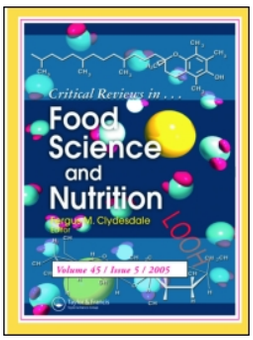

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Alginate gel particles–A review of production techniques and physical properties

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ABSTRACT

The application of hydrocolloid gel particles is potentially useful in food, chemical, and pharmaceutical industries. Alginate gel particles are one of the more commonly used hydrocolloid gel particles due to them being biocompatible, nontoxic, biodegradable, cheap, and simple to produce. They are particularly valued for their application in encapsulation. Encapsulation in alginate gel particles confers protective benefits to cells, DNA, nutrients, and microbes. Slow release of flavors, minerals, and drugs can also be achieved by encapsulation in gel particles. The particle size and shape of the gel particles are crucial for specific applications. In this review, current methods of producing alginate gel particles will be discussed, taking into account their advantages, disadvantages, scalability, and impact on particle size. The physical properties of alginate gel particles will determine the effectiveness in different application conditions. This review will cover the current understanding of the alginate biopolymer, gelation mechanisms and factors affecting release properties, gel strength, and rheology of the alginate gel particle systems.

KEYWORDS

Alginate; microgel; physical behavior; polysaccharide gel; particle size

Introduction

Alginate, a natural polysaccharide extracted from brown algae, is commonly used in the food industry to modify food properties such as rheology (thickening), water binding capacity, stabilizing emulsion, and film formation. Alginate is also able to gel by ionic crosslinking. One of the forms in which alginate gel is commonly used is in the form of gel particles.

The size (diameter) of alginate gel particles typically range from $>$ 1 mm (macro), 0.2 to 1,000 μm (micro) and $<$ 0.2 μm mm (nano). These gel particles, which typically hold high water content, have adjustable chemical and mechanical properties that are dependent on the type of crosslinking agent used. As a natural ingredient, alginate gel particles are attractive for biological applications because they are biocompatible, nontoxic, biodegradable, and relatively cheap (Orive et al., 2005; Andersen et al., 2012).

To date, the major application of particulate alginate gels can be found in the encapsulation of food, pharmaceutical, biomedical, and agriculture materials (Table 1). For example, numerous food ingredients have been encapsulated over the years. These food ingredients include oils/lipid (Chan et al., 2011), probiotic cells (Sohail et al., 2011), flavorings (Zhang et al., 2011), polyphenols (Zohar-Perez et al., 2004), vitamins (Abubakr et al., 2010), antioxidants (Belščak-Cvitanović et al., 2011), peptides (Hurteaux et al., 2005), enzymes (Hariyadi et al., 2012), etc. The jammed structure of gel matrix locks in the core materials and forms a protective barrier that limits diffusion of molecules based on their size and charges, or minimizes the degradation of the sensitive core materials due to outside environment (Tanaka et al., 1984; Oyaas et al., 1995). Undesirable sensory properties of food ingredients may also be masked by encapsulation in alginate gel particles. The wide application of alginate gel particles in a wide range of fields such as food, microbiology, pharmaceutical, and medicine has led to a proliferation of methods for producing alginate gel particles to suit each intended application.

In this review, we will attempt to update the development of various methods for producing alginate gel particles in the macro to nano (nanometer) diameter size range and their potential application. We will categorize these methods based on the size range of gel particles they produce. The physical characteristics of these gel particles and additional techniques to enhance their barrier properties are briefly discussed. The advantages and limitations of each method will be described and opportunities for further research will be outlined.

Alginate molecular structure and composition

The alginate polymer is made up of two monomeric units: β-(1→4) linked D-mannuronic acid (M) residues and α-(1→4)-linked L-guluronic acid (G) residues (Figure 1). The basic structure of alginates consist of linear unbranched units of polymers made up of monomers arranged in blocks of M and G residues interspersed with regions containing alternating M-G sequence within the structure (Donati and Paoletti, 2009; Draget, 2009).

Alginate derived from different brown seaweed species will contain different proportion and sequence of M and G residues that determines the molecular weight and physical properties of the alginate and their derived structures. The alginate

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Table 1. Summary of current methods of producing alginate gel particles.

Technique	Particle size range	Scalability	Application	Reference
Simple dripping (extrusion)	1000–2000 μm	−	Encapsulation of probiotics, yeasts, fish oil, drugs, proteins, enzymes, plant extract and flavors	Smidsrød and Skjåk-Bræk, 1990; Blandino et al., 1999; Fundueanu et al., 1999; Chan et al., 2000; Zohar-Perez et al., 2004; Chan et al., 2011; Zhang et al., 2011
Electrostatic potential	0.9–1.5 μm; 50–350 μm	+	Encapsulation of lipid nanoparticles, probiotics, animal and human tissue and enzymes.	Bugarski et al., 1994; Hsu et al., 1994; Klokk and Melvik, 2002; Nedovic and Wallaert, 2004; Suksamran et al., 2009; Strasdat and Bunjes, 2013
Vibrating nozzle	<20 μm; 200–5000 μm	+	Encapsulation of cells and proteins	Prüße et al., 2008; Yan et al., 2009
Jetcutting	200–5000 μm	+	Encapsulation of cells and proteins	Prüße et al., 1998; Prüße et al., 2008
Spinning disk	300–600 μm	+	Encapsulation of bacteria, yeast and proteins	Ogbonna et al., 1989; Ogbonna et al., 1991; Champagne et al., 2000; Senuma et al., 2000
Spinning nozzle	270 μm to 2 mm	−	Encapsulation of cells and proteins	Ryoichi et al., 2001; Haeberle et al., 2008
Spray nozzle	80–130 μm	−	Encapsulation of lipid nanoparticles, BCG vaccine, antigens, proteins and bacteria.	Kwok et al., 1991; Abraham et al., 1996; Cui et al., 2001; Yeo et al., 2001; Strasdat and Bunjes, 2013
Impinging aerosol	11–47 μm	+	Encapsulation of fish oil, lysozyme, ibuprofen, propranolol hydrochloride, probiotics, insulin	Bhandari, 2009; Hariyadi et al., 2010; Sohail et al., 2011; Hariyadi et al., 2012
Emulsification	100–1000 μm	+	Encapsulation of insulin	Poncelet et al., 1992; Chan et al., 2000; Poncelet, 2001; Zhang et al., 2006a; Reis et al., 2007
Microfluidics	50 to 70 μm	−	Production of non-uniform shaped (tail end, dimpled, mushroom-like, hemi spherical, red blood cell-like and disk-like) microgels; Encapsulation of cells (probiotics, mammalian); Janus particle formation	Liu et al., 2006; Zhang et al., 2006b; Zhao et al., 2009; Hu et al., 2012; Lian et al., 2012; Yang et al., 2012; Lin et al., 2013
Templating method	120–200 nm	−	Encapsulation of protein (BSA)	Hong et al., 2008; Nesamony et al., 2012; De Santis et al., 2014
Hydrid microgels	250–600 nm	−	Encapsulation of drugs (doxorubicin), cells (hemoglobin), insulin and herbicides	Rajaonarivony et al., 1993; Silva et al., 2005; Boissiere et al., 2006; Sarmento et al., 2006; Silva et al., 2011

polymer may contain regions consisting of exclusively one type of monomer (M-blocks or G-blocks) or an alternating sequence of M and G residues (MG-blocks) (Figure 2). Commercially, alginates are available in the form of sodium, potassium, or ammonium salts. Molecular weights of alginate typically range from 60,000 to 700,000 Daltons depending on the application (Draget et al., 1993).

Gel formation

Compared to other polysaccharides such as gelatin or agar, alginate is able to form gel independent of temperature. The formation of alginate gels can be achieved by two methods: ionic crosslinking with cations (ionic gels) or acid precipitation (acidic gels).

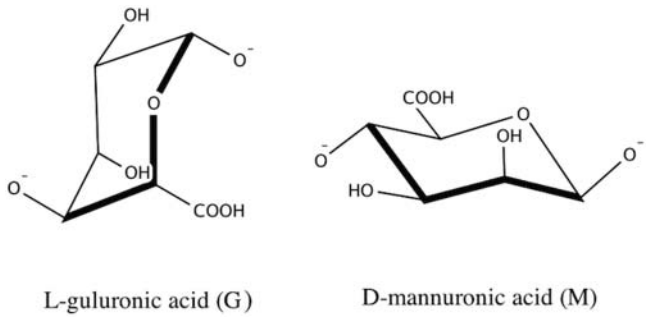

Figure 1. Chemical structure of alginate monomers: L-guluronic acid and D-mannuronic acid.

Ionic alginate gels

One of the most highly valued properties of alginate in the food industry is the ability to form ionic gel in the presence of multivalent cations. The gel formed by this interaction is widely utilized in the encapsulation of bioactives in the food industry, drugs in pharmaceutical industry and cell immobilization in the biotechnology industry. Binding of divalent cation to alginate is a highly selective process and alginate affinity to cation increases in the order of Mn < Zn, Ni, Co < Fe < Ca < Sr < Ba < Cd < Cu < Pb (Haug and Smidsrod, 1962; Mørch et al., 2006). Alginate affinity towards cations is directly dependent on the amount of G-blocks present in the alginate structure (Smidsrød and Skjåk-Bræk, 1990). For practical applications, the use of highly toxic cations such as Pb, Cu, and Cd is limited. The use of Sr and Ba, which are mildly toxic, has been reported in cell immobilization applications although only at low concentrations (Nedovic and Wallaert, 2004). Ca is a nontoxic and hence is widely used to form ionic alginate gels. In this review, we will focus only on Ca-alginate gels because it is the most common alginate gel used. However, a major limitation of Ca-alginate gel is that it is destabilized in the presence of Ca chelators such as citrates, phosphates, carbonates, and lactates (Schlemmer, 1989).

The gelation of alginate is brought about by a cooperative binding of divalent cations and the G-block regions of the polymer. By using competitive inhibition studies, it has been found that the mechanism involved is the dimerization of G residues. The addition of Ca ions into the alginate polymer causes the binding of two G chains on opposite sides. This alignment

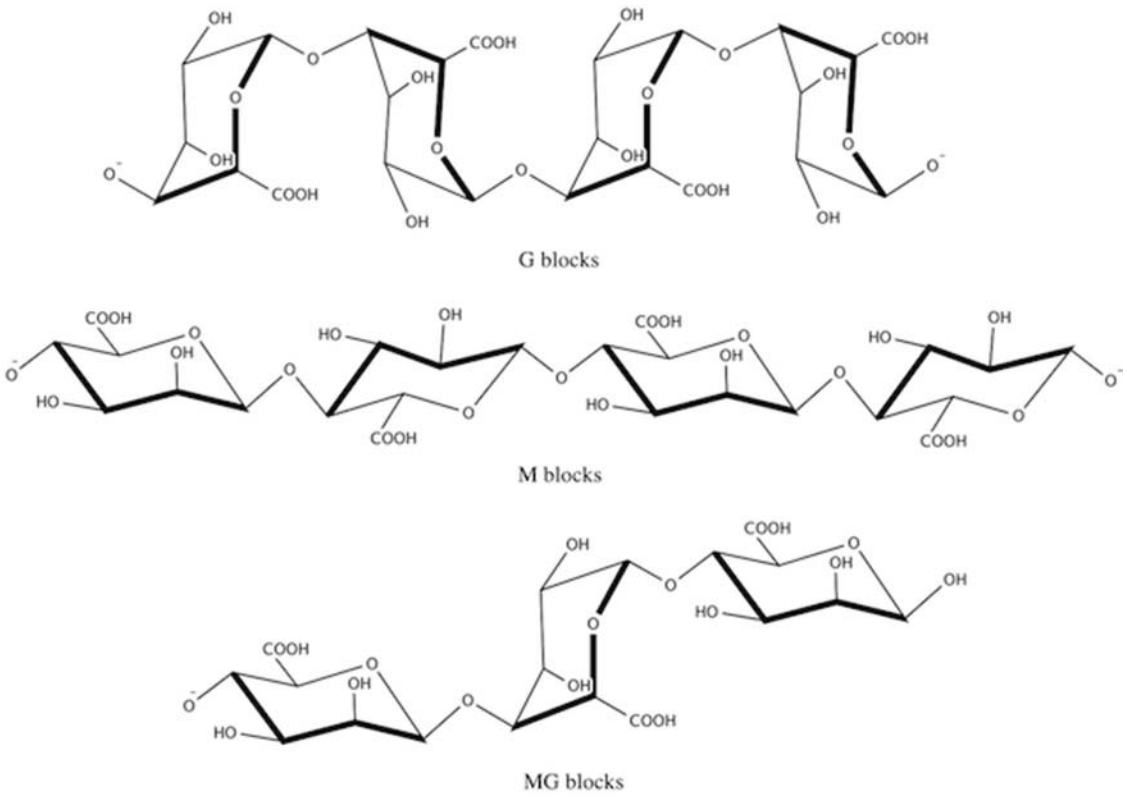

Figure 2. Possible sequences of L-guluronic acid (G) and D-mannuronic acid (M) residues in an alginate polymer. (Modified from Nussinovitch, 1997).

forms a diamond shaped hole consisting of a hydrophilic cavity that binds the Ca ions by multicoordination using the oxygen atoms from the carboxyl groups. This tightly bound polymer configuration results in the formation of a junction zone shaped like an "egg-box" (Figure 3). Each cation binds with four G residues in the egg-box formation to form a 3-D network of these interconnected regions (Clare, 1993). It has been shown that in the case of Ca, formation of a stable junction requires eight to twenty adjacent G residues (Donati and Paoletti, 2009).

Although it is generally recognized that most divalent cations are able to form alginate gels by the "egg-box" formation, it is unknown at this stage if the same gel formation mechanism is true for other divalent cations (Haug, 1961; Haug and Smidsrod, 1965; Jang et al., 1991; Gombotz and Wee, 1998). Past studies have shown that cations can also bind to different block sequence other than G-blocks in alginate. For example, binding studies have revealed that Ca is able to bind to G- and MG-blocks, Ba to G- and M-blocks, and Sr to G-blocks only (Mørch et al., 2006; Donati and Paoletti, 2009). Alginate is also able to gel in the presence of trivalent cations such as Al and Fe. The binding of trivalent cations with alginate is generally enhanced compared to divalent cations. Trivalent cations are able to interact with three carboxyl groups from different alginate biopolymer at the same time, forming a three-dimensional bonding structure that results in a more compact gel network (Yang et al., 2013).

The formation of gel particles can occur either by external or internal gelation. The methods differ in the way crosslinking ions are introduced to the alginate polymer. In internal gelation

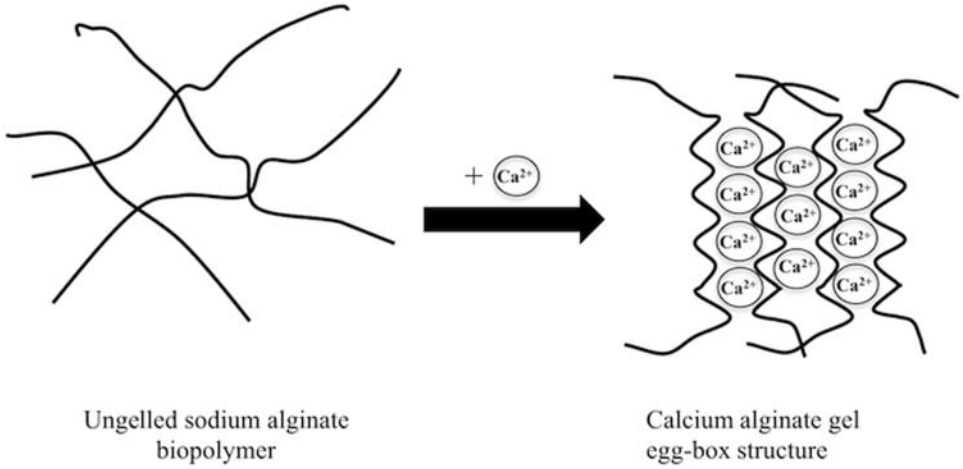

Figure 3. Formation of the egg-box structure during the ionic gelation of sodium alginate.

method, alginate exposure to cations is controlled to achieve a homogeneous distribution of alginate in the hydrogel. Gelation occurs simultaneously at a number of locations (internal and external of hydrogel particles) to give a homogeneous hydrogel structure (Draget, 2009). Inactive forms of Ca such as $CaCO_3$ or $CaSO_4$ is mixed with sodium alginate solution and extruded into oil (Liu et al., 2002). The mixture is then acidified to release Ca from these compounds. Acidification can be achieved either immediately, by direct addition of mineral acid such as glacial acetic acid, or in a controlled fashion using slowly hydrolyzing lactone such as D-glucono-δ-lactone (GDL) (Poncelet et al., 1992; Chan et al., 2006; Draget, 2009).

In the external method (diffusion controlled), cations diffuse from a higher concentration region into the interior of alginate particle. At the outermost layer of the hydrogel-cation layer, gelling kinetics is rapid and gel formation is instantaneous. This feature allows alginate to be used in cell or bioactive immobilization where the cell or bioactives are entrapped in singular alginate gel bead. Furthermore, rapid gel formation is also important in applications where certain size and shape of hydrogel is required. The diffusion method produces alginate gel that is inhomogeneous with a high cation and alginate gradient near the gel surface, which decreases as it approaches the core (Quong et al., 1998). As gel formation commences from the outer surface, cations are continuously diffusing towards the centre of the particle. This creates gelling profiles where the alginate and cation interaction will range from maximum, at the surface, to zero, at the core (Mikkelsen and Elgsaeter, 1995). Alginate also diffuses from the central nongelled region toward the region of zero activity (gelled zone) (Donati and Paoletti, 2009; Draget, 2009).

Alginic acidic gels

Alginic acid gels are formed when pH of the solution is brought down below the disassociation constant (pK_a) of the polymer (Donati and Paoletti, 2009). M and G residues have pK_a of 3.38 and 3.65, respectively. Hence, alginate is negatively charged across a wide range of pH (Draget, 2009; Helgerud et al., 2009). The rate of decrease in pH affects alginate solution in two ways. A rapid decrease in pH results in precipitation of alginic molecules in the form of aggregates while a slow and steady drop in pH results in the formation of a continuous alginic acid bulk gel (Draget et al., 2006). Unlike ionic gels, acid gels of alginate are stabilized by hydrogen bonding and M-blocks residues have been shown to play a part in gelation. On the other hand, acid gels are very similar to ionic gels in that gel strength is correlated to the G-block content in the polymer chain (Draget et al., 1994). Alginic acid gels are less studied compared to ionic gels due to its limited application (Draget et al., 2006). Nevertheless, alginic acid gel is commonly used as antacid to relieve gastric reflux heartburn (Smith and Miri, 2011).

Morphology of alginate gel particles

The morphology and size of alginate gel particles are dependent on the type of production method used. Gel particles that do not contain any entrapped material will have a continuous gel matrix structure. The presence of core materials can influence the morphology and other mechanical properties of the gel structure to a certain extent. Alginate gel particles can exist as single or multi cored system (Figure 4). In a single core system, the core material is concentrated in the middle of the gel surrounded by a layer of alginate gel matrix. An example of this system is when a single oil droplet or cell is encapsulated in a single gel particle. In a multi-cored system, the core material is dispersed throughout the gel matrix. This results in numerous cores within a single-gel particle (Fang and Bhandari, 2012).

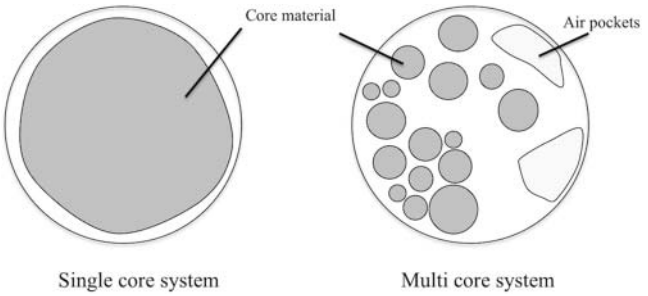

Figure 4. Possible internal arrangement of core material encapsulated in an alginate gel particle.

Scanning electron microscopy (SEM) has shown that the rough surfaces of alginate gel particles are composed of irregular large wrinkles. Although different core materials do not alter surface morphology significantly, the core materials (cells, drugs or emulsion) may be visible on the outer surface of the gel particles (Lee et al., 1998; Arica et al., 2005). Internally, the presence of core materials can disrupt the homogeneity of the alginate gel matrix resulting in the formation of secondary structures such as microchannels that proliferate inside the gel particle. Microchannels extend inwards from the surface and are formed when particulate core materials such as cells (chondrocytes, red blood cells) block the diffusion of Ca^{2+} ions from the gelling solution into the boundary of the alginate droplet (Aydelotte et al., 1998).

Methods for producing alginate gel particles

For most applications, the size of the alginate gel particles is an important consideration when choosing an appropriate encapsulation method. For instance, in food, microgels with an average diameter of 30 μm are preferred in order to minimize the sensory perception of powdery or graininess in food such as yoghurt and ice cream (Heidebach et al., 2012). This allows food manufacturers to improve the bioactive contents in their products while retaining the original taste and texture profiles. In biomedical applications, drug delivery through submicron nanogels allows drugs to be targeted at specific sites in the body. Nanogels (100–200 nm) used in cancer therapy experiments tend to preferentially accumulate in a number of cancerous tumors (Yih and Al-Fandi, 2006). Drug-filled nanogels (<500 nm) that are administered intravenously are also able to evade capture by macrophages in the blood stream and enter the intracellular space of fibroblasts and human stem cells (Boissiere et al., 2006; Torchilin, 2006).

Macrogels and microgels are defined as gel particles with diameter of above 1,000 μm and less than 1,000 μm, respectively. When the size of alginate gel particles falls into the

submicron range (usually less than 500 nm), they are called nanogels (Oh et al., 2008). In general, the complexity of the method is inversely related to the particle size of the resulting alginate gel particles. The main purpose of these methods is to break up the bulk alginate polymer into smaller particles during gelation. Gel particle formation can be achieved by two processes:

Continuous phase formation: A bulk alginate gel is firstly formed followed by the mechanical breakdown of the gel into particles of smaller dimensions. The alginate solution is brought up to a pre-gel state by the addition of cations and then sheared to break up the gel aggregates into smaller particles (Li et al., 2008). It is possible to achieve nano-sized gel particles with this method. Smaller spherical particles can be achieved by oscillatory shear conditions, while steady shearing conditions promote formation of larger elongated particles (Wolf et al., 2001; Burey et al., 2008).

Dispersed phase formation: In this process, the ungelled polymer solution is broken into discrete droplets before gel formation occurs. A number of methods exist to produce gel particles with this process. The variation between each method lies in the different techniques of droplet formation. Conditions during droplet formation and gelation determine the physical characteristics of the gel particles (Burey et al., 2008).

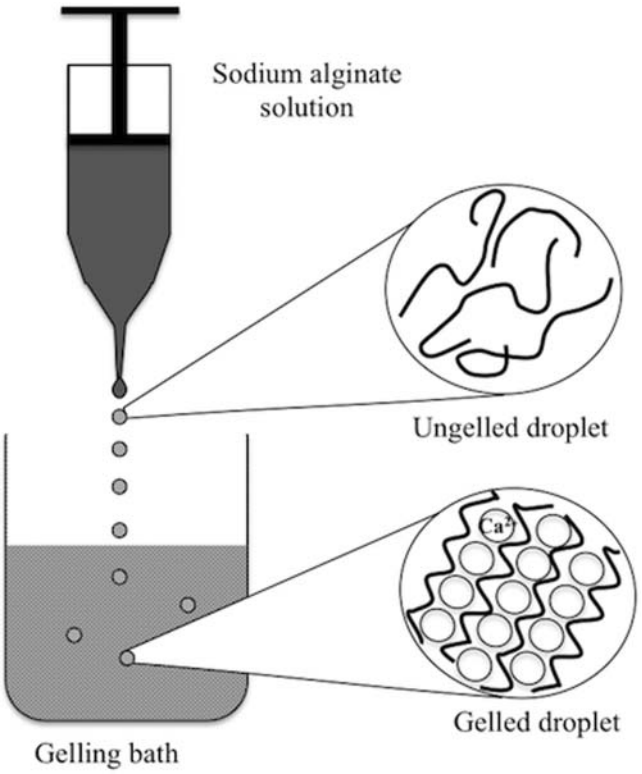

Figure 5. Alginate macrogel formation by a simple dripping (extrusion) setup.

Macrogel particles

Simple dripping (extrusion)

Simple dripping is the most common approach for producing alginate macrogels and has been widely reported (Thu et al., 1996a; Krasaekoopt et al., 2004; Krasaekoopt et al., 2006; Burey et al., 2008). The basis of this method involves the drop-wise extrusion of alginate droplets from a loaded syringe into a calcium gelling bath (Figure 5). When the alginate solution flows out of the syringe opening, a droplet is formed at the needle tip. The alginate droplet grows in size until the droplet detaches from the needle tip and fall toward the gelling bath. During this time, a spherical alginate droplet is formed due to the surface tension of the liquid.

Alginate macrogels produced by simple dripping are generally in the millimeter size range (1–2 mm) (Blandino et al., 1999; Chan et al., 2000; Chan et al., 2011). Examples of compounds encapsulated with this method include cells, oils, enzymes, flavors, and plant extracts (Smidsrød and Skjåk-Bræk, 1990; Fundueanu et al., 1999; Zohar-Perez et al., 2004; Chan, 2011; Zhang et al., 2011).

Gel particle size and morphology can vary depending on the viscosity of the alginate, diameter of the needle opening, exit flow rate of the alginate, and height of alginate exit point from the gelling bath (Ouwerx et al., 1998; Blandino et al., 1999). Particle sphericity is determined by the distance between the needle tip and the gelling bath. When the droplets hit the surface of the calcium gelling bath, their spherical shape can be deformed if the droplet viscosity and surface tension forces are unable to overcome the surface tension exerted by the gelling solution (Chan et al., 2009). However, within a distance of 7–10 cm, the liquid droplet is able to overcome the impact and drag forces to form spherical gel particles (Blandino et al., 1999). Gel particle uniformity can be improved by reducing the surface tension of the calcium gelling bath by the addition of surface active compounds (Thu et al., 1996a). Uniformity of the gel particles is also influenced by the flow rate of alginate. Extrusion by hand results in gel particles with low uniformity while extrusion by a pump will generally produce uniform gel particles. Lee et al. (2013) provides a more detailed account of the dripping extrusion method.

Although extrusion by syringe is the simplest method of producing uniform alginate gel particles, this method produces large gel particle size and scale-up difficulties limit this method to a lab scale setup only (Table 1). In addition, this method is also limited alginate solution with low viscosity (<200cP) due to pumping difficulties and needle blockage (Prüße et al., 2008). The large particle size also requires freshly made particles to be cured in the gelling bath for a period of time. Although the droplet surface gels instantaneously upon entering the gelling solution, a longer time is needed for the cations to diffuse into the interior of the droplet depending on gel particle size (Gacesa, 1988).

Microgel particles

Modified extrusion

Several modified extrusion techniques have been developed to overcome the shortcomings of the simple extrusion method and to produce micron size particles (Figures 6 and 7). These methods can be split into three general categories depending on the method of forming polymer droplet.

(1) Jet break up extrusion: In this method, a laminar jet of polymer is formed by forcing the solution through a

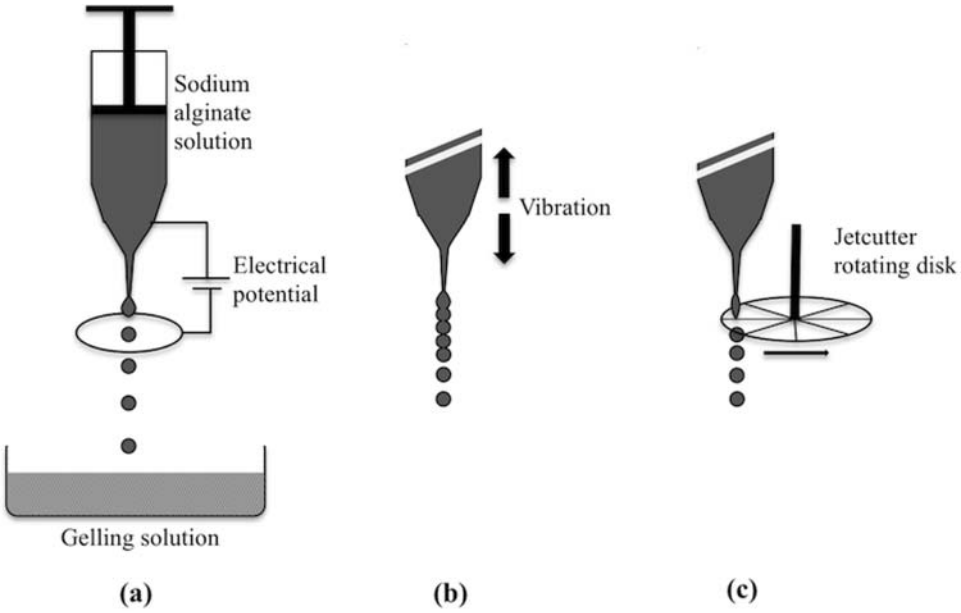

Figure 6. Alginate microgel formation by modified extrusion: (a) Electrostatic atomisation, (b) Vibrating nozzle and (c) Jetcutter.

nozzle tip. The jet is then broken into discrete droplets by electrostatic atomization (Watanabe et al., 2003), vibrating nozzle (Gotoh et al., 1991) or jetcutting (Senuma et al., 2000).

(2) Spinning disk: Droplet formation is achieved by the effect of centrifugal force acting on a flow of polymer solution across a spinning disk or a rotating nozzle.

(3) Atomization: Droplets are produced by pumping air and polymer solution concurrently at high flow rates into a nozzle. Fine polymer droplets are formed when the air and polymer solution come in contact with each other.

Jet break up extrusion. *Electrostatic atomization*: This method uses the effect of applied electric field on the hydrodynamic properties of a liquid jet to generate alginate droplets (Figure 6a). Alginate solution flowing out of a nozzle is subjected to an electric field. The electric field imposes an electrical charge on the liquid surface, which causes deformation and

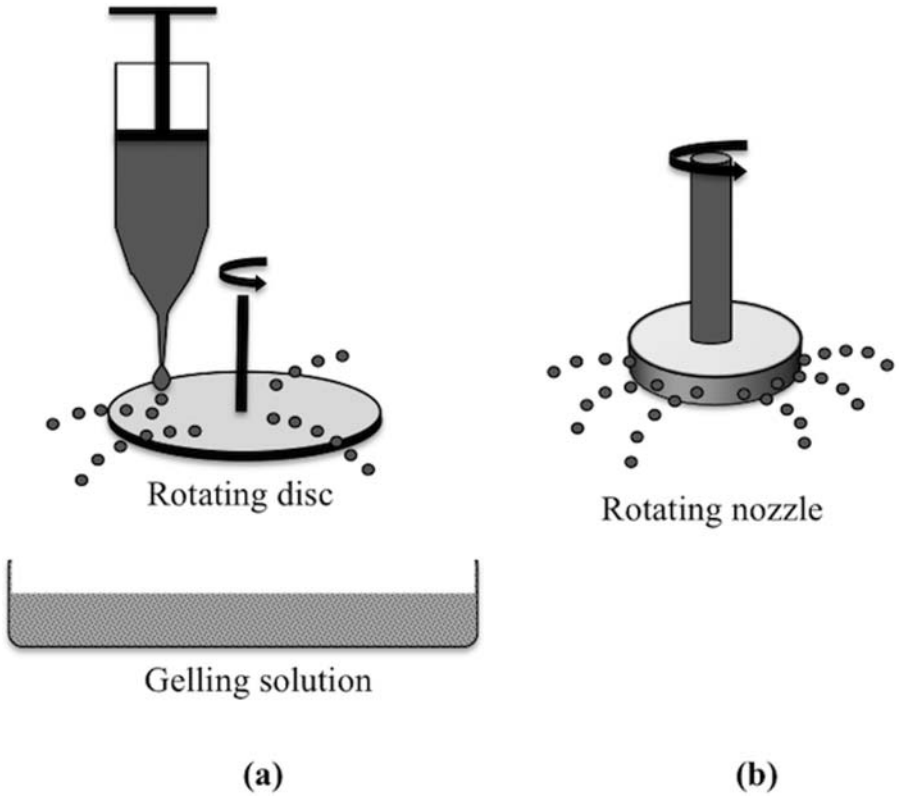

Figure 7. Alginate microgel formation by (a) Rotating disk and (b) Rotating nozzle.

elongation to the liquid flow. Droplets are formed when the deformed liquid is disrupted (Bugarski et al., 1994).

Gel particles produced by this method are usually ranged from 50–350 μm in diameter (Bugarski et al., 1994; Hsu et al., 1994). The strength of the applied electrical field contributes a significant effect on the size of the microgels produced. Most studies have reported that a potential difference in the range of 5–6 kV has been shown to produce spherical microgel particles in the range of 170–400 μm. However, by applying a potential difference of 18–20 kV, Suksamran et al. (2009) were able to produce submicron gels with a diameter of 0.9–1.5 μm. The high electric potential subjected on the liquid does not result in cell death (Halle et al., 1994; Manojlovic et al., 2006). Factors such as diameter of nozzle opening, electrostatic potential, field distance, as well as parameters including polymer surface tension, viscosity, flow rate, and density will influence the size and sphericity of the alginate microgel particles (Poncelet et al., 1999; Klokk and Melvik, 2002).

Vibration technique: In the vibration technique, the alginate fluid jet is subjected to mechanical vibration that is generated when a nozzle made of piezoelectric material vibrates in response to a transducer driven by a wave generator (Figure 6). Alternatively, droplets can be formed when the fluid jet is passed through a fused silica capillary that is being vibrated by a loudspeaker connected to a sine wave sound generator (Yan et al., 2009). The frequency from the wave generator determines the amount of vibrational energy that is generated while the movement of the nozzle disrupts the fluid flow out of the tip, which leads to the formation of discrete sodium alginate droplets.

This method produces alginate microgels with a large size range of 300 μm to 5 mm (Prüße et al., 2008), although particles as small as < 20 μm have also been reported (Yan et al., 2009). The size of the droplets is influenced by the frequency of the vibration, the applied voltage, nozzle diameter as well as the viscosity, flow rate, and surface tension of the alginate solution (Del Gaudio et al., 2013).

Jet cutter method: In this method, a rotating device comprised of small wires in a holder is placed beneath a nozzle (Figure 6). The rotation of the cutter disrupts the flow of the alginate fluid jet into cylindrical segments that transforms into discrete droplets of alginate as the droplet falls into a gelling bath. The rotating cutting action can result in the loss of alginate material (cutting loss) that occurs when the wire cuts through the alginate fluid. According to the inventors, this is estimated to be 5% of the staring material but can be recycled (Prüße et al., 2008).

This method produces microgels in the size range of 200 μm to 5 mm in diameter with high uniformity (CV = 7%). The size and morphology of the gel particles are dependent on the nozzle diameter, number of cutting wires, rotational speed of the cutting wire and flow rate of the alginate solution (Prüße et al., 1998).

The fact that these methods are performed under mild conditions without requiring toxic organic solvents has made these encapsulation methods widely used in the encapsulation of living organisms such as plant cells (Sajc et al., 1995), yeast (Nedovic and Wallaert, 2004; Manojlovic et al., 2006), and insect cells (Bugarski et al., 1994). The main drawback with all of the above methods is the requirement of a gelling bath, which makes them batch processes. In addition, these methods are often complex, tedious and only suitable for small volumes of biopolymer. The complexity in scaling up is compounded due to the increased need for electric energy for a method such as electrostatic atomization (Tran et al., 2011). Although some attempts of scaling up of these methods have been made [10 nozzle electrostatic generator (Strand et al., 2002); jet cutter method (Prüße et al., 2008)], these efforts have not reached a truly industrial scale up. Only the industrial scale up of the jet cutter method has been documented so far, which has claimed an output of 24 L/hr (0.5 mm particles) (Prüße et al., 2000).

Spinning disk/nozzle. The alginate solution can be atomized by a rotating disc or rotating multi-nozzle setup (Figure 7) (Ogbonna et al., 1989). In both setups, the alginate solution is fed directly onto a rotating disc or through rotating nozzles. Discrete particles are formed when the centrifugal force from the rotation disintegrates the fluid flow out of the nozzle or disc surface. The speed of rotation determines the morphology and size of the formed droplets (Ryoichi et al., 2001; Prüße et al., 2002). At slow rotational speeds, discrete droplets are formed directly at the perimeter of the disc or nozzle. At medium speeds, a continuous fluid ligament is formed upon exit from the disc or nozzle. After a certain length, the ligament disintegrates into discrete droplets. However, at high rotational speeds, a fluid film is formed when the solution leaves the disc or nozzle that causes rapid disintegration of the film into droplets (Prüße et al., 2002).

The rotating nozzle setup produces alginate microgels ranging from 270 μm to 2 mm (Ryoichi et al., 2001) while the rotating disc method produces microgels of 300–600 μm with a narrow distribution (CV = 6–27%) (Senuma et al., 2000). Factors such as flow rate and viscosity of the alginate solution also influence the microgel size and morphology. Uniform sized spherical microgels were produced using 2–3 wt% alginate. At sodium alginate concentrations below 2 wt%, pear shaped microgels are formed due to droplet deformation caused by the impact of the less viscous droplets against the surface of the gelling bath (Senuma et al., 2000).

A major shortcoming of this method is the occurrence of satellite microgels that are generated when the break-up of the fluid ligaments is not ideal (Prüße et al., 2002). The presence of satellite microgel particles is caused by nonoptimal production conditions and can constitute up to 50% the total mass of microgels produced (Senuma et al., 2000). As these microgels are significantly smaller (50–250 μm) than the rest of the microgel population, their presence will increase the size distribution of the existing samples. There have been attempts to scale up this process. Champagne et al. (2000) showed a large scale (up to 50 kg/hr) production of microgels using a system comprised of a rotating disk, which atomizes alginate droplets onto a rotating vortex-bowl containing a recirculating gelling solution. The vortex effect from the rotating bowl causes the gelled particles to be spun out of the bowl and into a collecting chamber.

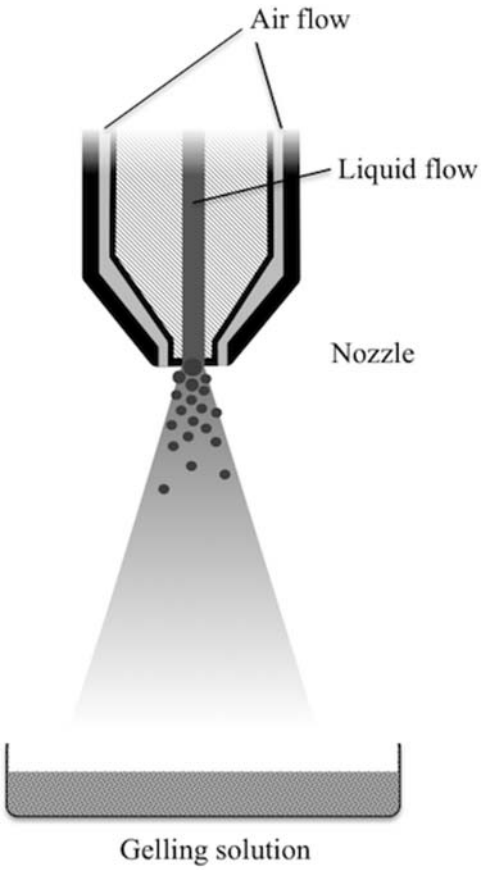

Figure 8. Alginate microgel formation by pneumatic nozzle.

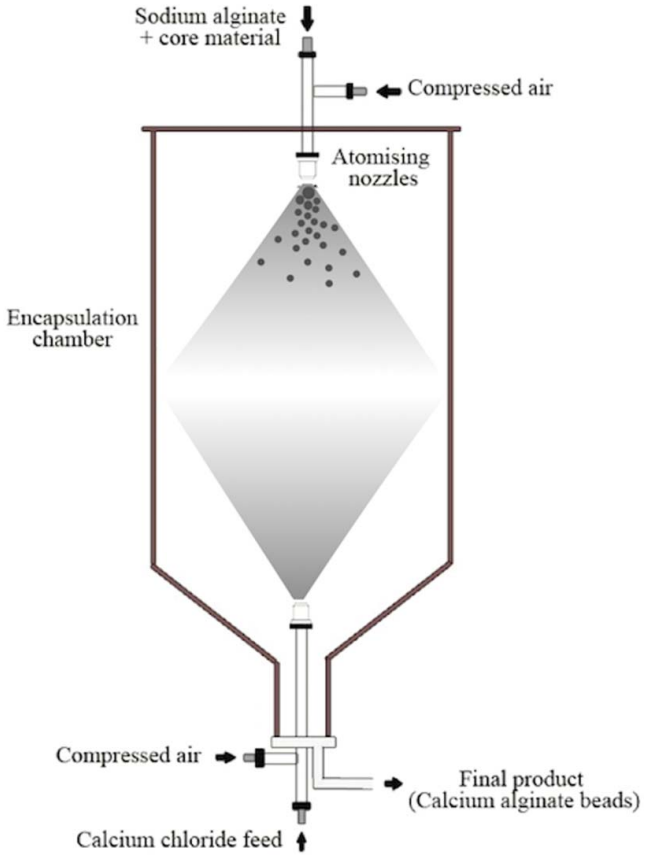

Figure 9. Setup and design of the spray aerosol system used for producing micron-sized calcium alginate beads. Atomized sodium alginate droplets containing a core material are gelled upon exposure to Ca2+ mist in the chamber. Gelled particles are formed instantaneously and are collected from the bottom of the encapsulation chamber.

Spray nozzle. Microgels can also be produced by pneumatic nozzles (Figure 8) (Kwok et al., 1991). Pressurized sodium alginate is passed through a nozzle orifice. As the alginate solution exits the orifice, it comes in contact with pressurized gas (usually air), which causes the atomization of sodium alginate. The sodium alginate droplets are then collected in a gelling bath. With stationary nozzles, the size of the alginate droplet size can be controlled by a number of parameters such as alginate concentration, rate of delivery, liquid pressure, air pressure, and spraying distance (Cui et al., 2001).

This method has been applied in the encapsulation of lipid nanoparticles, Bacillus Calmette–Guérin (BCG) vaccine, antigens, proteins, and bacteria (Kwok et al., 1991; Abraham et al., 1996; Yeo et al., 2001; Strasdat and Bunjes, 2013). The use of stationary nozzles such as the Turbotak air atomizing nozzle generally produces alginate microgels in the range of 80–130 μm in diameter (Cui et al., 2001; Yeo et al., 2001). However, smaller droplet size (5–15 μm) can also be produced with optimized production parameters (Kwok et al., 1991; Abraham et al., 1996; Yeo et al., 2001).

The use of stationary nozzles is more suitable for small scale production and is a batch process. The added disadvantage of this method is that blockages can occur in the nozzles especially with high viscosity polymer solution (Burey et al., 2008).

Impinging aerosol gel formation technique

The impinging aerosol method, recently patented (provisional) by Bhandari (2009), is a method of producing alginate microgels by oppositely atomizing alginate and cross-linking solution in a reaction chamber (Figure 9). Alginate solution is atomized using compressed air driven pneumatic nozzles into fine droplets. At the same time, a separate pneumatic nozzle located at the bottom of the chamber atomizes Ca solution in the form of a mist. The tiny droplets of atomized sodium alginate are instantaneously gelled upon contact with the $CaCl_2$ mist. The newly formed calcium alginate microcapsules settle and flow out of the chamber.

This method is effective in the encapsulation of drugs and probiotic cells. Sohail et al. (2011) showed that encapsulation of *Lactobacillus rhamnosus* GG (LGG) and Lactobacillus acidophilus NCFM was possible with this method, resulting in minimal cell loss. Hariyadi et al. (2012) showed that the bioactivity of lysozyme and insulin encapsulated by this method was retained (> 75%) after gastric treatment. The impinging aerosol system allowed easy modulation of microgel release kinetics and encapsulation efficiency using different Ca cation concentration (Hariyadi et al., 2012). Encapsulation of emulsion droplets was also possible with this method (Figure 10(c) & (d)). Lipid encapsulation has been previously reported but the oil-filled microgels from past studies were either large (mm-sized microgels produced by extrusion method)(Chan et al., 2000; Chan, 2011) or produced using a low throughput method (electro co-axial spraying) (Soliman et al., 2013).

The impinging aerosol method has numerous advantages over other current methods. As the sodium alginate and calcium

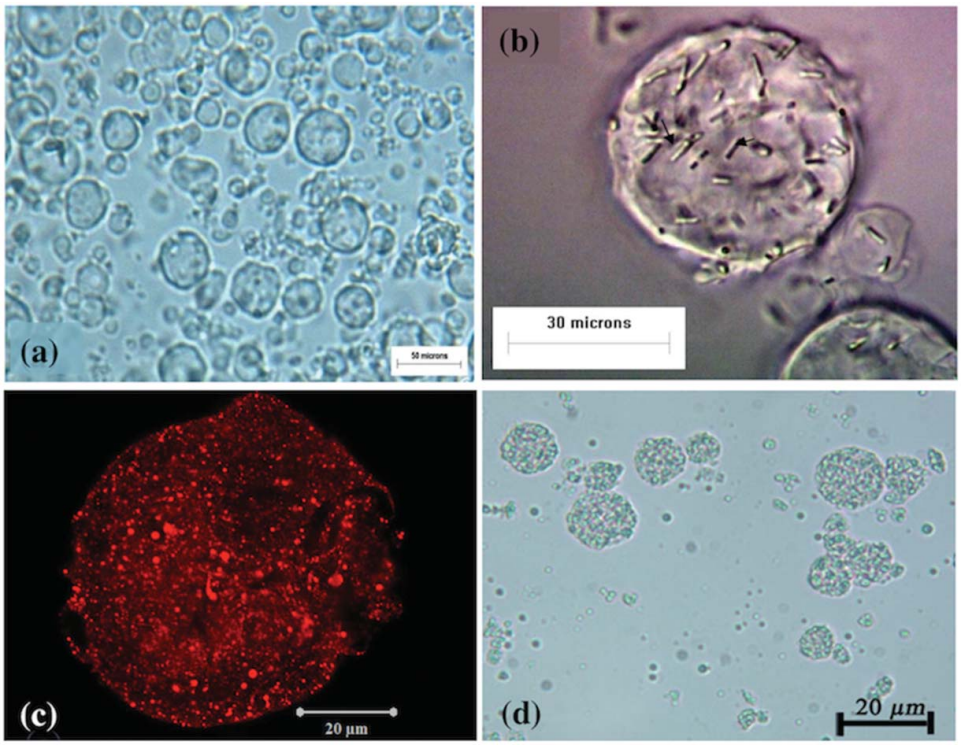

Figure 10. Optical micrograph of (a) Lactobacillus acidophilus NCFM (Sohail et al., 2011), (b) Lactobacillus rhamanosus (Sohail et al., 2011) and (d) vegetable oil nanoemulsion (unpublished data) encapsulated in alginate microgel particles by the impinging aerosol method; (c) Oil-filled microgel under confocal light scanning microscopy (CLSM) with oil droplets stained red (unpublished data).

chloride solutions can be fed into the reaction chamber continuously by a pump, this method is continuous. The modular design of the system permits different nozzle types to be used and tunable parameters such as air pressure, liquid pressure and distance between nozzles allows alginate microgel of narrow size distribution to be produced. Spherical alginate microgels in the range of 11–80 μm (with an average size of around 30 μm) can be consistently produced with this method. The lack of moving parts in the chamber also allows easy implementation of cleaning-in-process (CIP) procedures. Due to the lack of moving parts and electrical components, this method requires low maintenance and is easy to operate. The alginate microgels (<30 μm) produced by this method also means that application into food products will less likely affect key sensory properties such as texture and taste (Rao and Lopes da Silva, 2007).

Emulsification technique

In the emulsification method, alginate microgels are formed in a non-aqueous continuous phase. Alginate solution is dispersed in an oil bath and homogenized to produce a water-in-oil of emulsion (Figure 11). The gelling solution is slowly introduced into the emulsion by mixing. The droplets of alginate and gelling solution coalesce and gelling occurs. The emulsification technique is also often used in conjunction with the internal gelation technique described earlier (Ribeiro et al., 2005).

The particle size produced from emulsification method is generally in the range of 100–1000 μm (Zhang et al., 2006a), 0.2–1 mm (Poncelet, 2001), and 20–220 μm (Chan et al., 2000). Size distribution and diameter of the alginate microgels are influenced by factors such as emulsifier type and concentration, ratio of oil to sodium alginate and homogenization shear force (Reis et al., 2006). The use of emulsifiers in the emulsification technique is not essential although the presence of emulsifier will help decrease the mean diameter of the alginate microgels. Emulsifiers such as Span 80, Tween 80, Tween 85, sodium desoxycholate, poly(vinyl alcohol) (PVA), and Pluronic F68 in iso-octane have been reportedly used to produce microgels of 1 to 150 μm in diameter (Wan et al., 1992; Lemoine et al., 1998). An increase in the alginate solution to oil ratio will also lead to smaller alginate microgel diameters (Silva et al., 2005).

The advantage of the emulsification technique is that it is an economical technique for producing alginate microgels. The internal gelation method produces a more homogeneous gel structure. The main weakness of this method is that the random droplet coalescence in the method often leads to microgels that vary widely in size and shapes. This may cause poor reproducibility in controlled release studies (Fundueanu et al., 1999; Zhao et al., 2007). Nevertheless, process conditions can be controlled to produce alginate microgel with good uniformity (Poncelet, 2001). Although scaling up of this technique has also been shown possible, the technique is a batch process that is only suitable for producing microgels in the range of 200–1,000 μm (Poncelet et al., 1992). Furthermore, an extra procedure is needed for separating the microgel products from the oil phase. This usually requires light centrifugation of the mixture followed by a washing step with solvents to remove excess oil (Sultana et al., 2000). In addition, the high shear stress and heat generation in this method may cause denaturation to biological compounds and cells (Zhao et al., 2007).

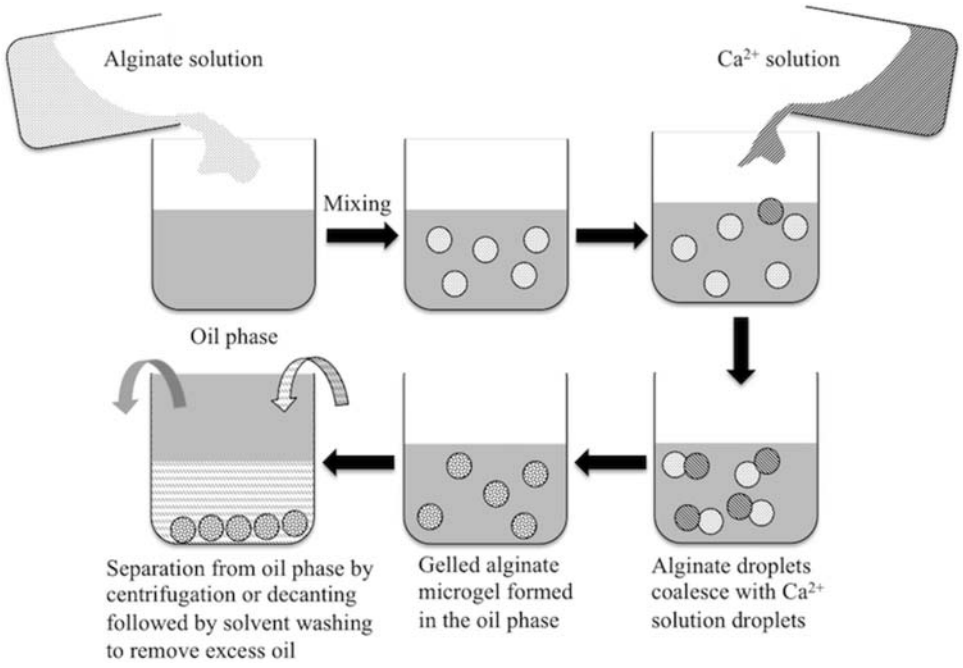

Figure 11. Alginate microgel formation by emulsification method.

Microfluidics

The field of microfluidics involves methods and devices that allow manipulation and control of fluids through sub-millimeter or sub-micron flow scales (Holmes and Gawad, 2010). Traditionally, microfluidic systems have been applied in the field of fluid mechanics, cell culture, DNA analysis, protein analysis, and PCR. Microfluidic devices are generally fabricated from elastomeric materials such as polydimethylsiloxane (PDMS) or polyurethane. Microchannels that allow the continuous flow of the different liquid phases are then etched onto the materials using soft lithography (Seiffert, 2013).

In the microfluidic approach, alginate microgels can be produced by internal gelation or external gelation (Figure 12). In external gelation, a central channel in a microfluidic device generates sodium alginate droplets by emulsification in a non-polar (soybean oil) continuous phase containing calcium acetate. As the sodium alginate droplets flow downstream, calcium acetate from the continuous phase diffuses into the droplet and releases Ca ions that initiate gelation (Tumarkin and Kumacheva, 2009). In internal gelation, droplets of sodium alginate containing calcium carbonate, a crosslinking agent precursor, is formed from the middle channel. A non-polar continuous phase containing acetic acid is introduced from the side channels. As the droplets moves downstream, the diffusion of acetic acid into the droplets liberates Ca ions calcium carbonate (Tan and Takeuchi, 2007).

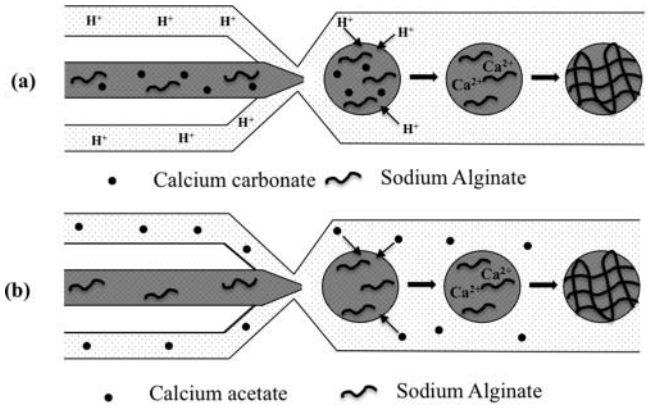

Figure 12. Alginate microgel formation in a microfluidic chip by (a) Internal gelation and (b) External gelation. (Modified from Tumarkin and Kumacheva, 2009).

Microfluidic systems enable precise control of the microgel droplet size and allows the production of monodisperse microgels with a defined size distribution for the encapsulation of biomaterials in the field of molecular biology, pharmaceutical, health, food, and cosmetics (Hu et al., 2012; Lin et al., 2013). Several groups have also reported on the intentional production of non-uniform alginate microgel of different shapes and sizes (tail end, dimpled, mushroom-like, hemi spherical, red blood cell-like and disk-like) by the microfluidic method coupled with internal or external gelation (Hu et al., 2012; Lin et al., 2013). The emergence of this trend is seen as a response to new evidence emerging from *in vivo* studies that have found drug pharmacokinetics and cellular functions to be influenced by the shape of the encapsulated particle (Christian et al., 2009; Venkataraman et al., 2011).

Alginate microgels produced by this method ranged from 50 to 70 μm in diameter with a narrow size distributions and polydispersity index of 2.8% (internal gelation) and 3.8% (external gelation) (Liu et al., 2006; Zhang et al., 2006b). Morphology of the microgels was influenced by various factors. With the external gelation method, microgels with capsular, gradient, and uniform structures could be produced by controlling the extent of Ca diffusion into the alginate droplets, concentration of calcium acetate in the continuous phase and residence time in the device (Tumarkin and Kumacheva, 2009). Uniform flat non-spherical (discoid) microgels could be produced with internal gelation by increasing residence time in the microchannels and

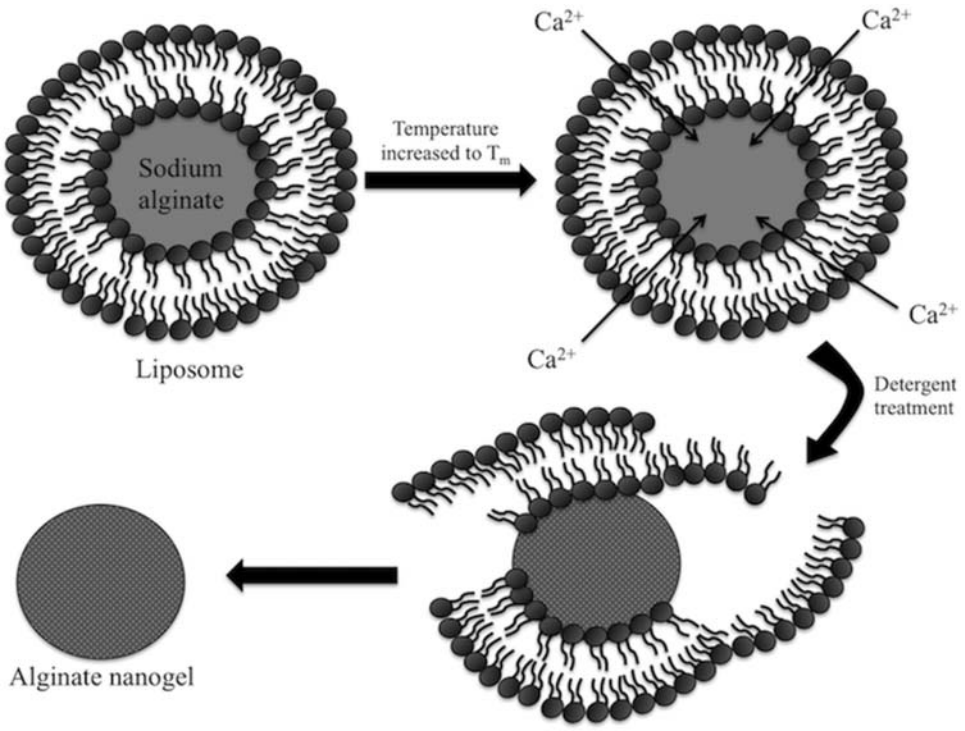

Figure 13. Alginate nanogel formation by liposomal template method. (Modified from Hong et al., 2008).

increasing the concentration of reactants (calcium carbonate and acetic acid) (Tan and Takeuchi, 2007).

Although this approach is suited to produce uniform alginate droplets, the complex fabrication process of the microfluidic device that involves computer-aided design and non-standardized process such as soft lithography, is expensive, time-consuming and not widely accessible (Tang and Whitesides, 2010). Specialized equipment such as pumps and valves that can handle minute liquid volume is also required. Because of the constrained geometry of the device, surface-surface interactions between the microgels and microchannel surface will influence the flow of particles through the microchannels (Fiddes et al., 2007). The scalability of this method is limited due to the complexity of the devices. Scalability has been attempted by incorporating a large number of devices working in tandem but it remains to be seen if this is scalable to industrial levels.

Alginate nanogel particles

Templating method

Nanogels can be created by using nanovesicles or emulsion droplets as templates. In micellar templating, sodium alginate solution is enclosed within liposomes (Figure 13). As the temperature is raised close to the melting point of the liposome lipid bilayer, the porosity of the bilayer increases and allows external Ca ions to diffuse into the liposome structure and alginate gelation begins. The introduction of a detergent removes the lipid bilayer and releases the newly formed calcium alginate nanogel (Hong et al., 2008). The size distribution of nanogels produced with this method is 120–200 nm. De Santis et al. (2014) further reported on the use of a polyion complex micelle (PIC) made up of poly[(N-isopropylacrylamide)-block- PAMPTMA] (PNIPAAM-b-PAMPTMA) polymers as nanovesicle templates for producing alginate nanogels in the size range of 80–100 nm.

Similarly, microemulsion can also be used as template for nanogels. Microemulsions are oil-in-water (O/W) or water-in-oil (W/O) systems comprised of water, oil, and amphiphile that are optically isotropic and thermodynamically stable (Hoar and Schulman, 1943; Danielsson and Lindman, 1981). Microemulsions spontaneously form when appropriate amounts of surfactants, oil and water are mixed together. When alginate is emulsified into a W/O microemulsion system, the addition of an emulsified Ca solution initiates gelation of the nanogels by the intermicellar transfer of Ca ions into the alginate W/O droplets. Subsequent washes with acetone removes the surfactant layer. Nesamony et al. (2012) described a method whereby sodium alginate is dispersed in a self-assembling microemulsion system with dioctyl sodium sulfosuccinate (DOSS) as surfactant and isopropyl myristate (IPM) as the oil (continuous) phase. This method yielded alginate nanogels with a mean diameter of 350 nm.

Although these methods have so far only been applied in the encapsulation of proteins, the ability to make nanogels opens up potential applications in cellular drug delivery (Nesamony et al., 2012). Nonetheless, these methods are complex and produce very low yields, making it unsuitable for scale up. For example, due to the low encapsulation efficiency of the liposome system, the nanogel yield from this method is less than 10% (Hong et al., 2008). Furthermore, the recovery of nanogels is difficult and often requires ultracentrifugation. So far, no application has been reported with this method. The use of harmful chemical and solvents also limits their use in biochemical, pharmaceutical, and food applications.

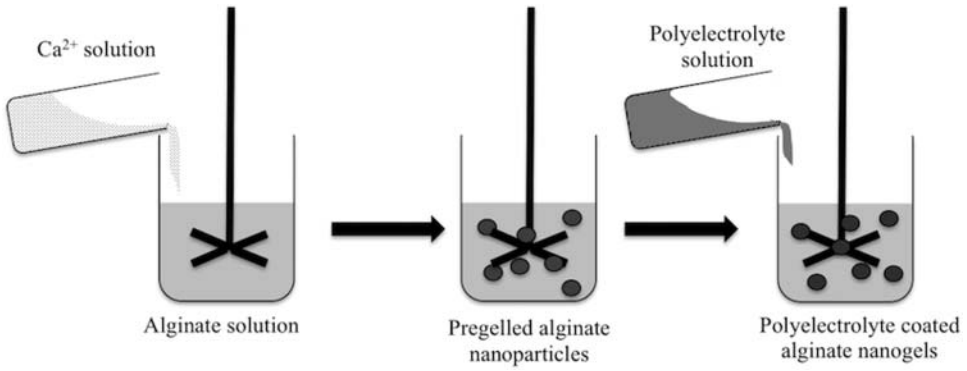

Figure 14. Formation of alginate-polycation hybrid nanogel particles.

Alginate-polycation hybrid nanogels

Alginate nanogels can be produced by utilizing the ability of alginate to form electrostatic complexes with polycations at low pH. Sarmento et al. (2006) described a process whereby low concentration (18 mM) calcium chloride solution was added slowly to a 0.0063 wt% sodium alginate solution under constant stirring (Figure 14). The addition of the dilute Ca solution initiates the cross-linking of sodium alginate while mechanical stirring prevents bulk gel formation by breaking the pregelled alginate into smaller aggregates. After 60 minutes, chitosan solution is slowly added into the pregelled alginate solution under constant agitation. The added chitosan forms a polyelectrolyte complex with alginate and stabilizes the pregelled microgel nucleus into individual sponge-like nanoparticles (Sarmento et al., 2006). Other polycation, such as poly-L-lysine, that forms polyelectrolyte complex with alginate has also been shown to be suitable in this method (Rajaonarivony et al., 1993).

This method produces irregular-shaped alginate nanoparticles with particle diameter as low as 635 nm (alginate/chitosan nanoparticles)(Silva et al., 2005; Silva et al., 2011), 280 nm (alginate/poly-L-lysine nanoparticles) (Rajaonarivony et al., 1993), and 250 nm (silica/alginate/poly-L-lysine nanoparticles) (Boissiere et al., 2006). Particle size is dependent on a number of factors such as polymer molecular weight, mixing conditions, and polymer viscosity (Wolf et al., 2001; Sarmento et al., 2007). A low viscosity alginate solution with polymer concentration as low as 0.063% can be used to make nano particles (Rajaonarivony et al., 1993). The mass ratio of polycation to alginate and order of Ca and polycation addition to the sodium alginate solution also determines the particle size. When alginate to chitosan mass ratio was decreased from 6:1 to 3.3:1, the mean size of the alginate-chitosan nanoparticles increased from 764 to 2209 nm (Sarmento et al., 2006). Alginate-poly-L-lysine nanoparticles of 280 nm was produced when Ca^{2+} was added to sodium alginate first followed by poly-L-lysine. However, when the order of addition was reversed, the nanoparticles produced were 850 nm in diameter. The addition of Ca leads to formation of a compact packing domain within the alginate molecules (egg-box structure). This forms very small aggregates of alginate molecules in the system. The polycation that is added later only acts to strengthen the aggregates into small and well-defined nanoparticles. If the polycations are added first, the formation of the compact egg-box structure is not possible as the polycation-alginate complex that are formed are not tightly packed and well organized (Rajaonarivony et al., 1993).

The application of this method has been explored as a carrier for drugs (doxorubicin) (Rajaonarivony et al., 1993), cells (hemoglobin) (Silva et al., 2005), hormones (insulin) (Sarmento et al., 2007), and herbicides (paraquat) (Silva et al., 2011). Although the potential application of the nanoparticles is very wide, the low nanoparticle yield and length of time required to produce these nanoparticles is a hindrance to scaling up.

Polyelectrolyte coating of alginate gel particles

As mentioned earlier in section 5.3.2, alginate gels are able to form complexes with strongly positively charged polycations such as chitosan, poly-L-lysine and polyethyleneimine. Strong ionic interactions occur between the carboxyl residues of alginate and amino terminals of polycations (Helgerud et al., 2009). This interaction is used to form an outer layer of polycation on the surface of alginate gel beads (Figure 15 and 16) that has been shown to confer protective properties to the content of the alginate gel particles. The polycation-alginate complex layer reduces alginate gel porosity and forms an additional barrier against molecule diffusion in and out of the gel particles (Gombotz and Wee, 1998; Heidebach et al., 2012).

In literature, the use of chitosan and poly-L-lysine as an additional layer has been widely reported to improve probiotic viability in gastric pH (Krasaekoopt et al., 2006), modulate enzyme and cell release (Zhou et al., 1998), improve encapsulated lipid stability (Gudipati et al., 2010), and as a novel drug delivery system (Ghaffari et al., 2011). While chitosan bound alginate beads have higher mechanical strength, poly-L-lysine bound alginate beads have been shown to be able to dissolve more readily in simulated intestinal fluid while conferring the same low pH protection behavior to the contents of alginate microgel. De and Robinson (2003) showed that methylene blue was released from poly-L-lysine-alginate nanogels twice as quickly as from chitosan-alginate nanogels at similar ionic conditions. However, the use of chitosan and poly-L-lysine in food is still limited due to regional legislation and toxicity issues.

As alginate gel is negatively charged across a large range of pH, positively charged contents can potentially bind with carboxylic acid sites on alginate by electrostatic interaction (Gombotz and Wee, 1998). This interaction has been reported with anionic drugs (Stockwell et al., 1986; Segi et al., 1989), proteins (Velings and Mestdagh, 1994; Kuo et al., 2007; Ching et al., 2014), hormone (Catarina et al., 2006), and DNA (Quong and Neufeld, 1999). Catarina et al. (2006) showed that electrostatic

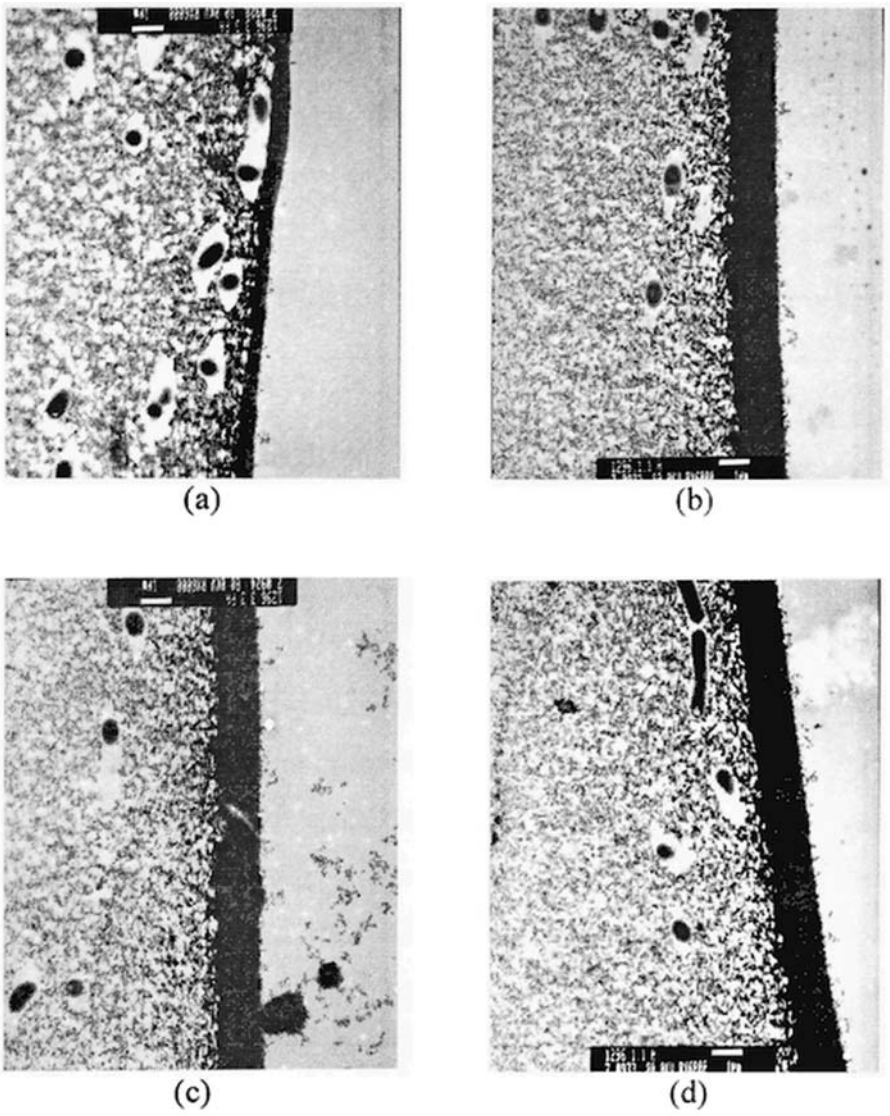

Figure 15. Transmission electron microscope (TEM) micrographs of alginate microgel surfaces with different polyelectolyte coating; (a) uncoated, (b) chitosan coating, (c) alginate coating, and (d) poly-L-lysine coating (Krasaekoopt, 2004).

attraction prevented release of the insulin from alginate beads. Anionic drugs were rapidly released from alginate beads compared to cationic small molecular drugs due to electrostatic interactions (Stockwell et al., 1986). The retardation of diffusion of positively charged bovine serum albumin (BSA) proteins from negatively charged alginate gel particles has also been observed (Tanaka et al., 1984).

Physical properties of alginate gel particles

Gel strength

Gel strength in calcium alginate gels is influenced by the G-block content of the alginate and the level of interaction of cations with alginate (Smidsrød, 1974). At a given cation concentration, a high G-block content gives high gel strength while a high M-block content gives medium strength gel with fewer tendencies for syneresis. The trade off for a strong gel is that the gels are brittle in high G-block gels while a high M-block gel will be more elastic (Chapman, 1980). Gel strength is also dependent on the degree of interaction of alginate with divalent cation, which is dependent on ionic radius. For example, Br, which has a high affinity for alginate and can bind to M- and G-blocks, produces stronger gel compared to Ca ions, which bind to both G- and MG-blocks, and which have less affinity towards alginate (Mørch et al., 2006). For multivalent cations, charge and ionic radius affect the interaction with alginate (Yang et al., 2013).

The concentration of gelling ions used in alginate gelation has a direct effect on gel strength. Draget et al. (1993) found that in gels with excess Ca ions, gel strength increased with the molecular weight (M_w) of alginate. However, this was only true for alginate with molecular weight up to 150 kDa. At M_w higher than 150 kDa, only a slight amount of increase in gel strength can be observed when Ca binding sites were saturated. When Ca levels were increased from 8 to 15 mM, gel strength of alginate gel was observed to increase exponentially (Draget et al., 1993). Addition of more Ca ions increased gel strength of high G-block alginate. However, excess Ca had little effect on low G-block alginate as all the G-blocks have already fully combined (Clare, 1993).

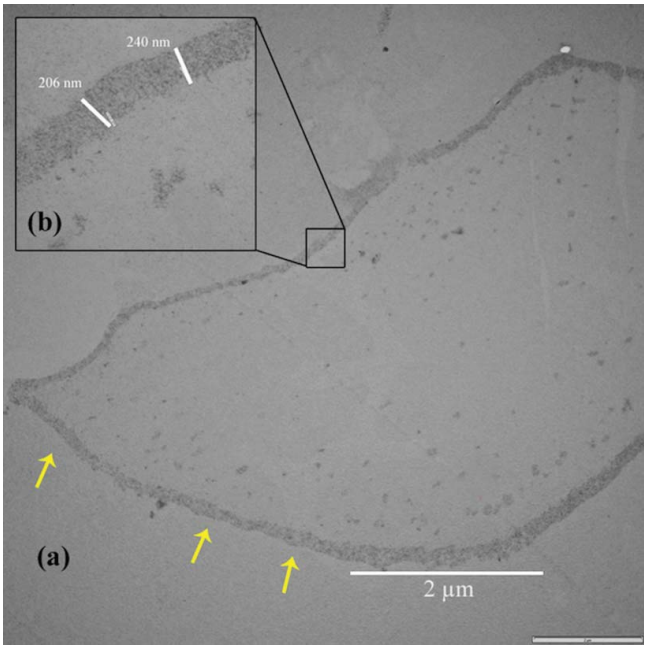

Figure 16. TEM image of sodium caseinate protein coating (yellow arrows) on the surface of an alginate microgel. (Ching et. al., 2014).

The presence and volume fraction of dispersed phase within a gel particle can result in dramatic changes in gel strength (Chen and Dickinson, 1999). Active fillers enhance the microstructure of the gel by interacting strongly with the gel matrix. Inactive fillers have little to none or repulsive interactions with the gel matrix and leads to a decrease in gel strength (Vliet, 1988). The most commonly studied filler particles are emulsion droplets (Kim et al., 1996; Chen and Dickinson, 1999; Dickinson, 2012; Lorenzo et al., 2013). The size of the dispersed phase also plays a part in influencing gel strength. Kim et al. (1996) showed that when the size of the filler particle (emulsion droplets) was larger than the average pore size of the gel, the droplets act to weaken the gel regardless of whether or not the particle interacts with the matrix.

Syneresis and swelling

When alginate gels are formed, water molecules that are bound to the internal structure of gel by hydrogen bonding are trapped within the gel matrix. Syneresis occurs when water molecules are exuded out of the gel matrix due to an external force that contracts the gel (Helgerud et al., 2009). For commonly used food hydrocolloid, hard and brittle gels are more susceptible to syneresis compared to more elastic gels (Mao et al., 2001). In alginate gels, Draget et al. (2001) showed that a linear relationship exists between gel syneresis, alginate M_w and the degree of flexibility of the elastic junctions. Syneresis was observed to be higher in alginate gels with higher proportion of alternating MG-blocks and lower molecular weight (M_w) (Donati et al., 2005). Draget et al. (2001) also showed that low molecular weight alginate creates rigid gel structure that resists deformation (contraction) forces that lead to syneresis while results from Donati et al. (2005) suggested that syneresis increased as storage modulus decreased in alginate gel with MG-MG junctions due to a partial collapse of the gel matrix network (Davidovich-Pinhas and Bianco-Peled, 2010). In a system where alginate gel is saturated with Ca ions, syneresis is shown to be negligible (Helgerud et al., 2009).

Alginate gels have the ability to increase in size under different conditions. The swelling behavior of alginate gels has been studied by numerous groups (Segeren et al., 1974; Moe et al., 1993; Draget et al., 1996; Pillay and Fassihi, 1999; Saitoh et al., 2000; Qin, 2008; Davidovich-Pinhas and Bianco-Peled, 2010). These studies showed that the swelling capacity of alginate beads reduces with an increase in Ca concentration. Moe et al. (1993) showed that dried alginate beads swell instantaneously to their original size when rehydrated in water. Pillay and Fassihi (1999) demonstrated that alginate beads size decrease at low pH and swell at high pH (>6.6). Darrabie et al. (2006) also found that swelling was reduced in alginate beads prepared with higher amounts of G-blocks. Most importantly, it was observed that gel particle swelling was dependent on the gelling cation. Ba ions induced gels showed significantly lower swelling capacity compared to Ca gels (Smidsrød and Skjåk-Bræk, 1990). This difference was attributed to the increased affinity of Ba towards the G blocks.

Rheology

Alginate gel particles are viscoelastic soft particles that are deformable in response to external stimuli due to the presence of water in their gel network. Although there are only limited studies on the rheological behavior of alginate microgel particle suspensions, it is highly likely that the rheological behavior will be analogous to polymeric or hydrocolloid microgel suspensions where rheological behavior is governed by suspension phase volume (ϕ), microgel particle modulus and inter particulate interaction (Adams et al., 2004; Liétor-Santos et al., 2011; Shewan and Stokes, 2012).

At low phase volume, the dilute microgel suspension follows the rheology of hard sphere suspensions in which suspension viscosity increases with an increase in phase volume (Wildemuth and Williams, 1984). As the phase volume reaches a maximum packing fraction, microgels are arranged in a close pack system and are sterically confined by neighboring microgels (Ketz et al., 1988). Suspension viscosity increases sharply towards infinity and rheology of the suspension deviates from the hard sphere suspension to a solid-like behavior with a yield stress (Ketz et al., 1988; Stokes, 2011).

At high phase volume, the microgels are close or in contact with each other and in the absence of interparticle interaction, the rheology of the suspension is determined by deformability of individual microgel particles. Shewan and Stokes (2012) showed using agar microgels that at the same phase volume, suspensions with softer microgels will exhibit a lower viscosity compared to suspensions with harder microgel. Individual particle modulus is influenced by cross-link density of the gel, biopolymer type, presence of embedded filler particles, and the interaction between the gel matrix and the filler particles (Kim et al., 1996; Dickinson, 2003; Liétor-Santos et al., 2011).

Porosity and permeability

The porous nature of alginate gel allows substrate to diffuse in or out of the gel particles and is essential for the immobilization

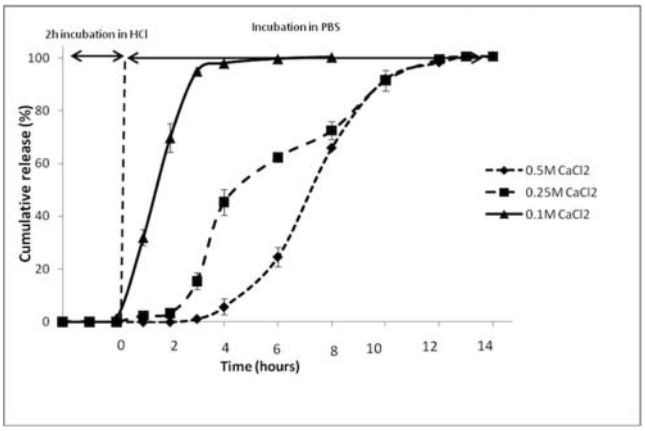

Figure 17. Release profile of ibuprofen from alginate microgels in a simulated gastric environment. Encapsulated ibuprofen was incubated in HCL (2 hrs) followed by PBS. The alginate microgels were gelled with either 0.1, 0.25, or 0.5 M CaCl2 solutions (Hariyadi et al., 2012).

characteristics of the gel. Small molecules that are soluble such as glucose and insulin are able to diffuse in and out of alginate beads. However, diffusion of larger molecules such as proteins is restricted by their molecular size and charges (Donati and Paoletti, 2009; Lanza et al., 1995).

By using methods such as electron microscopy, exclusion studies, and gel chromatography, various groups have showed that pore size of alginate gels is in the range of 5–200 nm (Andresen et al., 1977). The type of gelation mechanism (external or internal) influences the pore size of the gel. In externally gelled alginate gels, the inhomogeneous gelling profile resulted in a more constricted gel network on the gel surface leading to a pore size of 12–16 nm at the gel surface (Thu et al., 1996a). The homogenous gel structure obtained from internal gelation resulted in larger pore size. This difference in pore size could lead to significant difference in release profile. Liu et al. (2002) was able to show that the hemoglobin was able to diffuse into internally gelled alginate gel particles quicker compared to externally gelled gel particles. However, it is not known if porosity is affected by the overall size of the gel particle.

Pore size is also determined by the monomer composition of the alginate. Thu et al. (1996b) showed that BSA protein diffused out of alginate gel particles at a higher rate in gels with lower concentration of alginate. At the same time, porosity of gel particles increased when high G-block content alginate was used (Martinsen et al., 1991; Thu et al., 1996b) because the high G-block gel adopts a more open pore structure that is less susceptible to shrinkage (Thu et al., 1996a). The same study also showed that diffusion of hemoglobin from alginate gel particles was retarded by the addition of polycations such as poly-L-lysine layer. Poly-L-lysine binds electrostatically to the anionic surface of the alginate gel and this binding decreases surface pore size (Donati and Paoletti, 2009).

The concentration of gelling cation can also affect the permeability of the microgel. Hariyadi et al. (2012) showed that the release of drugs from alginate microgels could be controlled by using different concentrations of gelling cations (Figure 17). In a simulated gastric environment, the release of ibuprofen from microgels cross-linked with lower concentration of Ca (0.1M) was quicker compared to microgels made with higher concentration of Ca (0.5M). The difference in the rate of release of drug could be explained by a higher gel density, which reduced gel permeability (Hariyadi, 2011).

Release properties

Core materials are released from alginate gel particles by diffusion or erosion (Hariyadi, 2011). In general, solvent-soluble low molecular weight active ingredients such as drugs, vitamins and sugars that are smaller than the pore size of the alginate gel, are able to freely diffuse in and out of the gel particles (Tanaka et al., 1984). This leads to gel particles with low encapsulation efficiency and a rapid release kinetic of the encapsulated core materials (Pfister et al., 1986). Core materials release by erosion occurs when the alginate gel matrix disintegrate. Gel disintegration occurs at high pH or in the presence of cation chelators such as EDTA and citrate. Under these conditions, the alginate matrix swell up due to the dissociation of the gel matrix caused by ionic exchange of gelling cation with Na ions from the environment (Kikuchi et al., 1999). Core materials are released as the gel swells due to the reduction in crosslink density. The release is further accelerated as the gel matrix is eroded (Murata et al., 1993).

A number of factors affect the release properties of core materials from alginate gel particles. The type of crosslinking cation used influences diffusion. For example, Al-Musa et al. (1999) alginate gel formed with Ba or Al has smaller pores compared to Ca gelled alginate and the smaller pores retards the release of water soluble drugs. The gelation technique employed also determines the diffusion properties of alginate gel particles. Homogeneous gel particles structure made by internal gelation allows a higher rate of diffusion due to uniform pore size throughout the gel structure. Inhomogeneous gel made with external gelation with a higher crosslinking density on the outer surface delays diffusion of core materials (Al-Musa et al., 1999).

The crosslinking density of the alginate gel is also critical to the release of bioactives. Permeability of the gel matrix has been shown to be dependent on the concentration of gelling cations. Aslani and Kennedy (1996) showed that an increase in the concentration of gelling cations (Ca and Zn) from 0.1 M to 0.7 M, the permeability of the alginate gel to acetaminophen was decreased by five-folds. Hariyadi et al. (2012) showed a decreased release rate of lysozyme from alginate microgels when the gelling Ca concentration was increased from 0.1 M to 0.5 M.

Release properties are also determined by environmental factors and modifications to the gel microstructure. At low pH environment, viscous alginic acid gels that are formed at the surface of the microgel can act as a barrier to drug diffusion (Hariyadi et al., 2010).

Effects of temperature

Alginate generally forms thermostable gels over the range of 0–100°C (Oates and Ledward, 1990). The stability of alginate gels is influenced the by composition of the alginate. Oates and Ledward (1990) observed that M-block rich alginate gel was less

stable under heating compared to alginate gel with higher proportions of G-block residues.

Above 100–120°C, alginate gels undergo depolymerization. In thermal treated gels, Leo et al. (1990) showed that gel strength, as defined by critical compression force (N), peaked at 90°C and drop sharply as temperature increased to 120°C. Depolymerized chains lead to a more open structure of the gel and increased particle size and gel porosity (Leo et al., 1990). As a result, alginate gels tend to be less rigid as temperature increases (Gacesa, 1988).

Gels subjected to thermal treatment (boiling 100.0°C or steam 121.6°C) resulted in changes in textural attributes. Roopa and Bhattacharya (2010) found that heated gels were generally softer (mushy) and less brittle but nevertheless maintained gel structural integrity. Syneresis was also observed in steam treated gels, which caused shrinkage of the gel particles. As alginate gels are not thermoreversible, gel deformation was maintained after heat was removed (Roopa and Bhattacharya, 2010).

At temperature above the gel transition temperature (>180°C), thermal decomposition of the ionic alginate gel was observed as alginate gel melts (Oates and Ledward, 1990). Using thermogravimetry and differential thermal analysis methods, Said and Hassan (1993) postulated that the decomposition followed a 3-step reaction. Water molecules were liberated up to 200°C. Metal oxalates were formed above 200°C. At temperature above 350°C, metal oxide was formed (Oates and Ledward, 1990; Said and Hassan, 1993).

Effects of pH

Alginate gel particles undergo morphological and chemical changes under different pH levels. At low pH environment, gel particles shrink and pore size decreases. At pH above neutral, gel particles swell and gel pore size increases. Prolong exposure to high pH levels initiates dissolution of the alginate gel. Mumper et al. (1994) and Segi et al. (1989) observed a size reduction of alginate gel particles when pH was reduced from 4 to 1. Early work by Haug et al. (1963) showed that even at low pH, proton catalyzed hydrolysis of alginate can occur. This reduces the polymer molecular weight and makes the gel more prone to disintegration when pH is raised (Gombotz and Wee, 1998). The mechanism by which low pH causes gel shrinkage is unclear. However, it is known that low pH condition suppresses the disassociation of carboxyl groups in alginate molecules (Wu et al., 2010). Carboxyl groups that are protonated form a more compact gel network due to the reduced electrostatic repulsion between alginate polymers (You et al., 2001).

Observations on pH dependent swelling of alginate gels by Niedz and Evens (2009) suggest that swelling effect is partly dependent on the ionic concentrations of the environment rather than only pH levels. Presence of chelating agents such as EDTA, citrate or phosphate and high concentrations of Na^{+} or Mg^{2+} accelerate gel swelling, which is the precursor to gel disassociation.

Conclusion

Different methods of producing alginate gel particles (macro-, micro- and nano-sized) were reviewed in this paper. The proliferation of alginate gel particle production methods is in part due to current demands for the targeted delivery and controlled release of functional bioactives. Although alginate gel particles are already widely used in pharmaceutical, cosmetic, and chemical industries, their use in the food industry is still comparatively limited.

The current application research in food that is focused towards food texture modification, encapsulation, fat substitution, targeted digestive tract delivery, and satiety control shows that there are vast opportunities for the application of alginate microgels in the development of innovative products, especially in the rapidly expanding sector of functional foods. However, there are still gaps in understanding the interaction of alginate gel particles with a food system and their effects on textural and rheological behavior. The importance of creating consumer-acceptable texture profiles, and understanding how these textures come about, will be crucial in the widespread adoption of alginate gel particle systems in the health and food industry. For manufacturers looking to diversify their product range or to gain a foothold in an established market, the use of the microgel technology may be an innovative way to gain an edge over their competitors through enhancing the health benefits of their products.

The uptake of gel particles as a conventional tool in food manufacturing is still limited by cost. The importance of keeping costs down in the low margin food industry typically impedes the incorporation of novel technologies into manufacturers with firmly established brands and products. The main obstacle for widespread adoption is the lack of a truly commercially viable production method. Various techniques of producing alginate gel particles have emerged each with their own strength and weaknesses. While droplet-based microfluidic technique has emerged as a powerful technique to produce alginate microgels with unparelled control of shape, size, and uniformity, current production rates are limited to up to several hundred grams per hour. Furthermore, production rates of commercialized methods are limited to < 10 kg/hr. The need for calcium baths in the majority of other methods restricts manufacturing to a batch process. Although the batch process is suitable for small-scale productions, continuous manufacturing is preferable in the industrial scale when economy of scale becomes a key factor. In this aspect, the impinging aerosol method is seen as an attractive candidate for scaling up due to its continuous process, ease of use, lack of moving parts, tunable parameters and proven capability in the encapsulation of cells, drugs and lipid.

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