



Rheological Properties of Alginate/Whey Protein Composite Solutions and Gel Beads

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Abstract

This present work investigated rheological properties of whey protein/alginate composite solutions and gel beads. This information would be useful for designing encapsulated matrices as bioactive compound carriers in functional food applications. The composite solutions were 100% alginate, 62% alginate-38% whey protein, 50% alginate-50% whey protein and 100% whey protein. The rheological properties of the solutions were measured using a cone and plate rheometer. They exhibited shear-thinning behavior. Due to excellent hydrophilic property of alginate, the 100% alginate solution yielded the highest consistency index and flow behavior index while the 100% whey protein solution gave the opposite result. The composite gel beads were formed by dropping the solutions into a CaCl₂ solution. The rheological properties of the calcium-induced gel beads were measured using a material testing machine under relaxation testing. The gel beads exhibited viscoelastic behavior and could be explained by the modified Maxwell model. The gel beads containing alginate exhibited less relaxation time than the 100% whey protein bead. Alginate provided good ability of holding water in a gel matrix. The water facilitated the structure of the alginate composite beads; this resulted less elastic and elicited less relaxation times. Adding whey protein in the composition resulted dense gel beads, more elastic and long relaxation time.

Keywords: Rheology, Alginate-whey, Gel bead

1 Introduction

Novel functional foods incorporating diminutive particles for delivering bioactive compounds for supporting health and reducing risks of diseases have recently gained popularity (Chen and Subirade, 2006). The bioactive compounds can be sensitive to several factors during processing such as relative humidity, temperature, oxygen and light or in the gastro-intestinal (GI) tract due to pH and enzymes. Therefore, encapsulation technology has been developed to provide barriers against the degradation accentuated by these factors, especially for microspheres or microbeads. This also improves the viability and stability of the compounds during processing and storage and they are the most desirable delivery vehicles to accomplish sustained and targeted release in the gastro-intestinal tract (Chen and Subirade, 2006; Han *et al.*, 2008; Wichchukit *et al.*, 2013).

Alginate is a favorable biodegradable polysaccharide that has been used alone or in combination with others, especially whey protein isolates, to form a delivery matrix. Alginate is extracted from marine brown algae. This natural biopolymer is composed of two uronic acid blocks, 1,4-linked- β -D mannuronic acid (M) and α -L-

guluronic acid (G). It has an ability to form gels by dropping its solution into a divalent cation solution such as calcium chloride. The electrostatic interaction between G-blocks and divalent cations results in crosslinks forming an “egg-box” structure and inducing a hydrogel network (5, 46). Because of its non-toxic and excellent gel formation, alginate becomes a promising choice for an encapsulated matrix (Kukighi *et al.*, 1999).

Whey protein is a protein by-product obtained from cheese manufacturing. It has been extensively used in a variety of food products due to its high nutritional value and its ability to form gels, emulsions, foams and its water-blending properties (Chen and Subirade, 2006; Doherty *et al.*, 2011; Fang *et al.*, 2013). Whey protein isolates (WPI) are the common form of whey protein used for encapsulation. They contain approx. 90% of protein which are approx. 75% of β -lactoglobulin and 15% of alpha-lactalbumin (Fioramonti *et al.*, 2013). Whey protein has an ability to form gel by the “cold gelation” method. By heating whey protein solution typically about 70-80 °C, the protein is denatured. This process lets its molecules transform from compactly folded into an unfolded state, in which the protein dissociates changing from a dimer to a monomer, allowing protein-protein interactions.

hydrogen-bonding and sulfhydryl/ disulphide interchange reactions to occur. These actions consequently provide stiffer, stronger and more stretchable protein. Accordingly, heat denaturation results in aggregation, coagulation and finally ends with precipitation (Déat-Lainé *et al.*, 2012; Doherty *et al.*, 2011; Fioramonti *et al.*, 2013; Haper *et al.*, 2013; Rajam *et al.*, 2013). After the cooling step, the formation of the gel network of the whey protein occurs by associating with divalent cation interaction in a similar manner to alginate. (Chen and Subirade, 2007; Doherty *et al.*, 2011).

Wichchukit *et al.* (2013) proposed a release system made from whey protein/alginate gel beads in a xanthan- sucrose solution that mimics a food beverage. They investigated 4 proportions of alginate – whey gel beads on their physical and mechanical properties and their releases of riboflavin to the xanthan- sucrose solution. The gel bead proportions were 100% or pure alginate, 62%, 38% and 0% or pure whey protein. They found that the pure alginate gel solution exhibited Newtonian flow; adding whey to alginate solution resulted shear thinning behaviors of the gel solution. All four types of gel beads exhibited the viscoelastic property and the pure whey protein beads elicited the least relaxation response.

This present research aimed to extend the work of Wichchukit *et al.* (2013). However, this research had been conducted using different sources of materials and sample preparations and measurements were modified. Therefore, the objective of this work was to explore rheological properties of gel solutions and gel beads made from alginate and whey protein with the same final fraction as the study of Wichchukit *et al.* (2013). The information obtained from this study would be primary data used for the next step of the extending research in designing the delivery matrix in functional liquid foods.

2 Materials and Methods

2.1 Materials

Sodium alginate (Food grade) E 401 was received as a gift from Nutrition Sc. CO Ltd., Samparn, Nakhonpathom, Thailand. Instant Whey Protein Isolate 90 (WPI) was purchased from Davigo Foods International Inc., Le Sueur, MN, USA. Calcium chloride (MW 110.983) for the calcium chloride curing solution and Tween 80 added to the calcium chloride solution as a surfactant during formation of beads that contained whey protein, were all purchased from Fisher Scientific, Pittsburgh, PA, USA. Sodium azide as an antimicrobial agent was purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.1 Methodology

2.1.1 Gel bead preparation

An aqueous solution of 10% (w/w) WPI was prepared using distilled water at room temperature (25°C). The solution was allowed to stand overnight at room temperature to ensure complete hydration of the proteins. The solution pH fell within the range 6.80-6.84. The WPI solution was adjusted to pH 8.0 with 1 N NaOH and heated at 80 °C for 45 min to denature proteins completely (Tang *et al.*, 2013). The denatured WPI solution was cooled to 30 °C. Sodium alginate powder was dispersed in distilled water and stirred overnight to form a 1.5% (w/w) solution. Sodium azide was added at a final concentration of 0.02% (w/w) to WPI solution and Na-Alg solution.

Four polymer solutions were prepared by mixing sodium alginate solution with denatured WPI solution in the following proportions: 100% w/w Na-Alg or pure alginate (designated 100A/0W); 62% w/w Na-Alg and 38% w/w denatured WPI (designated 62A/38W); 50% w/w Na-Alg and 50% w/w denatured WPI (designated 50A/50W); and 100% w/w denatured WPI or pure whey protein (designated 0A/100W). The samples were stirred overnight until used.

To form beads, each polymer solution was extruded into 200 ml of 0.25 M CaCl₂ solution using a syringe pump. Tween 80 was added to the CaCl₂ at a final concentration of 1% to obtain spherical beads. The formed beads were allowed to harden for 45 min in 0.25 M CaCl₂ solution at room temperature and then rinsed with distilled water and wiped gently with a clean napkin.

The particle size of beads was also observed by an imaging method. For each proportion matrix bead, an image of fifteen randomized beads was taken using an Olympus digital camera (Olympus Optical Co., Ltd., Japan). Then, their Feret's diameters were measured and the mean diameters were analyzed using ImageJ software version 1.4. Moisture content and water activity of each composition were measured by using a vacuum hot air oven and Aqua Lab CX-2 instrument (Aqua Lab, Pullman, WA, USA), respectively.

2.1.2 Rheological measurements

Rheological properties of the polymer solutions without riboflavin were investigated using a rotational rheometer (CVO 50, Bohlin Rheometer,

Malvern Instruments Limited, UK) with a cone and plate (cone diameter = 60 mm, angle = 1°, gap = 0.052 mm. Configuration was performed at a controlled temperature of 20± 0.1 °C. The shear rate range was 5-100 s⁻¹.

The rheological properties of beads without riboflavin were studied using a texture analyzer (TA-XT2i, Texture Technologies Corp., NY, USA) equipped with a 2 kg load cell. Beads were compressed to 20% deformation and maintained for 60 s with a 5 cm flat. The automatic detection of the contact by the probe with the beads was carried out with a contact force of 0.01 g. Tests were performed at room temperature (25 ± 1°C). All rheological measurements were performed in triplicate.

2.1.3 Structural Analysis by FTIR spectroscopy

FTIR spectra of alginate powder, WPI powder and gel beads were recorded using a Spectrum Two spectrophotometer (Perkin-Elmer, MA, USA). A sample was placed in the sample holder with a scan spectral region of 450–4000 cm⁻¹ with 16 scans recorded at 4 cm⁻¹ resolution. Spectra were analyzed using Spectrum software (Version: 10.03.05.0099).

3 Results and Discussion

3.1 Rheological characteristics of polymer solutions

Polymer solutions could be considered as the first form of delivery vehicle for the liquid state of gel beads. Their rheological characteristics could be used for understanding the characteristics of gel beads and beads' structures. All 4 polymer solutions (100A/0W, 62A/38W, 50A/50W and 0A/100W) exhibited a non-linear relationship between shear rate and shear stress. That relationship fitted the power law model as shown in Eq. (1),

$$\sigma = K\dot{\gamma}^n \quad (1)$$

Where σ , K , $\dot{\gamma}$ and n are the shear stress, consistency index, shear rate and flow behavior index, respectively (Steffe, 1996). The rheological parameters obtained from the solutions are listed in Table 1. The 'n' values of solutions were in the range of 0.54–0.69, suggesting that the flow behavior of all solutions was shear thinning behavior. Pure alginate solution gave a different result from that of Wichchukit *et al.* (2013) and Pongjanyakul and Rongthong (2010); they found that the pure alginate

solution behaved as a Newtonian fluid. However, the present result was in agreement to that of Borgogna *et al.* (2010), who observed shear thinning behavior in pure alginate solution at 1 and 2% w/v. This suggested that the factors affecting the rheological properties of the pure alginate solution could be its concentration level and/or its intrinsic properties such as its molecular weight. Increasing the molecular weight of alginate would cause an increase in its solution viscosity (Lee and Mooney, 2012). Hence, it might induce changes in flow behavior of alginate solution as well.

When WPI combined in the solution, less consistency of solution was observed, as seen in Table 1; 62A/38W, 50A/50W and 0A/100W solutions exhibited lower consistency values than the pure alginate solution. The flow behavior index decreased with an increase of whey protein. This suggested that incorporation of whey protein would affect the liquid state structure and consequently affect flow behavior of the sodium alginate solution.

3.2 Bead characterization

Different combinations in polymer solution (liquid state) can affect the morphology of gel beads (solid state). As seen in Figure 2, the pure alginate beads were in regular sphere shape and transparent, while an increase in WPI tended to decrease the degrees of the sphere regularity and transparency. This result is in agreement with many studies (e.g. Wichchukit *et al.*, 2013; Tang *et al.*, 2013). This can be attributed to the combinability between whey protein and alginate in aqueous solution and their proportions in the formations. It can be said that alginate is a main contribution to the sphericity of gel beads (Sandoval-Castilla *et al.*, 2010) and also their transparency. The opacity of the gel beads containing WPI may be explained in the same manner of wet composite whey film. Harper *et al.* (2013) found that compared with the unheated whey film, the heated whey protein films appeared white in color and less translucent. They explained the change in protein structure by the denaturing of the WPI by the heating process. The heat induces an unfolding of the protein structure and therefore causes the loss of a tertiary protein structure and buried hydrophobic amino acid groups. The addition of calcium salt can lead to a decrease in surface charge and consequently result in the protein aggregation via a hydrophobic reaction. Thus, a calcium crosslinking process in gel beads, at

the same time, enhances the aggregation of the protein.



Figure 1 Pictures of gel beads prepared by using solutions: (a) 100A/0W; (b) 62A/38W; (c) 50A/50W and (d) 0A/100W.

The formations of an alginate- whey protein mixed solution also results in a wide range of size distribution. Here, diameter values of the beads varied between 2.81 and 4.53 mm as given in Table 2. A gradual increase in the diameters of the bead was observed upon increasing the concentration of whey protein in the formulation. Pure alginate beads showed the smallest diameter, while whey protein beads had the largest diameter. Alginate gel shrinks during forming beads in CaCl_2 ; adding other compounds may reduce mechanical stability (Sandoval-Castilla *et al.*, 2010) and results to less compact sizes. Statistical analysis showed that there were significant differences ($p < 0.01$) between the particle size of the pure alginate beads and those of the whey protein containing beads. Though smaller, there was no significant difference in the diameters of 62A/38W and 50A/50W beads ($p > 0.05$).

The moisture content and water activity were in the range of 83.49-93.52% and 0.96-0.97%, respectively (Table 3). The water activity of beads showed no significant difference ($P > 0.05$). Bead

moisture content decreased significantly ($P < 0.05$) with increasing proportions of whey protein. This result was similar to our previous studies (Wichchukit *et al.*, 2013). Because of its hydrophilic characteristics, pure an alginate bead can somehow trap more water inside its matrix. In contrast to whey protein, the denatured protein from heat induction has high exposure of hydrophobic patches on its surface (Fioramonti *et al.*, 2013), which produce a water barrier for the composite beads.

The integrity and mechanical stability of formed beads were observed by means of a force relaxation test. The relaxation curves of the beads are presented in Figure 2. The exponential decay showing in all curves elicits viscoelastic materials. Pure whey protein beads (0A/100W) exhibited higher force values compared to the other bead types, which indicated more solid-like behavior than the others (Wichchukit *et al.*, 2013) and greater resistance to flattening (Bhattacharya, 2010). The expansion of the gel solution during extrusion resulted in bigger beads than the others was also confirmed the viscoelastic behavior (Wichchukit *et al.*, 2013). Textural properties of the beads were affected by the biopolymers' composition and concentration (Sandoval-Castilla *et al.*, 2010). Pure whey protein beads exhibited soft but tough gel beads, while alginate composite beads (100A/0W, 62A/38W, and 50A/50W) were more flexible (Han *et al.*, 2008). The flexibility is obtained due to more water combining in the matrix; water acts as a lubricant (Bhattacharya, 2010; Bhattacharya and Narasimha 1997) and consequently decreases the compressive force values of the beads (Wichchukit *et al.*, 2013). The relaxation data were well fit with the modified Maxwell model, a single Maxwell element connected in parallel with a spring (Steffe, 1996) as in Eq. 2.

$$F = F_e + (F_0 - F_e) \exp\left(\frac{-t}{\lambda_{rel}}\right) \quad (2)$$

Where F is the decaying force, F_e is the residual force, F_0 is the initial force, and λ_{rel} is relaxation time. The fitting results for the model ($R^2 > 0.97$) are given in Table 3. Stress relaxation provides useful information for understanding of the internal structure of a material such as permanent cross-linking of polymers. Water in food materials plays an important role on relaxation time (Andrés *et al.*, 2008).

The gel beads containing alginate exhibited less relaxation time than the 100% whey protein bead. Alginate provided good ability of holding water in a gel matrix. The water facilitated the structure of the alginate composite beads; this resulted less elastic and

elicited less relaxation times. Adding whey protein in the composition resulted dense gel beads, more elastic and long relaxation time. Thus, whey protein increased mechanical strength of the composite beads

Table 1 Flow parameters of the solutions used to form gel beads

Bead type	K	n	R ²
100A/0W	1.38±0.04 ^a	0.68±0.01 ^a	0.98
62A/38W	0.38±0.02 ^b	0.69±0.01 ^a	0.99
50A/50W	0.38±0.03 ^b	0.59±0.01 ^b	0.99
0A/100W	0.88±0.09 ^c	0.54±0.02 ^c	0.99

Means ± SD in the same column identified by different superscripts are significantly different ($p < 0.05$)

Table 2 Physical characteristics, water activity and moisture content of gel beads.

Bead type	A _w	% MC	Diameter (mm)
100A/0W	0.97±0.01	93.52±0.88 ^a	2.81±0.07 ^a
62A/38W	0.97±0.01	86.50±0.50 ^b	3.81±0.14 ^b
50A/50W	0.97±0.01	85.28±0.61 ^{bc}	3.80±0.12 ^b
0A/100W	0.96±0.01	83.49±1.84 ^c	4.53±0.14 ^c

Means ± SD in the same column identified by different superscripts are significantly different ($p < 0.05$)

Table 3 Maxwell model parameters of gel beads.

Bead Type	F ₀ (g)	F _c (g)	λ _{rel} (s)	R ²
100A/0W	6.36±0.08 ^a	2.25±0.02 ^a	10.26±0.11 ^a	0.98
62A/38W	4.38±0.05 ^b	2.31±0.05 ^b	10.26±0.08 ^a	0.97
50A/50W	4.94±0.10 ^c	2.86±0.06 ^b	10.88±0.38 ^a	0.97
0A/100W	10.59±0.10 ^d	5.75±0.05 ^c	14.23±0.13 ^b	0.99

Means ± SD in the same column identified by different superscripts are significantly different ($p < 0.05$)

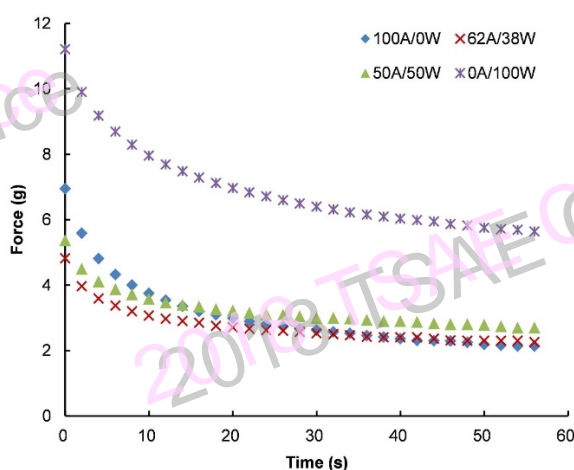


Figure 2 Relaxation curves for gel beads

3.1 Structural Analysis by FTIR spectroscopy

Molecular interaction of alginate powder, WPI, and gel beads were investigated using FTIR spectroscopy. FTIR spectra of alginate powder

showed the absorption bands around 3226, 1600, 1406, and 1030 cm^{-1} , corresponding to the stretching of O-H, COO- (asymmetric), COO- (symmetric), and C-O-C, respectively (Figure 3a). This result was in the neighborhood of the studies by Pongjanyakul and Puttipipatkachorn (2007) and Pongjanyakul and Rongthong (2010). The cross-linking process of alginate with calcium ions caused an obvious shift to higher wave numbers and an increase in intensity of the O-H stretching peak, and a decrease in intensity of the C-O-C stretching peak of alginate powder (Figure 3b). This confirmed the existence of an ionic bonding between calcium ions and carboxyl groups of alginate and a partial covalent bonding between calcium and oxygen atoms of either groups, respectively (Pongjanyakul and Puttipipatkachorn, 2007).

FTIR spectra of WPI powder showed the peaks at approximately 3267, 1628 and 1389 cm^{-1} (Figure 3f).

The 3600- 3000 cm^{-1} peak may represent NH stretching intensity that increases with molecular weight, while the 1600- 1700 cm^{-1} band, which is found in proteins, indicating the amide I region (Eissa *et al.*, 2006; Lacroix *et al.*, 2002; Sadeghi *et al.*, 2014; Sullivan *et al.*, 2014). Eissa *et al.* (2006) reported that the band at 1621 and 1634 cm^{-1} represent β sheets of β - lactoglobulin. The cross- linking process of denatured whey protein with calcium ions caused a slight shift to higher wave numbers and an increase in intensity of the NH and amide I stretching peak of whey protein powder (Figure 3e).

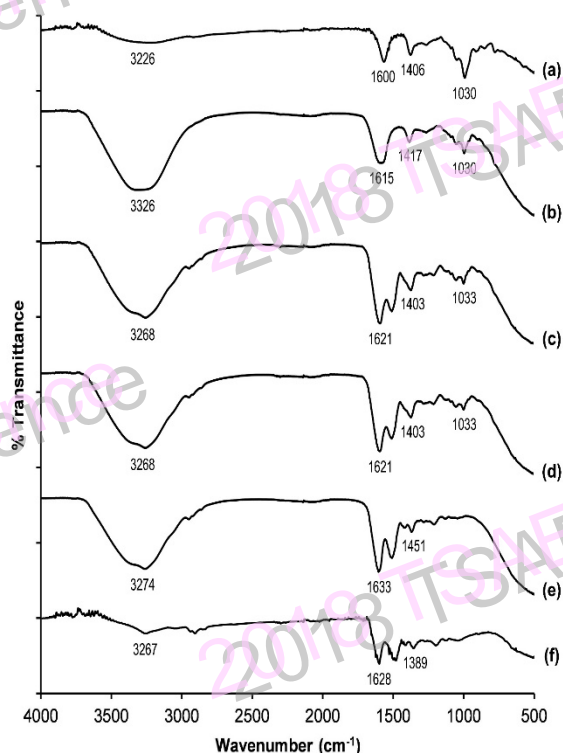


Figure 3 FTIR spectra of sodium alginate powder (a); 100A/0W (b); 62A/38W (c); 50A/50W (d); 0A/100W (e) and whey protein powder (f).

Based on pure alginate beads, incorporation of whey protein into the alginate beads obviously provided a shift to a higher wave number and a slightly lower intensity of the O-H stretching peak. It is possible to explain this phenomenon by the alginate and whey protein forming inter-molecular hydrogen bonding. The COO⁻ stretching peak at 1615 cm^{-1} shifted to 1621 and 1633 cm^{-1} (Figure 3c-e). It could be hypothesized that this indicated an ionic bonding between amide groups of whey protein and carboxyl groups of alginate. While the peak at 1030 cm^{-1} shifted to a higher wave number at 1033 cm^{-1} and the decrease intensity of C-O-C stretching

was observed, no difference was observed when whey protein was added in the range of 38-50% to the combination.

4 Conclusions

This study showed that there are some factors, such as sources of raw materials and preparation procedures, affect physical and structural characterizations of the alginate/ whey gel beads. Pures and combinations of alginate and whey protein resulted shear thinning behavior in their liquid state and yielded more solid-like viscoelastic behavior in their solid state. The FTIR result provided the proof of the molecular interaction between alginate and whey protein in the composite gel beads. The results derived from this research could be useful for developing biopolymer particles for not only functional foods applications, but also for other specific characteristics and applications. It would certainly be useful to carry out additional studies in the future.

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