Carbohydrate Polymers 76 (2009) 206–213

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Gel particles from spray-dried disordered polysaccharides

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ARTICLE INFO

Article history:
Received 30 July 2008
Received in revised form 9 September 2008
Accepted 8 October 2008
Available online 17 October 2008

Keywords:
Alginate
ι-Carrageenan
Gel particles
Swelling studies

ABSTRACT

The formation of gel particles from alginate and ι-carrageenan was studied through a novel pathway of formation via an amorphous spray-dried intermediate. Dried biopolymer particles were suspended in solutions of different Ca^{2+} concentration. Particle size ranges and microscopic observation demonstrated that a range of swelling behaviour could be induced, with lower calcium concentrations resulting in more expanded particles, until a lower limit is reached below which particles initially dissolve. For the same calcium charge stoichiometry, larger swollen gel particles were obtained for alginate than for ι-carrageenan. The ability to produce a range of swollen biopolymer gel particle sizes, on the order of 1–600 μm, is attributed to the balance between gelation and dissolution kinetics, with fast gelation kinetics and slow dissolution promoting production of small gel particles whilst fast dissolution with slow gelation leads to larger gel particles. By controlling the solution environment in which rehydration is carried out, it is therefore possible to produce particles with desired degrees of swelling from a single starting material.

1. Introduction

Biopolymers are widely utilised in food, agricultural, pharmaceutical and chemical industries and are particularly attractive for these applications as they are from natural sources and often biocompatible. One use of biopolymers of growing interest is in the form of gel particles (Burey, Gidley, Bhandari, & Howes, 2008; Gidley & Hedges, 1998; Hunik & Tramper, 1993; Klokk & Melvik, 2002; Lapitsky & Kaler, 2004; Wandrey, Espinosa, Rehor, & Hunkeler, 2003). These are useful for encapsulation or texture control within food (King, 1995; Malone & Appelqvist, 2003), pharmaceutical (Mukai-Correa, Prata, Alvim, & Grosso, 2004; Sriamornsak & Nuthanid, 1998; Wong, Lee, Chan, & Heng, 2002), agricultural, probiotic (Krasaekoopt, Bhandari, & Deeth, 2003; Sugiura et al., 2005), medical (Zimmerman et al., 2005) and cosmetic products (Burey et al., 2008; Gidley & Hedges, 1998), as well as in process reactors. Gelled particulate forms of polymers are prized for a range of applications as they combine macroscopic structure formation with an ability to flow and often have an attractive soft solid texture, all at high water contents (>95%).

Extensive studies have been carried out on the properties of bulk biopolymer gels, many of them several years ago (Braudo, Plashchina, Semenova, & Yuryev, 1998; Bu, Kjoniksen, Knudsen, & Nystrom, 2004; Burchard & Ross-Murphy, 1990; Djabourov, 1991; Labropoulos, Niesz, Danforth, & Kevrekidis, 2002a; Labropoulos, Niesz, Danforth, & Kevrekidis, 2002b; Luh, Flink, & Karel, 1977; Nickerson & Paulson, 2004; Papageorgiou, Kasapis, & Gothard, 1994; Sherman, 1982; Siew & Williams, 2005; Totosaus, Montejano, Salazar, & Guerrero, 2002; Zabik & Aldrich, 1965), however, by comparison, gelled biopolymer particles have received relatively little attention. Most studies of such particles focus on applications and formation methods (Gavini, Rassu, Sanna, Cossu, & Giunchedi, 2005; King, 1995; Liu et al., 2003; Madziva, Kailasapathy, & Phillips, 2005; Malone & Appelqvist, 2003; Mukai-Correa et al., 2004; Sriamornsak, Thirawong, & Puttipipatkhachorn, 2004; Sugiura et al., 2005; Wong et al., 2002; Zimmerman et al., 2005), but limited information is available on mechanisms of formation, and how to control these mechanisms to form gelled particles with varying size, strength and release properties (Blandino, Macias, & Cantero, 1999; Darrabie, Kendall, & Opara, 2006; Hills et al., 2000; Saunders & Vincent, 1999) .

Previously gel particles have been formed through a variety of techniques, but usually through two main mechanisms, continuous phase formation and dispersed phase formation (Burey et al., 2008). Continuous phase formation of gel particles consists of forming the gel or pre-gel first and then breaking up the gel/pre-gel into discrete gel particles. Coacervation and shear processes are most frequently used (Burgess & Carless, 1984; Gander, Blanco-Príeto, Thomasin, Wandrey, & Hunkeler, 2002; Hamberg, Wohlwend, Walkenstrom, & Hermansson, 2003).

Dispersed phase formation involves formation of a droplet prior to gelation; the droplet then becomes a discrete gel particle after gelation has occurred. This technique is particularly useful for sys-

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doi:10.1016/j.carbpol.2008.10.001

tems which gel via ionotropic gelation as droplets of the hydrocolloid solution can be formed prior to the introduction of ions to aid gelation (Glicksman, 1983; Hoefler, 2004; Nussinovitch, 1997). Examples of gel particle formation techniques which use this mechanism include extrusion and variations thereof, and emulsion formation (Adams, Frith, & Stokes, 2004; Blandino et al., 1999; Campbell, Taylor, Cayre, & Paunov, 2004; Cheng & Lim, 2004; Hunik & Tramper, 1993; Klokk & Melvik, 2002; Lamprecht, Schäfer, & Lehr, 2000; Malone & Appelqvist, 2003; Simeone, Tassieri, Sibillo, & Guido, 2005; Wolf, Frith, & Norton, 2001a; Wolf, Frith, Singleton, Tassieri, & Norton, 2001b; Zvitov & Nussinovitch, 2001).

There are several limitations of the techniques described above including: (1) the requirement for sophisticated equipment, (2) difficulties in scaling up, (3) expensive storage and transport costs due to the high level of water and (4) possible problems with spoilage caused by high water content, especially compared with dried powders.

Here we propose and demonstrate an alternative approach for formation of biopolymer gel particles, based on rehydration of spray-dried polysaccharides under gelling conditions. A dried intermediate is attractive in principle, as transport and storage costs are minimized, and hydrated products can be made at the time and place that they are desired (Burey et al., 2008). Spray drying is a well-established method for preparing low moisture biopolymer particles. If drying is carried out from a polysaccharide solution, then it is likely that solid particles will contain disordered (non-aggregated) chains. When these particles are introduced to an aqueous environment containing a gelling co-solute (e.g. calcium for alginate or ι-carrageenan), then there are two processes that may occur. One is hydration and dissolution of polysaccharide chains and the second is aggregation and/or network formation due to the gelling environment. Provided gelation is more rapid than dissolution, then gelled particles would be predicted to form with their size determined by the relative kinetics of the two processes (Fig. 1).

Type 1 particles are predicted to be formed when gelation kinetics are very fast compared with hydration-driven expansion of the dried hydrocolloid particle, such that polymer chains cross-link so rapidly that there is no time for significant particle expansion to occur. Type 2 particles are predicted to be formed when expansion is fast compared with gelation kinetics. In the limit of slow gelation and fast expansion, polymer chains would be expected to dissolve from expanded particles, forming a polymer solution. Depending on the solution concentration, hydrocolloid chains may not remain in solution but subsequently form a continuous bulk gel (Fig. 1).

These principles of formation of gel particles via use of a spray-dried intermediate are illustrated in this study using two calcium-mediated gelling systems – alginate and ι-carrageenan.

2. Materials and methods

2.1. Materials

ι-Carrageenan Viscarin SD 389 and alginate Protanal RF6650 were both supplied by FMC Biopolymer (Philadelphia, USA). The alginate powder had a guluronic acid content of 63%. Calcium chloride was from Ajax Finechem (Seven Hills, Australia).

2.2. Particle formation

2.2.1. Spray-drying

Spray drying was carried out on 2% w/w biopolymer solutions (on basis of powder as supplied), formed by high shear mixing at 70–80 °C using a Silverson L2R mixer (Silverson, Chesham Bucks, England) at maximum speed until all powder was dissolved, typically after 20–30 min. Solutions were kept at 70 °C before being pumped into the spray dryer.

The solutions were spray-dried using a Saurin SL 20 Pilot spray dryer (Saurin Enterprises Pty Ltd., Melbourne, Australia). Spray drying was carried out with an inlet temperature of 180 °C and an outlet temperature of 80 °C. The solution was pumped into the spray dryer using an attached peristaltic pump the flow rate of which was controlled by the spray dryer outlet temperature. Spray-dried particles were collected from the base of the cyclone and stored in airtight containers for further sample preparation. Two separate experimental runs of spray drying were carried out on each of the materials.

2.2.2. Rehydration

Spray-dried particles were rehydrated in solutions of varying calcium chloride concentration (0.001–1 M). Powders were rehydrated into a 1 w/w% suspension in sample sizes of 40 g. Samples were prepared by first mixing 0.4 g of spray-dried particles with 1.6 g of isopropanol and then mixing for 15 s using a UP 400s ultrasonic processor (Dr. Hielscher GmbH, Teltow, Germany) at a cycle of 1% and 100% amplitude prior to rehydration with calcium chloride solution. Isopropanol addition and ultrasonication were used

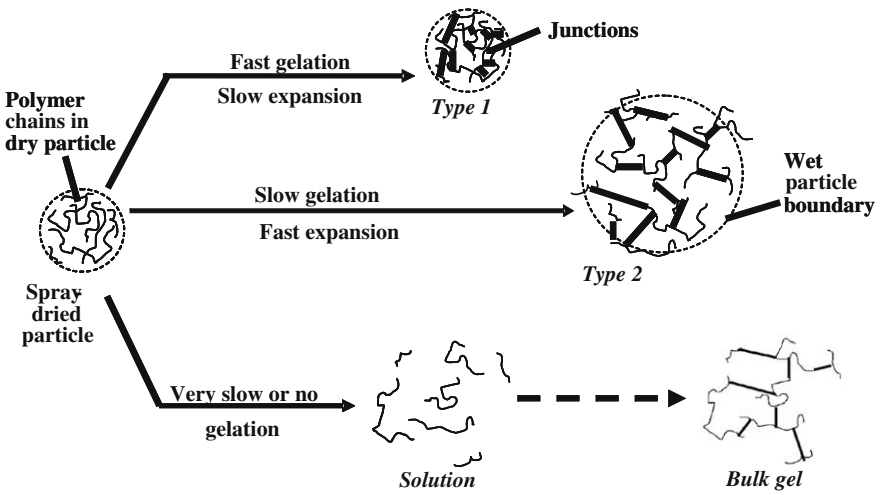

Fig. 1. Schematic of biopolymer particle rehydration, leading to different biopolymer forms. Type 1 represents slightly swollen particles, while Type 2 represents highly swollen particles. Solid lines represent typical formation pathways. Dashed lines describe a gel formation pathway which may occur slowly over a long period of time.

to prevent particle agglomeration. Calcium chloride solution of appropriate concentration was then added to the powder/isopropanol slurry to the amount of 38 g and the mixture was ultrasonicated for a further 15 s.

2.3. Particle size analysis

Particle size distributions (PSDs) were determined using a Mastersizer/E laser diffraction analyser (Malvern Instruments Ltd., Worcestershire, UK). Two different lenses were used, 100 and 300 mm, to ensure that the majority of the particle size distribution could be detected. Particles were analysed in calcium chloride solutions at their corresponding rehydration concentrations and were agitated during analysis to prevent agglomeration. PSD measurements were taken at the following times after addition of calcium chloride solution and ultrasonication: 1, 3, 5 min, 1 week and 1 month.

2.4. Light microscopy

Gel particles were observed by light microscopy using an Olympus BX61 light microscope (Pennsylvania, USA). A droplet of sample was placed on a glass slide and then covered with a glass cover slip for observation under the microscope.

2.5. Scanning electron microscopy

Spray-dried particle samples were prepared for scanning electron microscopy (SEM) by direct attachment to double-sided adhesive carbon tabs mounted on SEM stubs and then coating with platinum. The particles were observed with a JEOL 6400 (JEOL, Tokyo, Japan) SEM operating at an accelerating voltage of 6 kV.

3. Results

3.1. Spray-dried particles

SEM images of the spray-dried particles display a large number of very small particles along with some larger 'collapsed' particles (Fig. 2). The collapsed particles are likely to have been caused by skin formation during spray-drying followed by internal boiling (Masters, 1991). The morphology of the particles may have some influence on the appearance of the subsequent gel particles formed.

Alginate particles appeared to show a noticeable number of medium-sized particles along with a number of small particles, while ι-carrageenan showed a majority of small particles along with a few large and medium-sized particles. The difference in particle appearance was most likely due to variations in biopolymer solution viscosity prior to spray drying, as all other spray-drying conditions were kept constant across the two different materials. The alginate solution had a noticeably higher viscosity than the ι-carrageenan solution at the same w/w%, which, all other things being equal, led to atomization of the more viscous feed (alginate solution) producing bigger droplets and subsequently, bigger particles (Masters, 1991).

The spray-dried particles formed show a broad range of particle sizes for both alginate and ι-carrageenan (Fig. 2a and b). This is inherent to the process of spray-drying, where traditionally it is difficult to obtain monodisperse particle sizes (Alamilla-Beltran, Chanona-Perez, Jimenez-Aparicio, & Gutierrez-Lopez, 2005; Bhandari, 2007, chap. 9; Masters, 1991). One way to overcome this would be to develop a large-scale 'drop on demand' process which encourages formation of monosize droplets (Adhikari, Howes, Bhandari, & Truong, 2000; Feng, Fuh, & Wong, 2006; Wu, Patel, Rogers, & Chen, 2007).

Particle sizes ranged from 0.3-ca 100 μm for both alginate and ι-carrageenan particles. The D_{50} value was slightly smaller for ι-carrageenan (8.10 μm) than for alginate (9.86 μm), reflecting a slightly higher proportion of small particles (Fig. 2). This could have implications for the corresponding gel particle sizes obtained after rehydration.

3.2. Particle behaviour on rehydration in calcium chloride solutions

3.2.1. Alginate

Alginate gel particles displayed an increase in particle size as calcium chloride concentration was reduced (Fig. 3). The gel particles showed both spherical and non-spherical morphology and had a propensity to agglomerate during observation under the light microscope (Fig. 3).

Alginate gel particle size showed a measurable increase between samples hydrated at 1 M $CaCl_2$ and samples hydrated at 0.1 M $CaCl_2$, but increased dramatically between hydration condi-

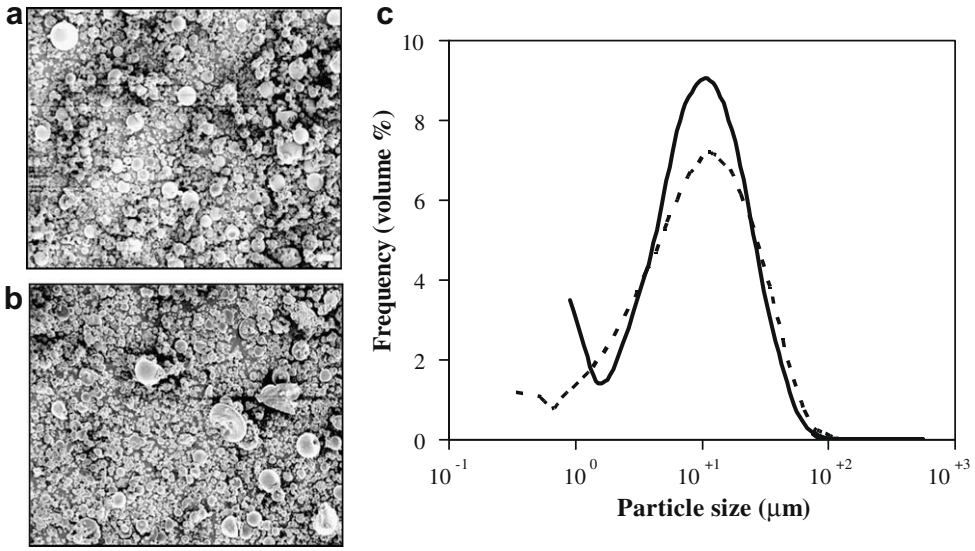

Fig. 2. SEM images of (a) spray dried alginate and (b) spray dried ι-carrageenan (scale bars are 10 μm) with (c) particle size distributions (PSDs) of spray-dried particles, solid line is for alginate, dotted line is for ι-carrageenan. PSD plots represent the frequency of particles within each size range measured by the Malvern E.

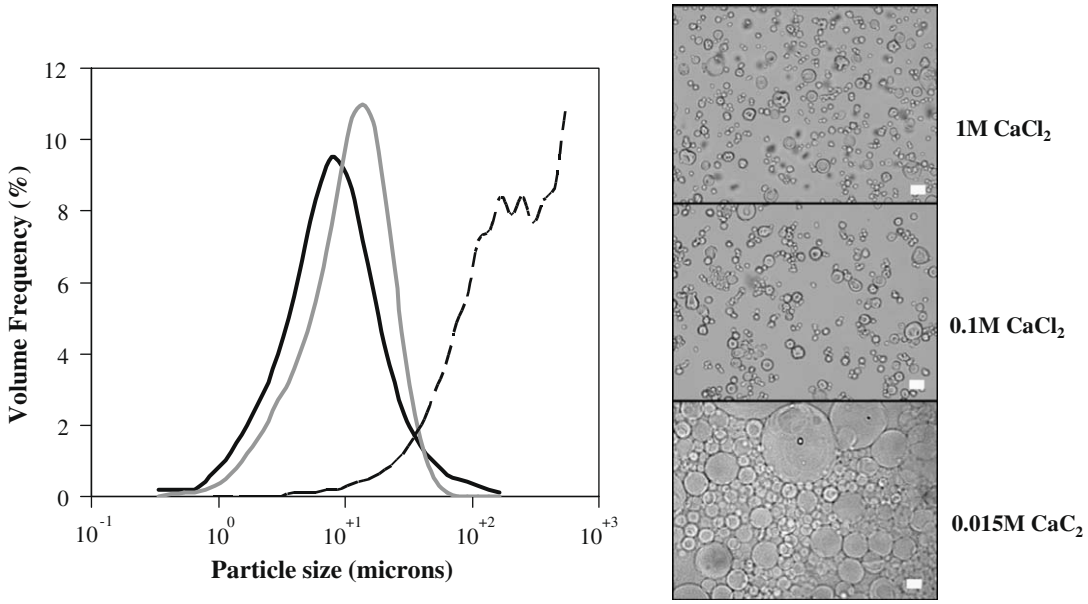

Fig. 3. Change in alginate particle size with calcium chloride concentration, shown through PSD and light microscopy (scale bars are 10 μm). The PSD curves are for samples hydrated at 1 M $CaCl_2$ (black line), 0.1 M $CaCl_2$ (grey line) and 0.015 M $CaCl_2$ (dashed line). PSD plots represent the frequency of particles within each size range measured by the Malvern E.

tions of 0.1 M $CaCl_2$ and 0.015 M $CaCl_2$ (Fig. 3). This provides a control window in which particle sizes can be modified for various applications, and the opportunity to investigate the effects of calcium concentrations within this range.

At 0.015 M $CaCl_2$ it was not possible to capture the particle sizes of the largest particles due to the limitations of the analysis equipment being used, which leads to the appearance of an incomplete particle size distribution (PSD) (Fig. 3); this was a reproducible trend. Because the analysis was based on a volume distribution, very large particles tended to skew the distribution to the larger end of the scale. In the corresponding light microscopy (LM) image, only a few particles are visible, due to increased absorption of calcium chloride solution, causing higher swelling, thus leading to the reduction in contrast between particles and surrounding medium, making it difficult to capture clear images of the extremely swollen particles.

At a concentration of 0.001 M $CaCl_2$ no gel particles were observed and it was found to be impossible to obtain a PSD, indicating that there were few, if any gel particles present. The sample had the solid-like characteristics of a bulk gel showing a resistance to flow not exhibited by gel particles formed at higher calcium concentrations.

3.2.2. ι-Carrageenan

ι-Carrageenan particles also showed an increase in size on rehydration with a decrease in calcium chloride concentration and displayed a round/spherical morphology (Fig. 4). The particles did not show as much agglomeration as alginate samples.

At a concentration of 0.001 M $CaCl_2$, similarly to alginate, no ι-carrageenan particles were visible and a PSD could not be obtained. The sample had the characteristics of a continuous but weak gel in that there was a resistance to flow not found for particles formed at higher calcium concentrations. The extent of the increase in ι-carrageenan particle size with calcium concentration was not as great as that of the alginate particles.

At 0.0095 M $CaCl_2$, the LM image shows gel particles, which are noticeably more swollen than ι-carrageenan particles at 0.1 M $CaCl_2$, but are not so swollen as to become difficult to view, due to reduced contrast between particles and medium, as was the case with highly swollen alginate particles (Fig. 4).

3.2.3. Process reproducibility

Alginate and ι-carrageenan particles were formed in two distinct batches, spray-dried on different days and rehydrated under the same conditions. The PSDs were similar between the two batches (Fig. 5) showing an acceptable level of reproducibility. This provided a good indication that an appropriate model for particle size, and related properties such as strength and rheology could be developed. Furthermore, hydrated gel particles appeared to form very rapidly (in less than 1 min) and had essentially stable PSDs after several weeks' storage, showing that any agglomeration processes were minor. No evidence for bulk gel formation (e.g. an increased resistance to flow) was found after storage, again consistent with hydrated gel particles being effectively stable once formed.

3.3. Swelling behaviour and calcium stoichiometry

In order to properly compare gel particle formation behaviour between alginate and ι-carrageenan particles, the swelling ratio, Q, (i.e. the ratio of increase in size from spray-dried particle to hydrated particle) was determined for both biopolymer systems using the following formula

$$Q = D_{50,x}/D_{50,Usp} \quad (1)$$

where $D_{50,x}$ is the median particle diameter at x mol L^{-1} $CaCl_2$ and $D_{50,sp}$ is the median particle diameter of the corresponding spray-dried biopolymer.

Q was determined for alginate and ι-carrageenan particles hydrated at concentrations including 1, 0.1, 0.05, 0.04, 0.03, 0.02, 0.015 and 0.0095 M $CaCl_2$. Stoichiometric balance, based on charge neutralisation, occurred at the following: alginate, 0.029 M, ι-carrageenan, 0.022 M $CaCl_2$.

Both alginate and ι-carrageenan showed similar very small swelling ratios at high calcium concentrations i.e. at 1 and 0.1 M (Fig. 6). However, despite similar hydration conditions for both systems, below a concentration of 0.1 M $CaCl_2$, the swelling ratios began to differ markedly (Fig. 6). At concentrations lower than 0.015 M $CaCl_2$ for alginate and 0.0095 M $CaCl_2$ for ι-carrageenan, at least some of the polysaccharide was dissolved initially and sub-

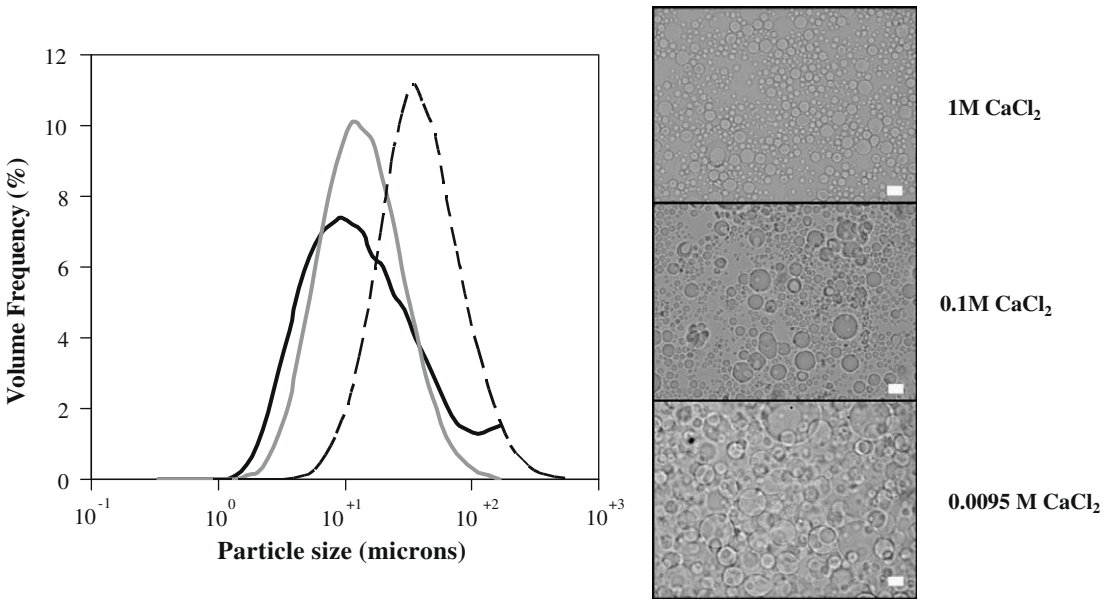

Fig. 4. Change in ι-carrageenan particle size with calcium chloride concentration, shown through PSD and light microscopy (scale bars are 10 μm). The PSD curves are for samples hydrated at 1 M $CaCl_2$ (black line), 0.1 M $CaCl_2$ (grey line) and 0.0095 M $CaCl_2$ (dashed line). PSD plots represent the frequency of particles within each size range measured by the Malvern E.

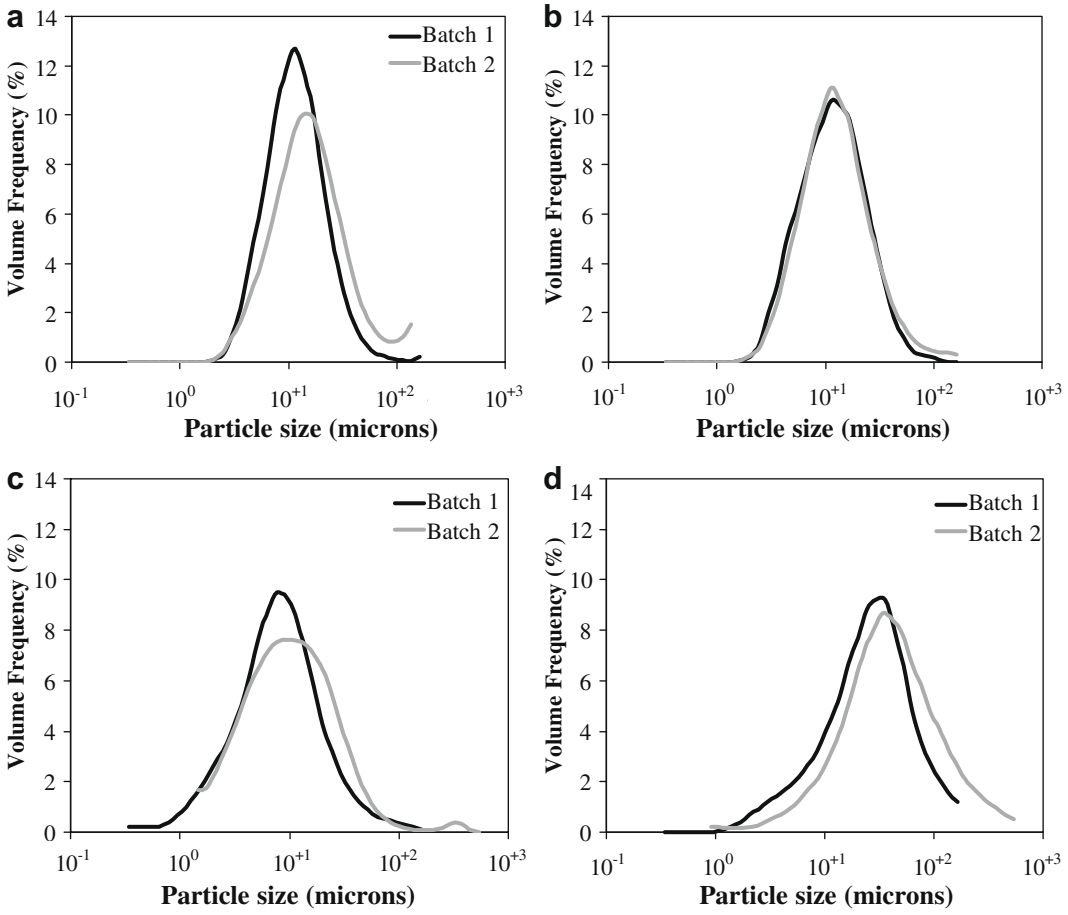

Fig. 5. PSD curves for different batches of ι-carrageenan hydrated with (a) 1 M $CaCl_2$, (b) 0.04 M $CaCl_2$ and alginate hydrated with (c) 1 M $CaCl_2$, (d) 0.04 M $CaCl_2$. PSD plots represent the frequency of particles within each size range measured by the Malvern E.

sequently formed a bulk gel. In order to determine whether differences between the two polysaccharides were due to differences in charge balance, swelling ratios were also plotted against the stoichiometric ratio (SR: mols of Ca^{2+} ions to mols of charged biopolymer expressed on a monosaccharide residue basis). Results are shown in Fig. 7. It is clear that charge stoichiometry alone is not

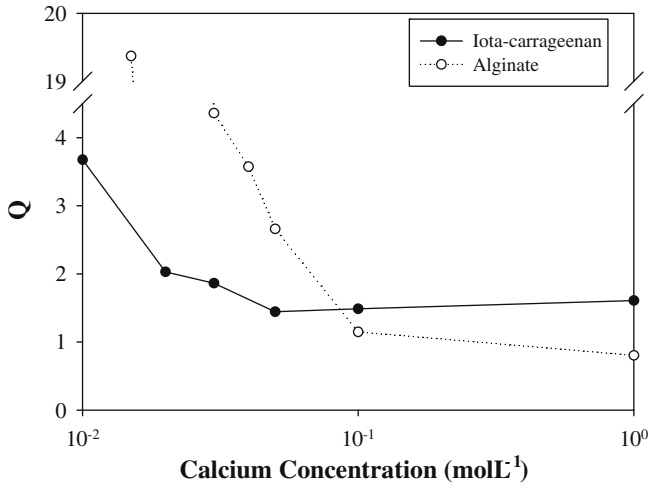

Fig. 6. Swelling ratio dependence on calcium concentration for alginate (♦) and ι-carrageenan (◇).

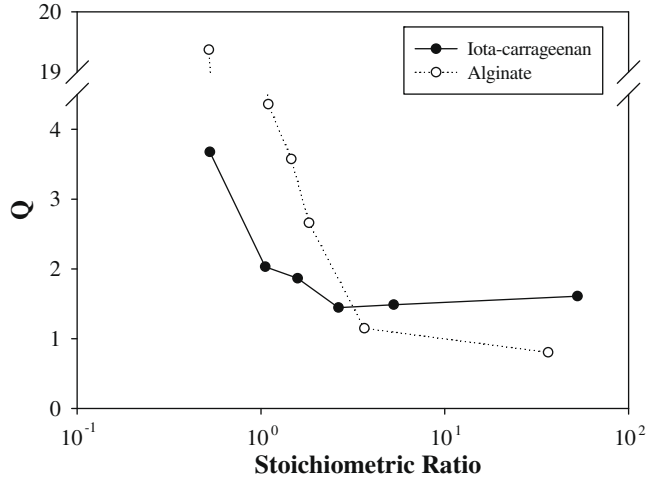

Fig. 7. Swelling ratio dependence on stoichiometric ratio (charge balance) for alginate (♦) and ι-carrageenan (◇).

sufficient to explain the different swelling behaviours of the two systems.

4. Discussion

4.1. Spray-dried disordered polysaccharides: Useful intermediates for the controlled formation of hydrated gel particles

The advantage of the approach used here for gel particle formation is that rehydration of a common spray-dried intermediate under varying hydration medium conditions leads to the formation of a variety of biopolymer microstructures, demonstrated by the systems studied here. Both alginate and ι-carrageenan systems formed a range of structures including small particles, large particles and bulk gels, depending on the concentration of calcium ions within the hydration medium, as depicted in Fig. 1.

The ability of the biopolymers to produce different structural products on rehydration is due to the interplay between gelation and dissolution kinetics. Conditions which promote fast gelation kinetics (e.g. calcium concentrations of 0.1 M or greater for alginate and 0.05 M or greater for ι-carrageenan) lead to the formation of small (ca. 10 μm) particles, whilst at the other extreme (e.g. 0.001 M calcium), slow gelation conditions lead to the formation of a biopolymer solution, which forms a weak but continuous gel after an extended period of time at cool conditions (e.g. 4 °C) (BeMiller & Whistler, 1996; Draget, 2000; Hoefler, 2004; Imeson, 2000). Selection of appropriate rehydration conditions therefore provides the opportunity for control of hydrated particle size. The swelling of particles due to uptake of solvent, prior to gelation, would be expected to have implications for other particle properties such as mechanical behaviour and molecular release if used as an encapsulant. Control of this swelling leads to the opportunity to produce particles tailored to various applications from a single starting dried particle size.

Factors which may affect gelation and expansion kinetics include hydrocolloid source (Wandrey et al., 2003), hydrocolloid concentration (Blandino et al., 1999), the presence of ions (Bajpai & Sharma, 2004; Ramakrishnan & Prud'homme, 2000), temperature (Lootens et al., 2003; Marcotte, Hoshahili, & Ramaswamy, 2001) and pH (Lootens et al., 2003).

In this study the control parameter was calcium ion concentration and it was found that, particularly below 1:1 charge stoichiometric ratio, that particle sizes could increase significantly (up to a swelling ratio approaching 20 – Figs. 6 and 7).

4.2. Similarities and differences between alginate and ι-carrageenan

Alginate and ι-carrageenan behaved in a qualitatively similar manner in this study displaying the three types of hydrated structure illustrated in Fig. 1. Of particular relevance to understanding mechanisms involved, it was found that for calcium concentrations too low to prevent dissolution, gelation occurred subsequently. This shows that gelation can be slow compared with dissolution at low concentrations, and that the rates of both processes need to be taken into account in rationalizing observed microstructures.

There were also notable quantitative differences in behaviour between these two calcium-sensitive polymers. The increase in alginate gel particle size was greater than that of ι-carrageenan particles at equivalent calcium concentrations (Fig. 6) or stoichiometric ratios (Fig. 7), and hydrated alginate particles appeared less round/spherical. These two differences are consistent with alginate particles undergoing more rapid expansion due to hydration than ι-carrageenan particles at equivalent calcium or SR.

The alternative limiting explanation would be that gelation kinetics of ι-carrageenan are faster than those for alginate under equivalent conditions, thereby allowing less expansion prior to effective locking of particle microstructures. This seems less likely, as guluronate-rich alginate such as used in this study is considered to gel very rapidly with calcium in comparison with ι-carrageenan, and has been observed to be the case for the samples studied here at equivalent calcium concentration (data not shown).

On this basis, we propose that it is more likely that the greater expansion of particles of alginate is due to more rapid hydration/dissolution. A constant drying process, similar charge density and identical rehydration conditions suggests that the initial particle hydration process would be expected to be similar for the two polymers. We therefore propose that the key difference between the two polymer systems is in the rate of the swelling of hydrated polymer chains, with alginate being faster than ι-carrageenan. Further work is required to determine more precisely the mechanisms involved.

5. Conclusion

This study has shown that it is possible to produce a range of hydrated physical forms from a single spray-dried biopolymer

intermediate, depending on the balance of gelation and dissolution kinetics. Not only can varying hydration conditions play a role in determining particle properties, but the choice of biopolymer can also have an effect, as shown by the differences in particle sizes formed by alginate and ι-carrageenan.

Although a common principle for the production of gelled particulate forms of both alginate and ι-carrageenan has been demonstrated, the different quantitative responses of the two systems to calcium concentration demonstrates that desired particle size characteristics can be obtained by choice of an appropriate combination of gelling polymer and gel-inducing agent – in this case calcium concentration.

Acknowledgement

This work was supported by an Australian Research Council Discovery grant.

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