC and 2.5% GA for 30 min at 80°C. Arrows indicate some examples of type A and type B particles, as defined in the text. Different panels show the effect of preheating WPI and/or SC for 30 min at

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80°C prior to mixing with GA. Note that panels A and B are show images from different locations on the same TEM grid.

3.5 Covalent bonding among proteins

Error! Reference source not found. shows the effect of preheating on protein aggregation, using SDS-PAGE. Heating WPI caused extensive polymerisation of β-lactoglobulin, bovine serum albumin (BSA) and α-lactalbumin (**Error! Reference source not found.**A lanes 1 and 2). Aggregation was fully reversed by the reducing agent (**Error! Reference source not found.**B), which shows that aggregates were held together by disulphide bonds. Comparing lanes 3 and 4 of **Error! Reference source not found.**A (1% SC unheated or heated) shows a difference in overall intensity. However relative band intensities within each lane are approximately the same, and no aggregated material appears in the stacking gel, so the difference is probably due to slight differences in loading volume or dilution, rather than covalent aggregation. When WPI was heated in the presence of SC, aggregation was slightly less than when the two were heated separately (compare lanes 5 and 6 of **Error! Reference source not found.**A), and aggregates in mixtures of WPI and SC were again disulphide bonded. Preheating had a limited effect on particle size in the absence of GA (**Table 1**).

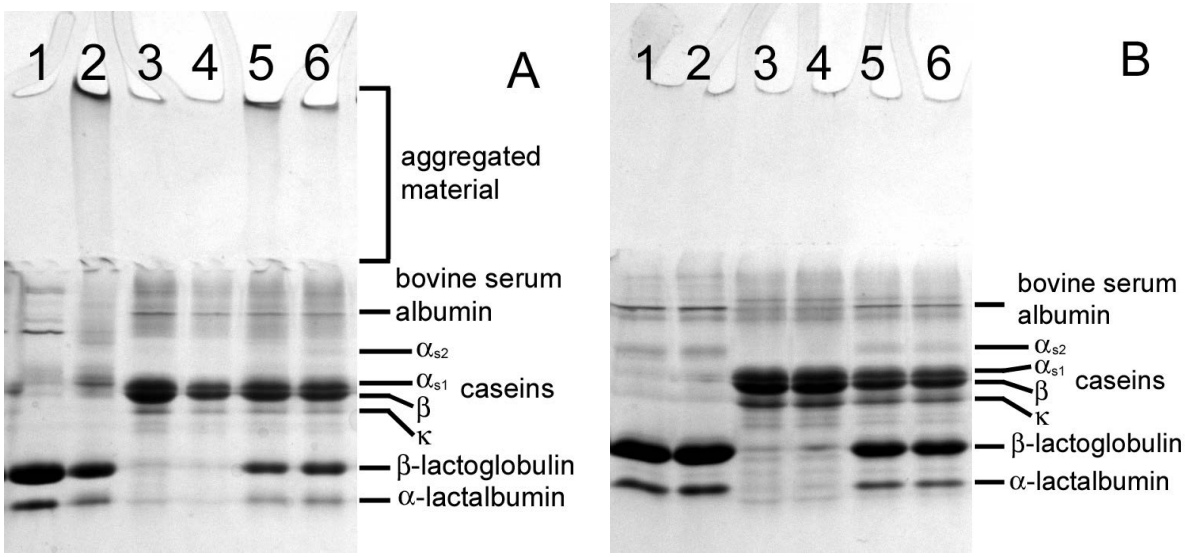


Figure 5. SDS-PAGE (A) and reduced SDS-PAGE (B) of WPI and SC before and after preheating. 1: 1% WPI, 2: 1% WPI preheated, 3: 1% SC, 4: 1% SC preheated, 5: 0.4% WPI and 0.6% SC preheated separately, 6: 0.4% WPI and 0.6% SC preheated together.

Centrifuging colloidally-aggregated suspensions of 0.2WPI|0.3SC|2.5GA (with or without protein preheating) produced a large white pellet and transparent supernatant, and these were analysed with SDS-PAGE (**Figure 6**). The pellet dispersed in sample buffer was diluted 10-fold more than uncentrifuged sample or supernatant to account for much higher protein concentration.

In the absence of reducing agent (**Figure 6**A), a substantial amount of material did not enter the stacking gel. Even supernatant lanes showed stained material remaining in wells, indicating that some aggregated material was not sedimented by centrifuging. Reduced SDS-PAGE (**Figure 6**B) showed that β-lactoglobulin, α-lactalbumin, BSA and α_{s2}-casein were

preferentially accumulated in the pellet (i.e. the colloidal particles), and that preheating WPI and SC together did not alter the protein composition of colloidal particles.

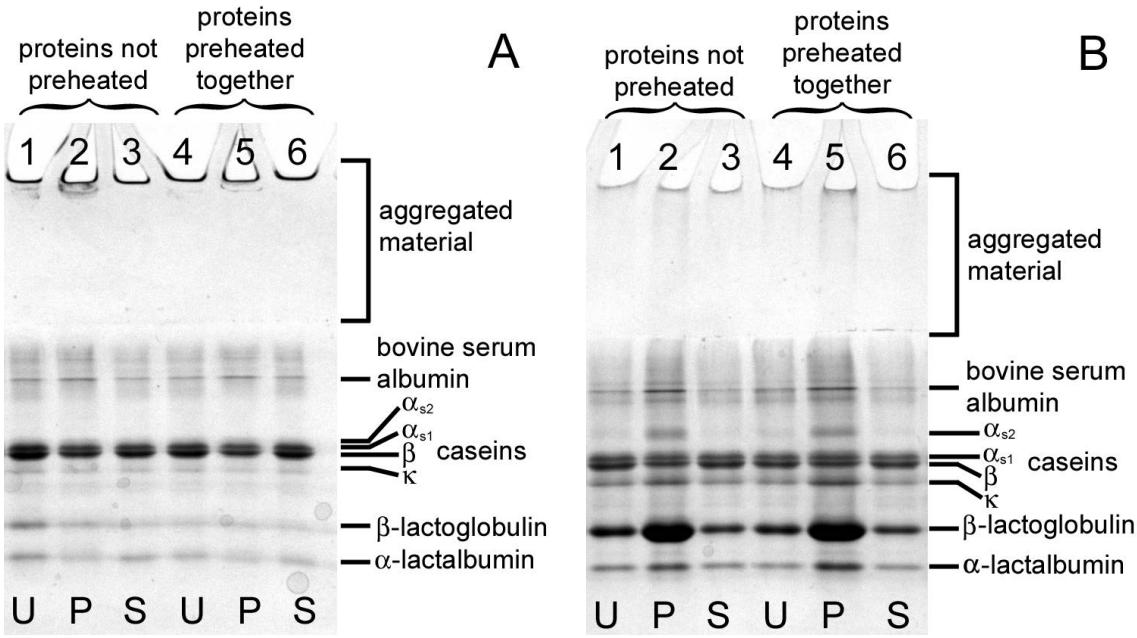


Figure 6. SDS-PAGE (A) and reduced SDS-PAGE (B) of 0.2% WPI + 0.3% SC + 2.5% GA mixtures (with or without protein preheating) separated by centrifuging. 1-3: not preheated, 4-6 WPI and SC preheated together; U: uncentrifuged, P: pellet, S: supernatant.

Complexation with GA slightly depleted whey proteins from the supernatant (**Figure 6**B), but most of the whey protein remaining in the supernatant was present as disulphide-linked aggregates (**Figure 6**A), which were apparently too small or too stable sediment under the centrifugation conditions. Complete separation of small aggregates from protein monomers clearly requires a stronger centrifugation treatment.

3.6 Effects of GA concentration on complexation and aggregate morphology

The amount of GA in solution had a strong impact on aggregation of 0.3WPI|0.2SC solutions. Increasing GA from 0.5% to 2.5% produced larger particles with less negative ζ-potential (**Figure 7**A and B), and this gave solutions a more turbid appearance, or led to partial precipitation (**Figure 8**). At pH 7, both GA and SC have a ζ-potential of approximately -30 mV (Ye et al., 2006) and the ζ-potential of the major whey protein - β-lactoglobulin - is approximately -20 mV (Schmitt et al., 2009).

The effect on particle size was small between 0.5% and 1.5%, although DCR did increase over this range **Figure 7**C), suggesting an increased number of particles. Further increases in GA led to substantial increases in particle size, and precipitation in some cases. This pattern was seen in all protein preheat treatments, but was more marked when WPI was preheated, though this was somewhat mitigated when SC was also present during preheating.

TEM images of 0.3% WPI + 0.2% SC + 0.5-2.5% GA confirmed the effect of GA on particle size (**Figure 9**), especially at 1.5 - 2.5%. The presence of more GA also drove a tendency towards more elongated particles with smoother, more rounded borders, especially within clusters. In all cases, particles were present as both loose clusters and separate individual particles.

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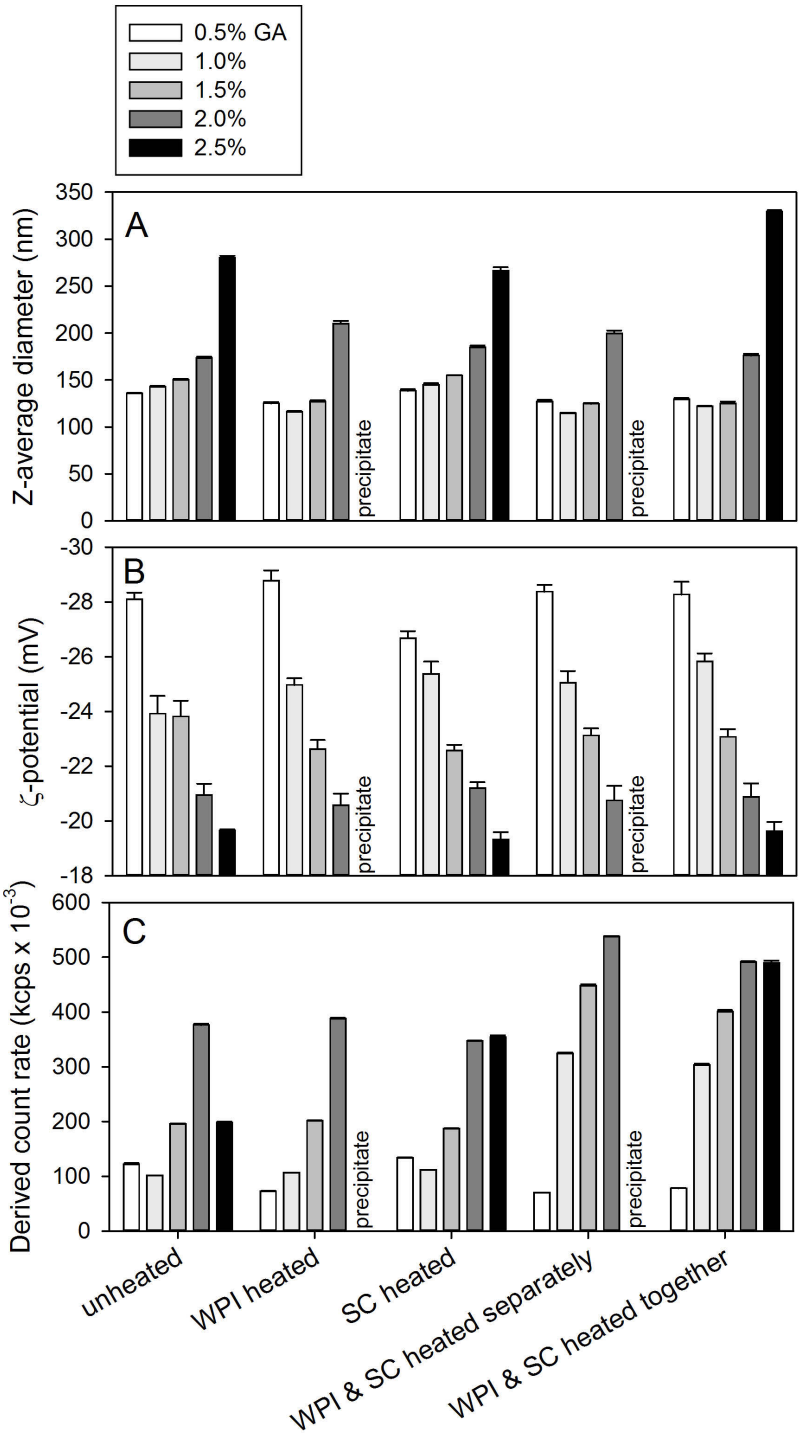


Figure 7. Effect of GA level and preheating treatment on particle size (A), ζ-potential (B) and derived count rate (C) in mixtures of 0.3% WPI + 0.2% SC + X% GA (where X ranges from 0.5-2.5 %) heated at 80°C for 30 min. Vertical bars are standard errors from four replicate measurements.

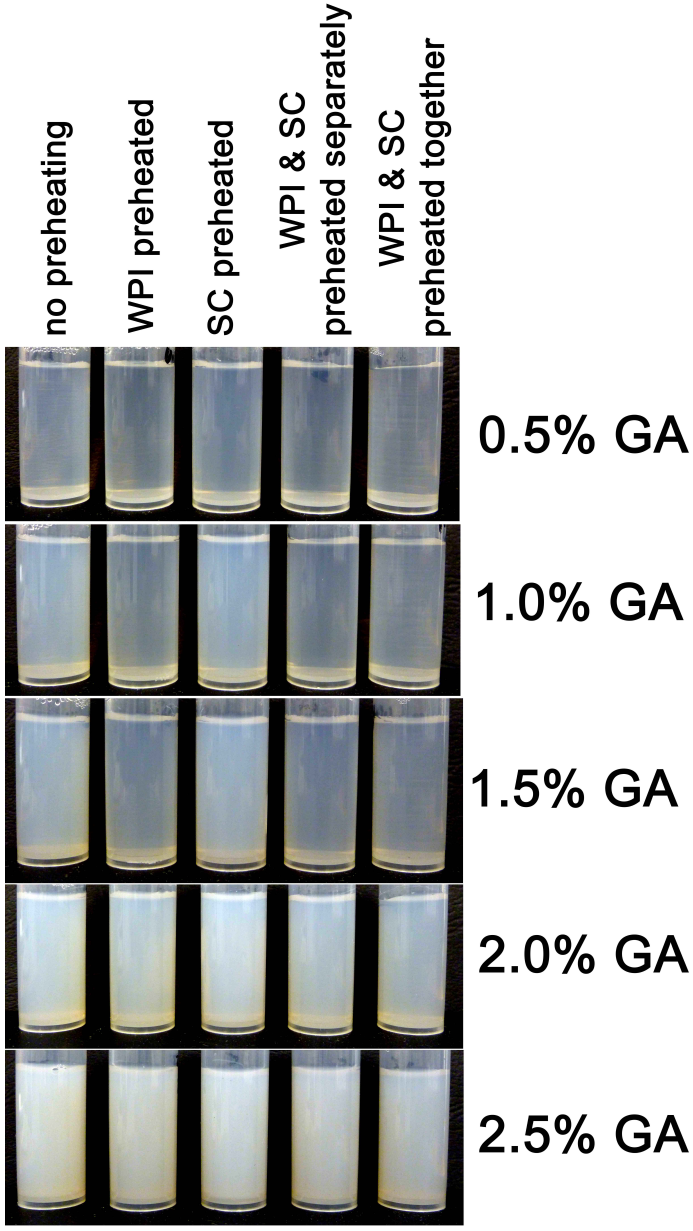


Figure 8. Appearance of 0.3% WPI + 0.2% SC +X% GA (where X varies between 0.5 and 2.5) after heating at 80°C for 30 min.

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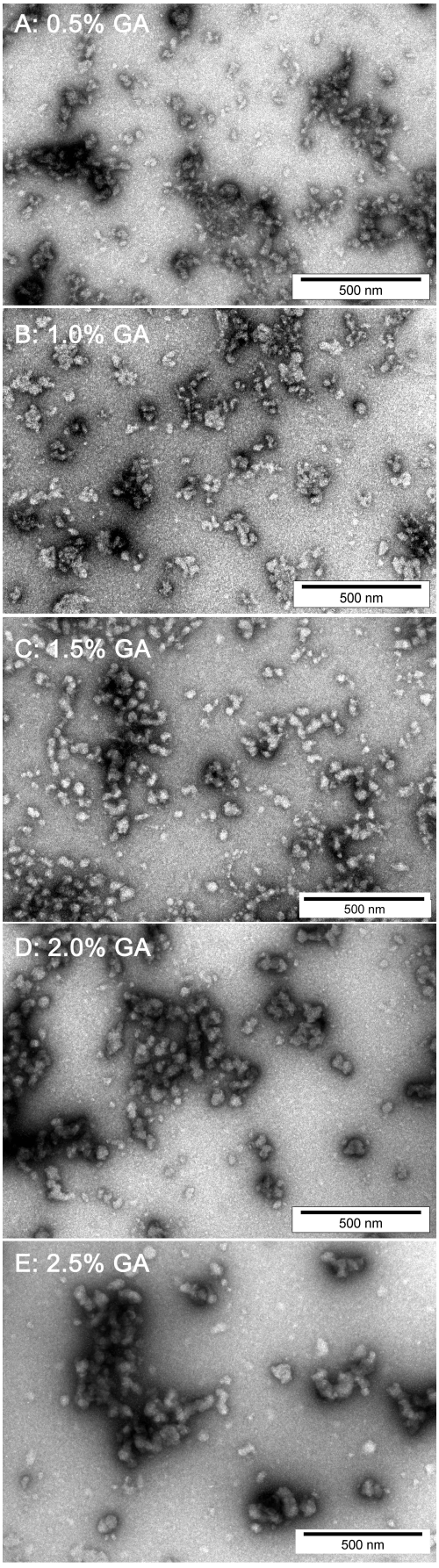


Figure 9. TEM images of 0.3% WPI + 0.2% SC +X% GA (where X varies between 0.5 and 2.5) after heating at 80°C for 30 min.

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4 Discussion

4.1 Effect of protein preheating and protein type

Whey proteins can polymerise through both covalent bonds (especially disulphide bonds) and noncovalent bonds (hydrophobic and electrostatic interactions, hydrogen bonding) (Havea, Watkinson, & Kuhn-Sherlock, 2009), and this polymerising ability was probably responsible for the increasing size of aggregates, higher turbidity and higher tendency to precipitate with increasing proportions of WPI in mixtures of 0.5% protein and 2.5% GA. This trend was particularly evident when WPI was preheated prior to mixing with SC and GA, which would have denatured proteins and exposed internal interaction sites.

The presence of SC during preheating of WPI decreased these tendencies, which is consistent with the chaperone-like effects of caseins reported in other studies on heat-induced protein aggregation (Kehoe & Foegeding, 2011; Yong & Foegeding, 2010). Preheating SC had no effect on the size of aggregates, which can be attributed to the reversible nature of hydrophobic heat-induced aggregation (Loveday et al., 2013; Ye et al., 2012) and the fact that caseins are intrinsically unstructured (Farrell, 2011). SDS-PAGE results confirmed that disulphide bonding played a role in aggregation of WPI|SC|GA mixtures, and complemented earlier observations (Loveday et al., 2013) that WPI|SC|GA mixtures aggregated irreversibly on heating, while aggregation of SC|GA at 80°C was reversed by cooling.

The differences in morphology with preheating treatment (**Error! Reference source not found.**) were too subtle and variable to be unequivocal, but the 'denser' appearance of particles when WPI was preheated may be attributable to more disulphide bonds within aggregates as a result of more extensive denaturation during preheating.

Reduced SDS-PAGE analysis of centrifugally separated 0.2WPI|0.3SC|2.5GA samples (**Figure 6**B) demonstrated the presence of all proteins in sedimented particles, but a lower relative concentration of α_{s1}- and β-casein. These caseins lack cysteine residues, which may explain why they did not aggregate with cysteine-containing caseins and whey proteins. Preheating apparently did not change the protein composition of aggregates.

The distribution of protein between aggregates and supernatant cannot be directly calculated from SDS-PAGE results, but there appears to be a significant amount of protein remaining in the supernatant after centrifuging. This may be in the form of soluble species, or aggregates that were too small or too stable to be sedimented during under the applied centrifuge treatment. Comparing the 'uncentrifuged' and 'supernatant' lanes in **Figure 6**B shows that supernatants were slightly depleted of BSA and β-lactoglobulin, and this is consistent with the prominent appearance of these proteins in pellets. A more quantitative investigation is needed to ascertain the yield of aggregates, and a faster centrifuging step would sediment small aggregates more effectively.

4.2 Effect of Gum Arabic Concentration

Increasing concentrations of GA at constant protein content produced larger particles with slightly less negative ζ-potential. However the conductivity of mixtures (as measured with the zetasizer) consistently increased in a stepwise fashion as GA concentration was increased, e.g. from 322 ± 0.4 $\mu S.cm^{-1}$ to 796 ± 1.2 $\mu S.cm^{-1}$ with no protein preheating. To put this in perspective: adding 6 mM NaCl increased conductivity by approximately 800 $\mu S.cm^{-1}$. Increasing conductivity suggests increasing ionic strength, and there are several possible explanations.

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Any polyelectrolyte dried from solution will contain adsorbed counterions, and may also contain free salts, depending on the purification process. GA solutions were somewhat acidic after hydration (pH below 6), and were neutralised with NaOH prior to mixing, incidentally increasing ionic strength. The effect of GA concentration was therefore statistically confounded with the effect of ionic strength. Decoupling these effects requires further experiments involving dialysis of GA before mixing with proteins.

It is useful here to compare our results with investigations at similar pH but lower biopolymer concentration, where the effect of salts in stock solutions will be less marked. Our earlier work (Ye et al., 2012) at 0.1% SC showed that increasing GA concentration from 0.1% to 0.5% dramatically enhanced heat-induced aggregation. Thus, while salts in GA solutions will have a stronger effect in the present work (0.5 – 2.5% GA), they are not the only factor driving aggregation with increasing GA. We suggest that GA molecules may be able to link smaller aggregates, e.g. separate hydrophobic regions on a given GA protein moiety could interact with proteins in two or more smaller aggregates.

Here we have shown how the makeup of whey protein-casein mixtures (proportions and prior denaturation/aggregation state) affects the properties of heat-induced aggregates with GA at neutral pH. Extensive disulphide bonding occurs both in preheating of proteins alone and during subsequent heating with GA, and disulphide-bonded aggregates do not dissociate on cooling. The size and charge of milk protein-GA particles can be tailored by choosing appropriate ratios of WPI, SC and GA.

In **Figure 10** we have schematically illustrated how the different bonding abilities of whey proteins and caseins influence the structure of aggregates with GA. With the exception of minor components, caseins are largely unable to polymerise *via* disulphide bonds, and noncovalent bonding alone is not strong enough to hold together heat-induced aggregates with GA on cooling. The covalent and noncovalent bonding abilities of whey proteins make for stronger networks that persist on cooling, and caseins can become entrapped within whey protein-GA aggregates.

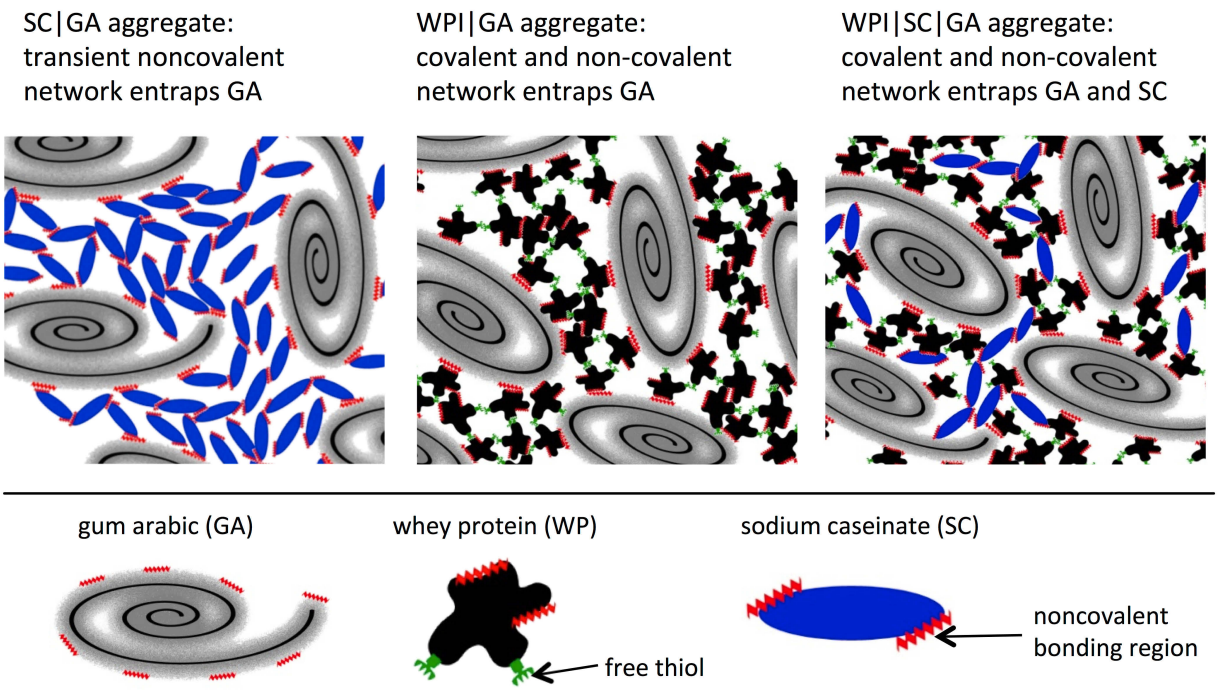


Figure 10. Schematic illustration showing the hypothetical structure of protein-gum arabic aggregates.

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The literature is equivocal on whether GA glycoprotein contains cysteine residues: some analyses have found none (Goodrum, Patel, Leykam, & Kieliszewski, 2000; Mahendran, Williams, Phillips, Al-Assaf, & Baldwin, 2008), whilst others report cysteine-containing fractions of gum (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). Disulphide bonding between gum arabic and proteins is a possibility, but has not been reported to date.

5 Conclusions

Our investigations have laid the groundwork that will allow the tailored fabrication of colloidal protein-polysaccharide particles with the appropriate size and physicochemical properties for food and/or pharmaceutical applications. For example, careful control of particle surface charge density is important for ensuring stability throughout processing, and size distribution will have a large impact on pharmacokinetics in bioactive delivery applications. The natural, food-safe and cost-effective materials used in these particles make them a good candidate for replacing synthetic alternatives. However further information about particle stability under a range of chemical and physical conditions is needed before this potential can be realised.

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