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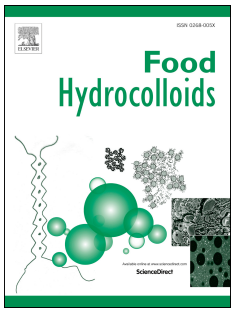

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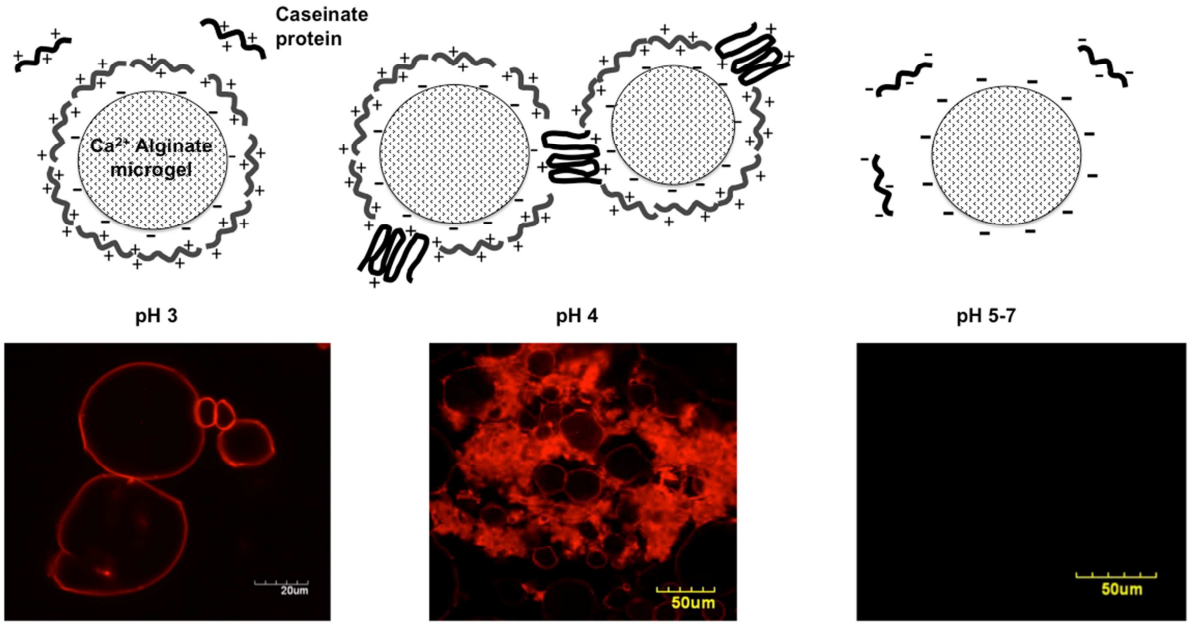

Visualizing the interaction between sodium caseinate and calcium alginate microgel particles

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Abstract

In this study, the pH dependent adsorption of sodium caseinate onto the surface of micron-sized calcium alginate microgel particles (20-80 μm) was evaluated by electrophoretic mobility measurements (ζ-potential), microscopy, protein assay and a protein dye binding method. ζ-potential measurements and protein assay results suggested that protein adsorption occurred due to electrostatic complexation between sodium caseinate and calcium alginate and was pH dependent. Results of protein dye binding method were in agreement with those of protein assay and ζ-potential measurements. Confocal laser scanning and fluorescence microscopy confirmed the presence of protein layer on the surface of alginate microgel particles at pH 3 and 4. Micrographs from transmission electron microscopy revealed a protein coating with a thickness of ~ 206-240 nm on the gel particle surfaces.

Keywords

Calcium alginate microgel; protein polysaccharide complexation; alginate caseinate interaction.

1. Introduction

Protein-polysaccharide interactions have been extensively studied over the years due to their wide range of applications in the food industry. Protein-polysaccharide interaction forms the basis of layer-by-layer deposition where multiple biopolymer coatings are electrostatically deposited onto the surface of a non-colloidal core, such as an emulsion droplet (Guzey & McClements, 2006). Alginate is a widely used polysaccharide and is made up of β-D-mannuronate and α-L-guluronate monomers. In the presence of divalent cations such as calcium ions, the carboxyl groups from the

guluronate monomers to bind to the calcium ions forming a gel network. Alginate as its sodium salt, sodium alginate, is able to form complex with common food proteins such as β-lactoglobulin (Harnsilawat, Pongsawatmanit, & McClements, 2006), lactoferrin (Tokle, Lesmes, & McClements, 2010), and whey proteins (Perez, Carrara, Sanchez, & Rodriguez Patino, 2009). However, the interaction of caseinate with calcium alginate gel has not been reported to date.

Although protein-alginate complexes are formed by a number of different non-covalent intermolecular interactions such as hydrogen bonding, van der Waal forces, hydrophobic interaction and ionic bonding, the mechanism of protein-alginate interaction is dominated by non-covalent electrostatic interaction (Doublier, Garnier, Renard, & Sanchez, 2000; McClements, 2006). The negatively charged carboxyl (-CO_2^-) groups contribute to the overall anionic charge of the ungelled biopolymer, which allows electrostatic binding with cationic proteins. Thus it is only logical to assume that alginate gel will also be negatively charged. Polycations such as chitosan and poly-L-lysine have been shown to adsorb onto the surface of calcium alginate gel (Gåserød, Smidsrød, & Skjåk-Bræk, 1998; Strand et al., 2002).

Common methods used to characterise and identify protein-polysaccharide interactions include electrophoretic (ζ-potential) measurements and scattering techniques (Doublier et al., 2000). Microscopic techniques such as transmission electron microscopy (TEM) and confocal light scanning microscopy (CLSM) can provide visual evidence of interactions based on changes in morphology, layer thickness, shape and distribution of colloidal particles (Podskoçová, Chorvát, Kolláriková, & Lacík, 2005). Weber et al. (1999) and Vandenbossche, Van Oostveldt, and Remon (1991) showed the possibility of using dye-labeled alginate gels to visualize its interaction with poly-L-lysine using CLSM. However, the covalently bound dye may alter the charge and solubility of the polymer (Strand, Morch, Espevik, & Skjåk-Bræk, 2003)

To further explore the use of microscopy techniques in protein-alginate gel studies, we attempt to visualize the interaction between a model protein and the calcium alginate gel. A natural ingredient that is widely used in the food industry, sodium

caseinate, was chosen as a model protein. Calcium alginate gel in the form of spherical microgel particles were produced by the novel spray aerosol method developed in our laboratory. The caseinate-calcium alginate interaction was evaluated by ζ-potential measurements, microscopy techniques, protein assay and dye-binding method.

2. Materials and Methods

2.1 Materials

Calcium alginate microgel particles were produced with sodium alginate (GRINSTED® Alginate FD 155, Danisco, Australia) and calcium chloride. Spray-dried sodium caseinate (NatraPro) was provided by Murray Goulburn Nutritionals (Australia). Rhodamine-B (Sigma Aldrich, Australia) was used to stain protein. Bradford reagent (Sigma Aldrich, Australia) was used for protein assay. Bovine serum albumin (BSA) (Sigma Aldrich, Australia) was used to construct a protein standard curve for the Bradford protein assay. Deionised water was used as sample diluent throughout the experiment.

2.2 Calcium alginate microgel particles preparation

The calcium alginate microgel particles used in this study were produced by the spray aerosol method as described in International Patent No. 062254, 2009 (Bhandari, 2009) and Sohail et al. (2011) (Figure 1). A fine aerosol mist of 0.1 M calcium chloride solution was created in the cylindrical encapsulation chamber using an air atomising nozzle operated at liquid and air pressure of 1.5 and 2 bars. Pressurised (0.5 MPa) 2% (wt/wt) sodium alginate solution was counter currently atomised in the chamber using compressed air at 0.5 MPa. The resulting alginate microgel particles (20-80 µm diameter) were collected from an outlet at the base of the encapsulation chamber. Alginate microgel particles were filtered (Advantec 5C filter paper) (<5 µm pore size) under vacuum and washed twice with deionised water to remove excess Ca^{2+} ions.

2.3 Sample preparation for ζ-Potential measurement

A stock solution containing 1% (wt/wt) sodium caseinate was prepared in deionised water. An alginate microgel dispersion was prepared by suspending 10% (wt/wt)

filtered alginate microgel particles in deionised water. The protein and alginate microgel stock solutions were further diluted into five 20 mL aliquots each of:

(1) 0.02% (wt/wt) sodium caseinate solution;

(2) 0.10% (wt/wt) alginate microgel solution; and

(3) 0.02% (wt/wt) sodium caseinate+0.10% (wt/wt) alginate microgel mixture

The aliquots were adjusted to the intended pH (3, 4, 5, 6 and 7) by adding 0.1 M NaOH or HCl.

2.4 ζ-Potential measurements

The ζ-potential of the samples was determined using NanoS Zetasizer (Malvern Instruments Ltd., UK). The Smoluchowski model was used to calculate ζ-potential. The sample refractive index and absorption was set at 1.33 and 0.01 respectively. Three readings were obtained for each sample and the experiment was repeated thrice. Preliminary trials showed that the excess caseinate molecules (if present) did not significantly affect the ζ-potential measurements. Hence the samples were not centrifuged and washed prior to ζ-potential measurements to remove excess caseinate. The samples were measured without any dilution because initial trials showed that the sample ζ-potential values did not change up to a dilution factor of 1:100.

2.5 Protein determination

Protein concentration was determined using Bradford micro assay (Bradford, 1976). The protein and alginate microgel stock solution were diluted as in Section 2.4. The protein and protein-alginate microgel aliquots were adjusted to the intended final pH (3 to 7) by the addition of 0.1 M NaOH or HCl solutions and centrifuged at 2500 *g* for 5 minutes. The supernatant of each sample was diluted 40 times with deionised water. 1 mL Bradford reagent was added to 1 mL diluted supernatant in a disposable cuvette. The mixture was incubated at room temperature for 5 min and the absorbance measured at 595 nm in a UV-Vis spectrophotometer (Pharmacia Ultraspec III, U.S.A). A protein standard curve was constructed using known concentrations (2.0-10.0 µg/mL) of BSA. The experiment was repeated thrice. The statistical significance of difference between protein concentrations was assessed by one-way ANOVA using Tukey's test at 95% confidence level (SPSS Ver. 20).

2.6 Microscopic Analysis

2.6.1 Confocal Laser Scanning Microscopy (CLSM)

CLSM was carried out using an Olympus Fluoview FV1000 BX2 upright confocal laser scanning unit with a 60x oil immersion objective lens. An air-cooled Ar/Kr laser (514 nm) was used as the source of excitation. Sodium caseinate was stained with 0.1% (wt/wt) Rhodamine B solution.

2.6.2 Light (LM) and fluorescent (FM) microscopy

Bright field and fluorescence micrographs of alginate microgel samples were obtained using an Olympus BX51 microscope with a 60x oil immersion objective lens. Sodium caseinate was stained with 0.1% (wt/wt) Rhodamine B solution.

2.6.3 Transmission electron microscopy (TEM)

Samples were suspended in 10% bovine serum albumin made up with phosphate buffer solution (PBS) in a membrane carrier (100 µm) and frozen in a high-pressure freezer (Leica EMPACT 2). Freeze substitution of frozen samples was done by suspending samples in 1% osmium tetroxide, 0.5% uranyl acetate and 5% water in acetone solution and allowing them to come to -20^{o}C over 1.5 h while agitating on an orbital shaker (McDonald & Webb, 2011). Samples were then brought quickly to room temperature and washed in acetone. Samples were embedded in EPON resin (standard recipe) and polymerised at 60^{o}C for 2 days. Thin sections (50-60 nm) were cut using an ultramicrotome (Leica Ultracut UC6) and picked up on formvar coated copper grids. Mounted samples were viewed in a transmission electron microscope (JEM-1010, JEOL, Tokyo) operated at 80 kV.

2.7 Particle size measurements

Particle size of alginate microgels was measured using the Malvern Mastersizer 2000 (Malvern Instruments, UK), which was capable of detecting particles of 0.02 to 2000 µm. Samples were under constant agitation (2000 rpm) during measurement. The sample refractive index and absorption was set at 1.33 and 0.01, respectively. An average from three readings was taken for each sample.

3. Results and Discussion

Preliminary experiments showed that 0.10% (wt/wt) of alginate microgels was the minimum concentration required to give a consistent ζ-potential reading. In a separate experiment, 0-0.05% (wt/wt) of sodium caseinate was allowed to interact with 0.10% (wt/wt) of alginate microgels at pH 3. From the ζ-potential values, it was found that 0.02% (wt/wt) sodium caseinate was the minimum amount required to completely coat the microgel surface. Thus, this concentration was chosen in this research work.

3.1 Determination of protein polysaccharide interaction by ζ-potential measurement

Alginate microgel particles were negatively charged across all measured pH ranging from 3 to 7 which was as expected from polyanions (Figure 2). At the same time, ζ-potential values decreased from -21.30 to -29.04 mV as pH increased from 3 to 7. The ζ-potential values for the microgel particles we obtained were comparable to values from other authors: -22.8 to -23 mV (Silva et al. 2011), -21.9 mV (Saeed et al. 2013) and -34 mV (Aynie et al. 1999). In comparison, the ζ-potential of sodium alginate solution has been shown to be close to -60 mV (Pallandre, Decker, & McClements, 2007). The difference in charge is likely due to the cation-induced gelling mechanism in the alginate gel. The negative charge of the alginate polymer originates from the negative carboxyl ($-CO_2^-$) groups (Donati and Paoletti, 2009). In the formation of calcium alginate gel, Ca^{2+} ions interact with the negatively charged carboxyl groups from the guluronic blocks of the alginate to form the “egg-box” structure (Mørch, Donati, & Strand, 2006). As more Ca^{2+} ions interact with the available guluronic blocks on the alginate polymer strand, the number of free carboxyl group decreases, resulting in a lower charge density. Hence the ζ–potential of the microgel particles, which are attributed only to the carboxyl groups from the manuronic residues, is likely to be lower.

In the sodium caseinate solutions, the charge reduced from 31.92 to -38.73 mV as pH was increased from 3 to 7 (Figure 2). Isoelectric point (pI) of sodium caseinate was estimated to be around 4.1, which falls into the pI range of pH 3.8-4.6 as reported in previous studies (Grigorovich et al., 2012; Pallandre et al., 2007). The pI of sodium caseinate exists in a range because different sources of sodium caseinate proteins can differ structurally in terms of the number of carboxyl and amine groups present in the protein structure (Ma et al., 2009).

In samples containing a mixture of sodium caseinate and alginate microgel particles, the ζ-potential (23.80 mV) of the mixture at pH 3 (at pH < pI) was lower relative to the ζ-potential (31.92 mV) of the pure protein solution (Figure 2). This decrease in ζ-potential suggests that there is an interaction between sodium caseinate and calcium alginate, which leads to a net increase in the microgel particle surface charge. Comparable observations by Pallandre et al. (2007) showed that sodium alginate was able to complex with the interfacial proteins from sodium caseinate-stabilized oil emulsion at pH 3 and 4. Complexation between the biopolymers is the result of electrostatic attraction between the amine ($-NH_3^+$) groups of the proteins and the carboxyl ($-CO_2^-$) groups of the polysaccharide (Benichou, Aserin, & Garti, 2002).

At pH 4 (Figure 2), sodium caseinate was close to its isoelectric point and was partially precipitated as indicated by a ζ-potential of 1.14 mV. In the presence of sodium caseinate, the ζ-potential value of the alginate microgels (-23.80 mV) increased to -9.46 mV at pH 4. This suggests that the weakly cationic sodium caseinate protein below its pI was still able to be adsorbed onto the anionic microgel particle surface. This is a strong indication that electrostatic attraction is still occurring between exposed patches of amino ($-NH_3^+$) groups of the protein and carboxylate ($-CO_2^-$) groups of the alginate gel. In the past, other researchers have reported similar observations of electrostatic attraction between anionic polysaccharides and cationic proteins in oil emulsions at pH below the pI of proteins (Dickinson, 1995; Fang and Dalgleish, 1997).

At pH 5, 6, and 7, ζ-potential of sodium caseinate-alginate microgel particles mixture was no different than that of the protein solution (Figure 2). This suggests that at these pH conditions, the charge of sodium caseinate-alginate microgel mixture is dominated by the more negatively charged sodium caseinate and that no interaction has occurred between sodium caseinate and alginate microgel particles. As the pH conditions were above the pI of the protein and pKa of the polysaccharide, the strong electrostatic repulsion between the protein and polysaccharide will prevent complexation.

3.2 Determination of protein-polysaccharide interaction by protein assay

As sodium caseinate alone did not separate by centrifugation at 2500 *g*, only sodium caseinate bound to the heavier alginate gel particles will be removed from the supernatant after centrifugation. Hence, an assay of the residual protein levels in the supernatant can be used as evidence to support the observations from the ζ-potential measurements. After centrifugation, protein content in the supernatant of sodium caseinate-alginate microgel particle mixture was compared to the original amount of protein (0.02% wt/wt) added initially (Figure 3).

At pH 3, protein content in the supernatant was almost negligible (0.01 mg/mL) (Figure 3). The low protein concentration in the supernatant of the mixture was attributed to the complete adsorption of sodium caseinate onto alginate microgel particle surface and no excess protein was present. This confirms observations from preliminary experiments that showed the protein concentration was sufficient to completely coat the microgels. A similar reduction in protein levels was observed at pH 4, where protein precipitation had started to occur as the pH of the mixture was close to the pI of the protein. Centrifugation caused separation of these flocculates and thus, reduced the amount of protein left in the supernatant from 0.13 mg/mL to 0.03 mg/mL. The reduction in protein level at pH 4 was attributed to both complexation with alginate microgel particles and protein aggregation. At pH 5, 6, and 7, no significant differences ($p > 0.05$) were detected between the protein content of the supernatants of sodium caseinate solution and sodium caseinate-alginate microgel mixtures. These results suggest that no protein adsorption onto the microgel particles occurred at these pH levels, as the supernatant protein level was similar to the amount initially added into the mixture (Figure 3). This result demonstrates that measuring the amount of unbound protein can be used as a quick and effective method for determining the protein-polysaccharide interactions.

3.3 Determination of protein-polysaccharide interaction by microscopic techniques

The samples containing alginate microgel particles and sodium caseinate at pH 3 to 7 were further studied using different microscopic techniques (Figure 4). Micrographs from FM and CLSM confirmed the presence of adsorbed protein on the surface of microgels at pH 3. A well-defined, smooth and continuous protein layer was observed

under FM and CLSM. TEM images further confirmed the presence of a homogeneous protein coverage layer on alginate microgel particles surface at pH 3 (Figure 5). From the same TEM images, the protein layer was estimated to be around 206-240 nm thick. Dalgleish, Srinivasan and Singh (1995) reported that caseinate monolayer electrostatically adsorbed onto latex particles have a thickness of 10-12 nm thick while caseinate monolayer at the oil/water interface of oil/water emulsion droplets have been shown to be 10-15 nm thick (Dalgleish, 1993, Fang and Dalgleish, 1993). Hence, the thickness observed in this study may represent a multi protein layer on the surface of alginate microgel particles.

Flocculation of alginate microgel particles occurred at pH 3. CLSM images showed that when one or more microgel particles were in close proximity, an intense colouration occurred at their point of contact. The increased colour intensity indicates a higher concentration of protein, which suggests the presence of a weak inter-particle linkage or overlap of protein layers from separate microgel particles. It was observed that these flocculates were easily redispersed under light manual shaking and the mild shear forces present during particle size analysis. Volume weighted mean (D[4,3]) diameter of the coated microgels at pH 3 (61 µm) was slightly higher than the control samples (57 µm). The presence of a protein layer may have contributed to the slight increase in microgel size.

There are two possible explanations for these inter-particle linkages. Firstly, although there is sufficient protein to completely saturate the microgel surface, complete surface saturation did not occur rapidly. The adsorption of sodium caseinate proteins onto the microgel particle surface occurred less rapidly than microgel-microgel collision resulting in bridging flocculation (Figure 6) (Dickinson, Golding, & Povey, 1997). Secondly, it is postulated that microgel flocculation can be due to depletion flocculation. In the alginate microgel- sodium caseinate mixture, unabsorbed sodium caseinate in the continuous phase may lead to microgel flocculation due to the increase in osmotic pressure when free sodium caseinate is excluded from the small region surrounding each microgel particle (Eliot, Radford, & Dickinson, 2003).

At pH 4, FM and CLSM images confirmed the occurrence of complexation from the presence of adsorbed protein on surface of alginate microgel particles (Figure 4).

However, the protein layer was observed to be of uneven thickness, non-continuous and interspersed by aggregates of precipitated protein, which appears as a fuzzy mass. LM images indicated the occurrence of flocculation. Flocculation occurred because of weak electrostatic repulsion forces between microgel particles, due to low surface charge (-9.46 mV) (McClements, 2005). The presence of precipitated proteins also leads to bridging flocculation of microgel particles through the binding of precipitated proteins onto the surface of one or more microgel particle (Vincent and Saunders, 2011). As a result, D [4,3] of microgels increased to 120 µm compared to 54 µm for the control microgels at the same pH. At pH 5, 6 and 7, alginate microgel particles appeared as discrete particles under LM (Figure 4). Micrographs from FM and CLSM did not reveal any protein adsorption on the surface of the microgel particles at these pH conditions (Figure 4).

Fluorescent microscopy techniques (CLSM and FM) were able to show the distribution of the caseinate on the microgel surface. From the micrographs, it was apparent that florescence microscopy techniques can reveal a lot about the surface topology and distribution of the coated microgels. Because the labeling of the protein coating can easily be done, this technique can be used to study protein binding in other polymeric gel particles. Furthermore, TEM allows quantification of protein layer thickness. Future work can be done to find out if the protein thickness can be controlled and if so what will be the impact be on gel properties such as porosity. Although light microscopy was able to show clear indication of flocculation in some samples, it could not be used to detect protein-alginate interaction. Micrographs did not reveal any features in the coated mcirogels that were different from the uncoated microgels.

The porous alginate gel allows substrate to diffuse in or out of the gel beads and is essential for the immobilization characteristics of the gel. Pore size is generally in the range 5-200 nm (Andresen et al., 1977, Thu, Smidsrød, & Skjåk-Bræk, 1996b). CLSM and TEM micrographs showed that the caseinate only binds to the periphery of the alginate microgels. This is likely due to the fact that the pore size of the gel is too small to allow caseinate to freely penetrate into the microgel (Thu et al., 1996a). The optical sectioning feature of CLSM provides additional information on the internal

characteristics of microgels and has previously been used to study polymer distribution and protein release kinetics of alginate microgels (Strand et al., 2003)

The technique of polycation coating of alginate microgels has been shown to reduce the gel surface pore size and thus improve the stability of encapsulated core materials such as lipids and probiotics against oxidation and harsh pH conditions (Krasaekoopt et al., 2006, Gudipati et al., 2010). However the polycation commonly used such as poly-L-lysine and chitosan is not yet widely accepted as safe for human consumption (Zuidam and Shimoni, 2010). The use of caseinate, a common food derived protein, as coating will improve the applicability of encapsulation techniques in food products.

3.4 Determination of protein-polysaccharide interaction by protein dye-binding method

During the microscopy work, it was noticed that binding of a protein-specific dye, Rhodamine B, gave sodium caseinate a pink colour. Figure 7a shows clear differences in the pellet colour between samples where protein adsorption has occurred on the surface of alginate microgel particles (pH 3 and 4) and samples where no adsorption has taken place (pH 5, 6, and 7). Centrifuged pellets from pH 3 and 4 had an intense pink colour whereas samples from pH 5, 6, and 7 were colourless. However, the colour in pH 4 pellets was more intense than the pH 3 sample. This difference was attributed to the fact that at pH 4 (pH close to the pI of protein), sodium caseinate had started to partially precipitate as discussed in the previous sections. The increase in surface area in the protein aggregates led to an increase in dye binding that translated into an increase in colour intensity.

When the pellets were resuspended in water at their corresponding original pH levels, colour difference between the complexed and un-complexed samples were still evident. These resuspended pellets were subjected to 4 cycles of washing and subsequent centrifugation-suspension. It was further observed that colour intensity was retained in the complexed alginate microgel particles during these washings (Figure 7b). These results confirmed that protein dye binding is an effective visual method for determining the protein-polysaccharide interactions.

4. Conclusion

The results from this study showed that microscopic techniques such as TEM, FM and CLSM could be used to provide a definitive confirmation of protein-polysaccharide interaction. Results obtained showed that sodium caseinate protein and gelled alginate were able to form protein-hydrocolloid gel complex by electrostatic interactions. This mechanism is likely to be similar to the complex formation between caseinate and ungelled sodium alginate, which has previously been shown. Results from ζ-potential measurements and protein assay showed the protein-alginate gel interaction was pH dependent. The micrographs from TEM, FM and LM supported the results obtained from ζ-potential measurements and protein assay and clearly showed a 206-240 nm protein coating deposited on the surface of the alginate microgels at pH 3. Additionally, a dye-binding method of studying protein-polysacchairde interactions was briefly explored. Although further work needs to be done to better understand the effect of the properties of the adsorbed protein layer on the microstructure of alginate microgel particles (porosity, charge characteristics, and molecular weight) and possible preferential protein binding of alginate to specific proteins from the sodium caseinate, this work has shown that microscopic techniques that are non-destructive and simple can be used as a supporting tool to more established methods in the characterisation of protein interactions with polymeric microgels.

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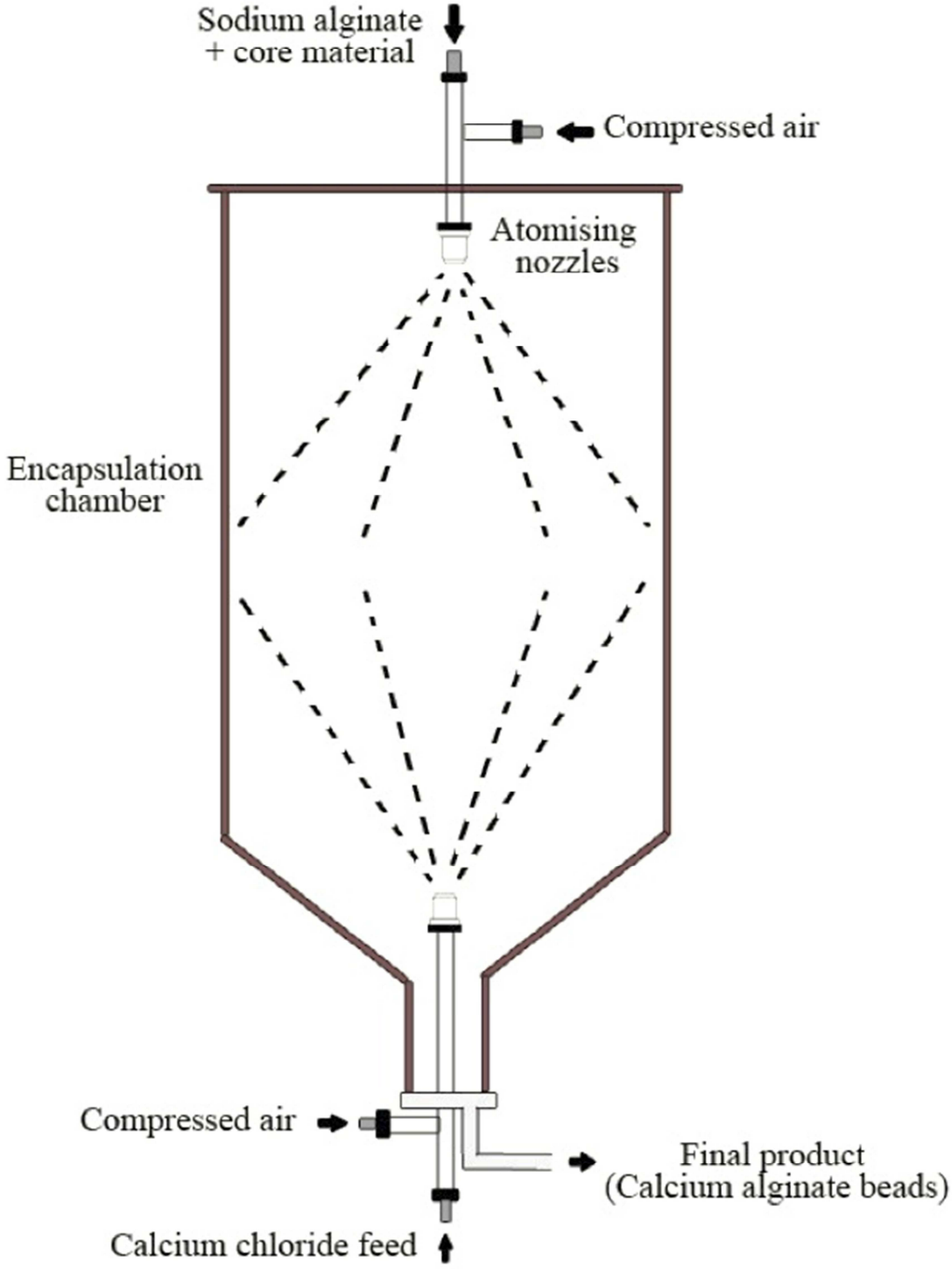
Figure	Caption	Filename and format
Figure 1.	Spray aerosol method of producing micron-sized alginate microgel particles. Modified from Bhandari (2009).	Figure 1.tiff
Figure 2.	Influence of pH on ζ-potential of 0.1% (wt/wt) alginate microgel particle solution (■), 0.02% (wt/wt) sodium caseinate solution (▲) and sodium caseinate-alginate gel particles mixture (●). Values represent a mean of three measurements and are expressed as mean ± SD.	Figure 2.xlsx
Figure 3.	Influence of pH on protein concentration in the supernatant of sodium caseinate (▧) and sodium caseinate-alginate microgel particle mixtures (□) after centrifugation at 2500 *g* for 5 minutes. Values represent a mean of three measurements and are expressed as mean ± SD. Columns that do not share the same alphabet are significantly different ($p<0.05$)	Figure 3.xlsx
Figure 4.	Influence of pH on microstructure of sodium caseinate-alginate gel particles mixture observed under light microscopy (LM), fluorescence microscopy (FM), and confocal light scanning microscopy (CLSM). Sodium caseinate is stained with Rhodamine-B and appears red under FM and CLSM.	Figure 4.tiff
Figure 5.	Observation of (a) sodium caseinate layer (yellow arrow) adsorbed onto an irregular shaped alginate microgel particle surface at pH 3 with TEM. (b) Protein layer is estimated to be 206-240 nm thick (inset).	Figure 5.tiff
Figure 6.	Illustration of the possible interaction between caseinate protein and alginate microgel particle at different pH levels. Proteins are electrostatically bound to the surface of microgel particles at pH 3 and 4. Precipitated proteins at pH 4 may bind to one or more microgel particle causing bridging flocculation. At pH 5 to 7, repulsion forces acting on the proteins prevent surface binding.	Figure 6.tiff
Figure 7.	Difference in colour intensity of (a) the centrifuged pellet of the caseinate-alginate gel particles mixture and (b) the washed resuspended pellets compared to the original 0.02% (wt/wt) sodium caseinate solution (CS) at pH 3, 4, 5, 6, and 7.	Figure 7(a).tiff Figure 7(b).tiff


Sodium alginate
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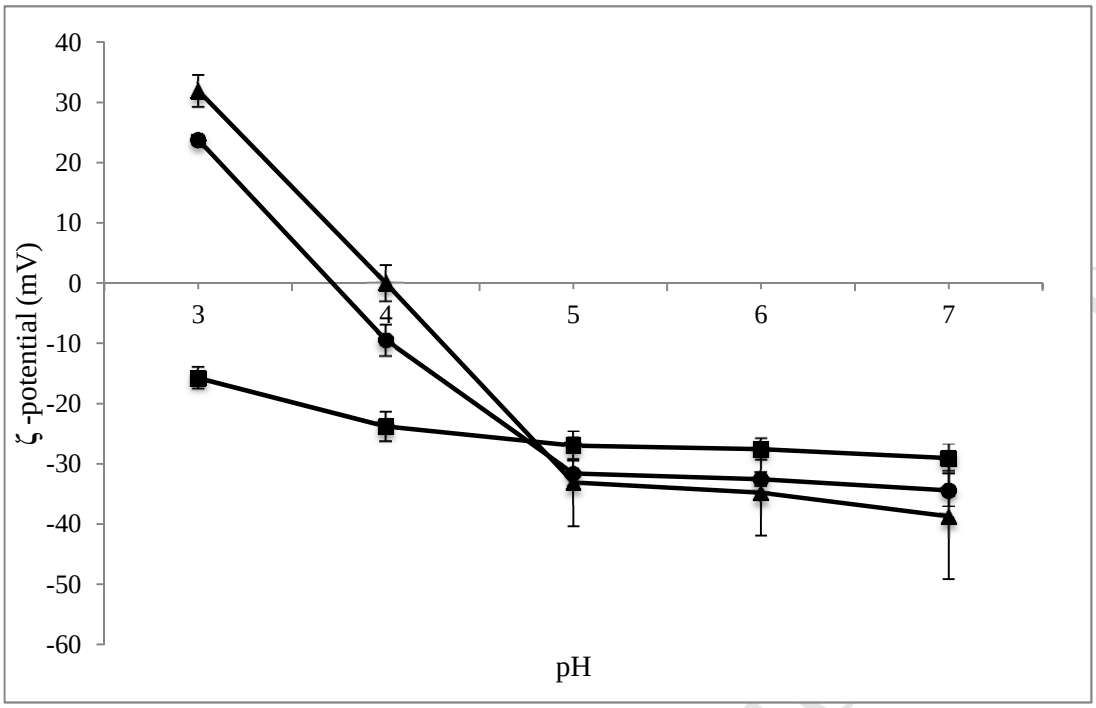
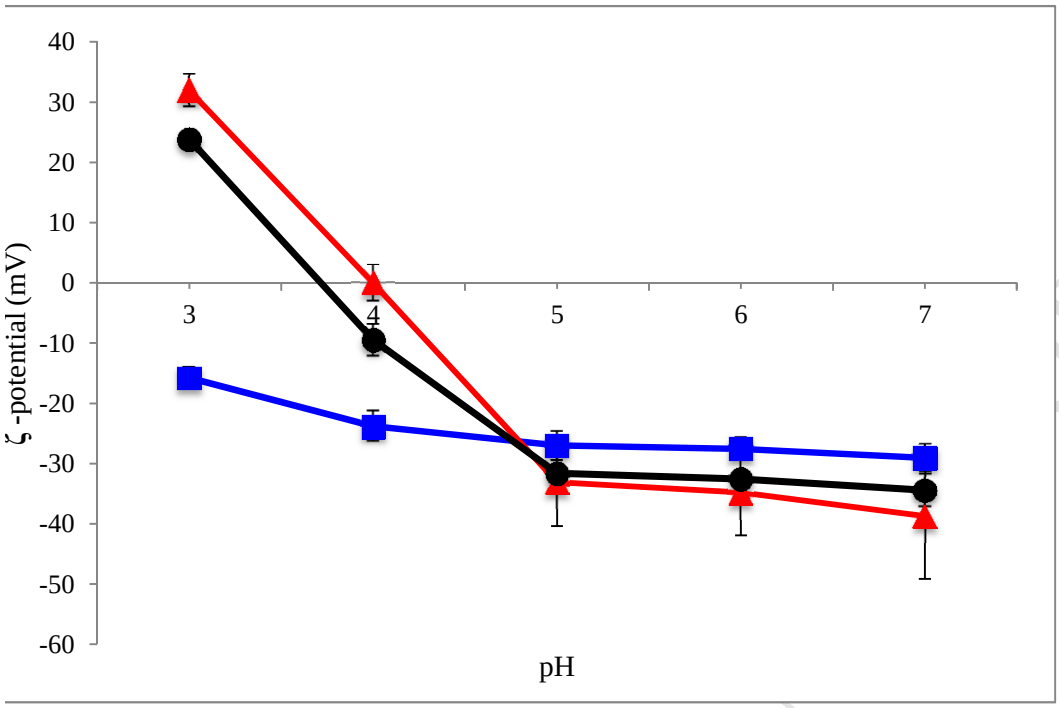
Compressed air
Atomising
nozzles
Encapsulation
chamber
Compressed air
Final product
(Calcium alginate beads)
Calcium chloride feed





		replicate 1		replicate 2		replicate 3		Total
	pH	average	stdev	average	stdev	average	stdev	average
0.1% Ca A	3	-17.67	1.23	-14.17	1.45	-15.50	2.19	-15.78
	4	-25.13	3.73	-25.30	0.92	-20.97	1.46	-23.80
	5	-28.73	4.45	-27.83	1.86	-24.33	0.31	-26.97
	6	-27.13	1.00	-26.03	3.93	-29.47	1.82	-27.54
	7	-31.53	3.35	-27.27	3.50	-28.33	6.47	-29.04
0.02% NaC	3	34.73	0.83	29.47	0.83	31.57	2.12	31.92
	4	-3.36	3.35	2.30	0.22	1.12	0.27	0.02
	5	-24.67	0.95	-37.37	1.88	-37.20	0.46	-33.08
	6	-26.80	0.35	-37.50	2.00	-40.17	1.40	-34.82
	7	-26.80	0.35	-43.50	4.81	-45.90	1.28	-38.73
0.1% CaAl	3	24.23	0.15	23.00	0.44	24.17	0.45	23.80
	4	-11.37	0.76	-10.47	0.90	-6.54	0.36	-9.46
	5	-33.80	3.47	-29.73	1.56	-31.27	0.85	-31.60
	6	-33.37	1.08	-31.30	3.82	-33.00	1.21	-32.56
	7	-37.50	2.01	-32.50	2.76	-33.27	1.82	-34.42

stdev
1.766457
2.455153
2.324507
1.753198
2.220444
2.651275
2.986487
7.284713
7.074236
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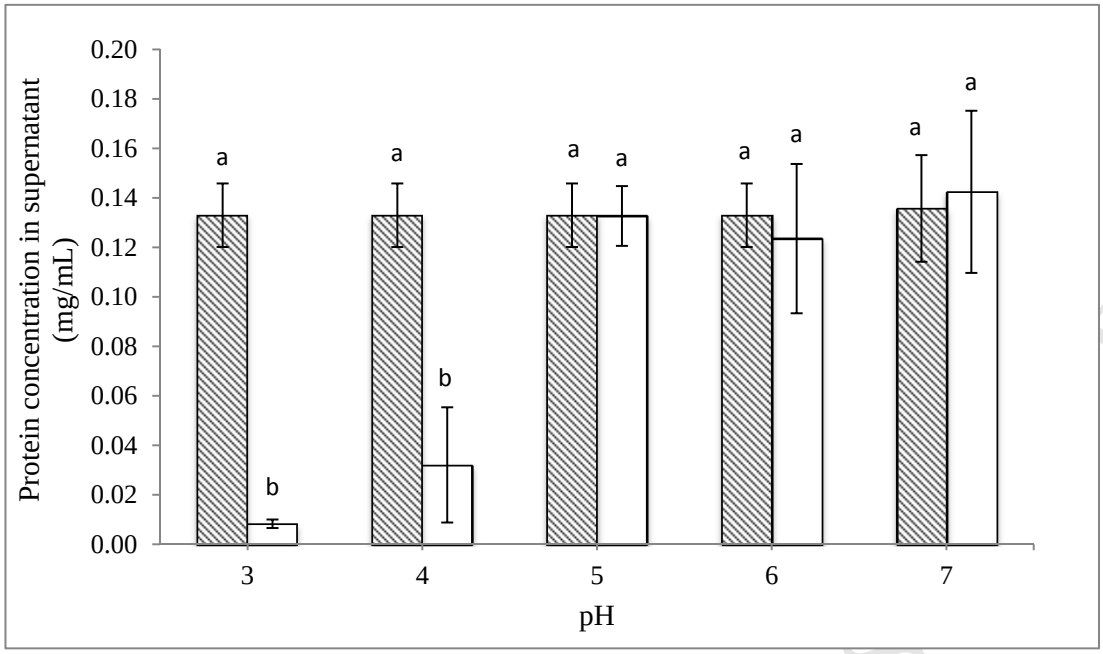
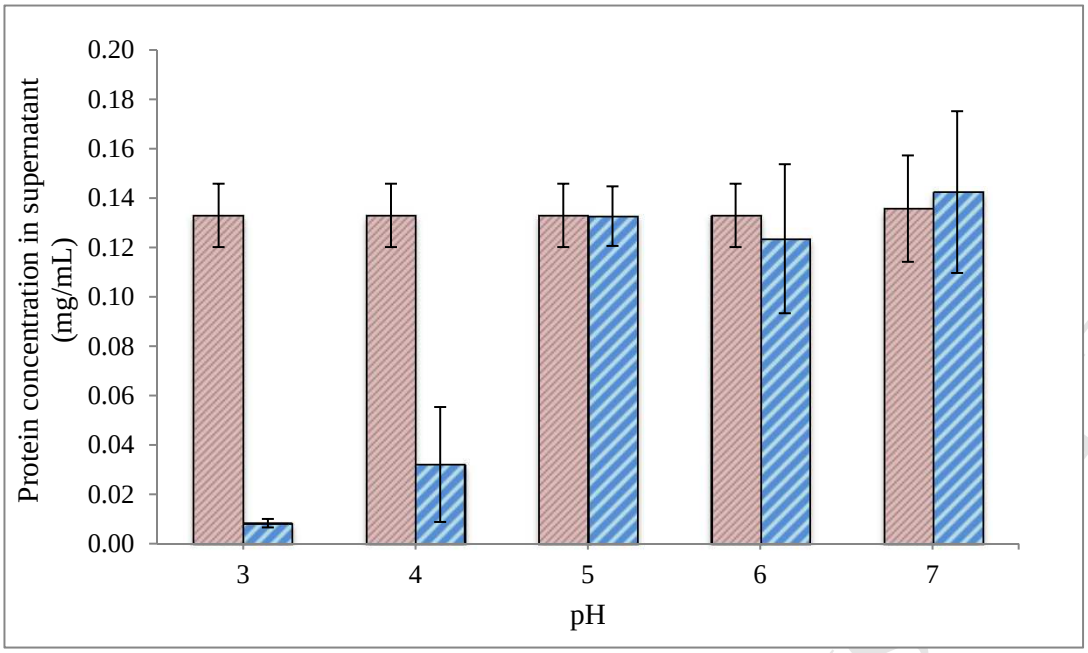
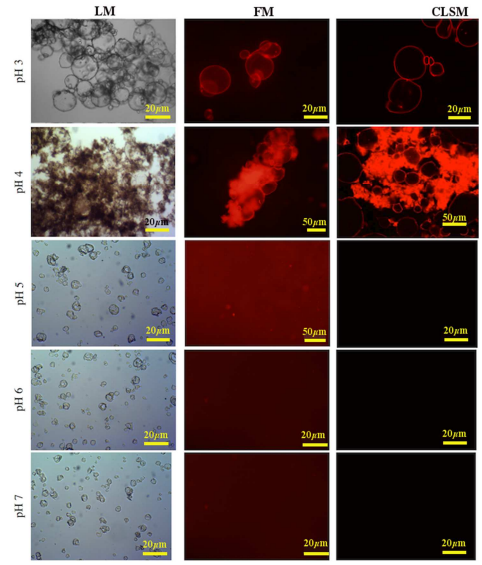
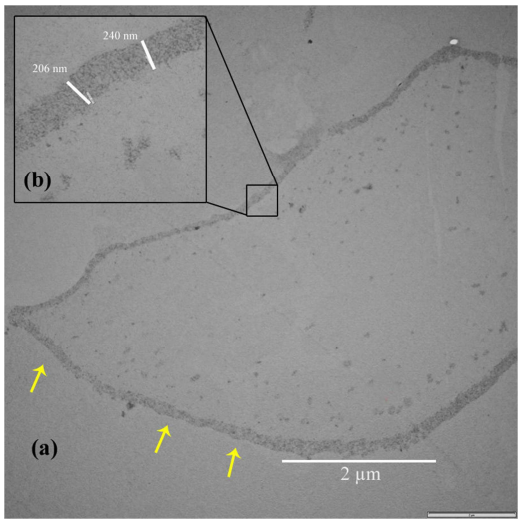
1.10269
2.692857

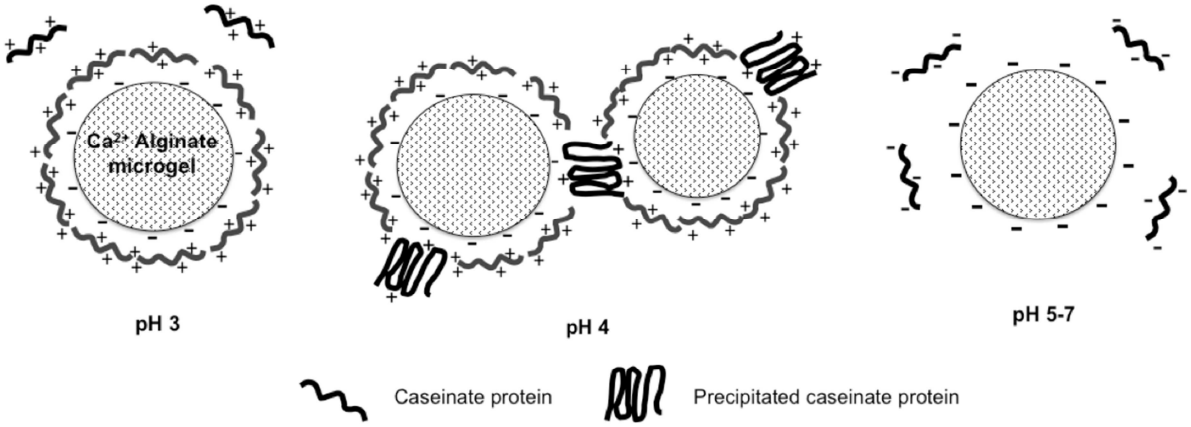


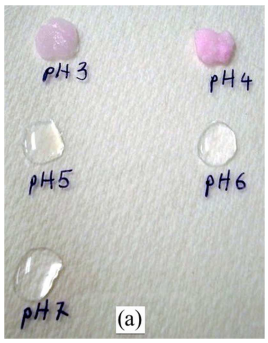


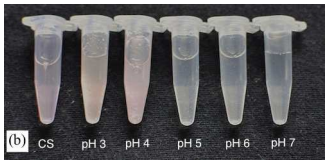
pH		0.02% NaCas average	0.02% NaCas stdev	0.10% Ca Alg + 0.02% NaCas average	0.10% Ca Alg + 0.02% NaCas stdev
3	0.10% Ca A	0.13	0.013	0.01	0.002
4		0.13	0.013	0.03	0.023
5		0.13	0.013	0.13	0.012
6		0.13	0.013	0.12	0.030
7		0.14	0.022	0.14	0.033











Highlights

- Caseinate-alginate microgel interaction was visualised with microscopy techniques.
- Caseinate adsorb onto alginate microgel particle through electrostatic interaction.
- The interaction of caseinate proteins with alginate gel particles was pH dependent.
- A dye-binding protein-alginate interaction detection method was described.