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Preparation of alginate microgels in a simple one step process via the Leeds Jet Homogenizer

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ABSTRACT

Fine calcium alginate microgel particles, down to particle sizes lower than 100 nm, were produced using a Jet Homogenizer previously developed in the School of Food Science and Nutrition (University of Leeds, Leeds, UK) consisting of highly turbulent mixing of two liquid streams of sodium alginate and calcium chloride solution. The final mean particle size, *d*, depended on the alginate to calcium ratio. From 0.5 to 2 wt.% alginate in the presence of 1–10 and 20 mM Ca^{2+}, *d* was lower than 5 μm and higher than 20 μm, respectively. However, *d* was not so significantly affected by the homogenization pressure above 150 bar at room temperature (20 ± 3 °C) or the volume ratio of the sodium alginate to calcium chloride solutions, within the limits 1:9 or 9:1. The particles initially exiting the homogenizer appeared to be slightly aggregated since sonication produced a further decrease in size. The particles were negatively charged (−31.7 mV ± 3.1 mV at pH 8) and inclusion of a suitable globular protein (lactoferrin but not lysozyme) of opposite charge led to a further reduction particle size and a slight decrease in particle ζ-potential. It is suggested that some degree of protein adsorption to the particle surface occurred, akin to a surfactant, which helped to control the particle size. In addition, some lactoferrin may also be incorporated inside the microgel particles during their formation, highlighting the potential of this technique to encapsulate various materials within microgel particles formed from Ca^{2+} cross-linked biopolymers.

1. Introduction

Alginate is a high molecular weight polysaccharide extracted from brown seaweeds of the phylum *Phaeophyceae*. The polysaccharides are monomers of β-D-mannuronic and α-L-guluronic acids linked together by α(1→4) glycosidic bonds and arranged according to different sequences with molecular weights ranging between 50 and 465 kDa, depending on the alginate sources (Stokke et al., 2000). Alginate is applied as a stabilizer and thickener in a wide range of food and pharmaceutical products, but also for the preparation of hydrogel beads or (micro)gel particles for the encapsulation of functional ingredients like vitamins and probiotics (Anal & Singh, 2007; Kailasapathy, 2006). Other applications of alginate encapsulation for controlled drug release and wound dressing management were reviewed by Goh, Heng, and Chan (2012).

Calcium alginate beads are quite simple to prepare and the mechanism of gelation via calcium ions forming egg-box junction zones is a widely known and exhaustively studied phenomenon (Fang et al., 2007). The calcium cross-bridging is so strong that simply dripping or spraying (prilling) alginate solution into a calcium ion solution will give 'instantaneous' gelation of the alginate droplet in the calcium solution (Brun-Graeppi, Richard, Bessodes, Scherman, & Merten, 2011; Quong, Neufeld, Skjåk-Bræk, & Poncelet, 1998). At the concentrations of alginate and Ca^{2+} (0.1–0.5 mol dm^{-3}) generally used for the prilling method, alginate chains bind quickly to Ca^{2+} and associate into dimers then multimers of increasing size. This rapidly leads to a growing number of chain entanglements within a dense alginate gelled network (Jørgensen, Sletmoen, Draget, & Stokke, 2007). Such methods have been reviewed by Shilpa, Agrawal, and Ray (2003) and more recently by Paques, van der Linden, van Rijn, and Sagis (2014). Once they have been formed, the gel particles can be extremely resilient, e.g., to boiling and shear (BeMiller & Whistler, 1996). They are therefore very attractive as encapsulating materials for oral delivery of protein or peptide drugs (George & Abraham, 2006), with

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the added advantage of being stable to acid, e.g., in the gastric phase of digestion. However, such simple preparation methods generally give rise to particles that are too large (typically no smaller than 25 μm) for some applications. There are disadvantages of being too large in terms of settling out of the particles, blending them into other ingredients and their organoleptic effects in foods. More advanced variations of the prilling technique have been developed with improved yield and smaller beads/particles possessing a narrower size distribution. These variants generally involve modification of the spraying nozzles and shear fields in the receiving calcium bath, or modification of the forces between them via electric fields and/or mechanical vibration. The minimum gel particle size formed by these methods still tends to be of the order of tens of microns (Paques, van der Linden, van Rijn, & Sagis, 2013). Smaller beads have the advantage that release is more rapid if this is based on diffusion out of them or their surface erosion – the specific surface area being larger. There are other advantages of small size in terms of ease of mixing and blending, lower tendency to settle or aggregate, plus their access to narrower capillaries and junction zones, or the relative ease to cross other biopolymer barriers, such as the mucin layers coating the gut and other epithelial surfaces (Bajka, Rigby, Cross, Macierzanka, & Mackie, 2015).

It is not easy to control the spraying of alginate solution, which is rheologically complex and thus it is difficult to control and reduce the droplet size consistently before it contacts the calcium-rich phase. Various other methods have been developed to produce particles of decreasing size. One obvious route is to prepare two separate water-in-oil (W/O) emulsions or microemulsions in which alginate is already dissolved in the aqueous phase of one emulsion and calcium dissolved in the aqueous phase of the other emulsion and then mix the two (micro)emulsions (Machado et al., 2012). Microemulsion droplets spontaneously exchange but require considerable amounts of surfactant to form the droplets, whereas the W/O emulsion route requires some method to initiate the slow release and diffusion of calcium ions between the droplets to gel the droplets containing the alginate (Amici, Tetradis-Meris, Pulido de Torres, & Jousse, 2008; Poncelet et al., 1992). Recently Paques et al. (2014) described a method where calcium nanoparticles dispersed in the oil phase act as the source of cross-linking ions under relatively neutral pH (pH 6), resulting in particles of around 1 μm and even as low as 200 nm.

In the present paper our aim is to describe a relatively simple new method that can be used to produce micron or sub-micron alginate particles, or other microgel particles that rely on rapid or confined exposure of the polysaccharide to a cross-linking agent such as Ca^{2+}. The calcium alginate microgel particles were prepared using a high pressure Jet Homogenizer (University of Leeds, Leeds, UK). The instrument has typically been used to make fine (O/W or W/O) emulsions as described in numerous publications from this research group (Burgaud, Dickinson, & Nelson, 1990). Here, however, the instrument has been used as a kind of high shear microreactor (Johnson & Prud'homme, 2003), as previously performed by Casanova and Higuita (2011), to prepare $CaCO_3$ microparticulates.

Microgel particles are also just one type of novel food particle that might be exploited to stabilize Pickering emulsions (de Folter, van Ruijven, & Velikov, 2012; Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014) – emulsions stabilized by an adsorbed layer of particles as opposed to molecules. Traditionally, the particles in Pickering emulsions are particles of solid material that do not deform on adsorption. However, as long as they maintain a size and contact angle sufficient to secure their interfacial attachment, deformable particles may also stabilize via the Pickering mechanism, so that the term 'Mickerings' emulsions has been coined by Schmidt et al. (2011) to describe protein microgel-particle-stabilized emulsions. Improvements regarding the production of truly nanoscale protein microgel particles of well-defined size or shape were reported by Sağlam, Venema, van der Linden, and de Vries (2014). Many methods rely on heating globular proteins in relatively dilute solution and/or at pH values far enough from their isoelectric pH so that they are highly charged. Whey protein has been particularly intensively studied (Schmitt & Ravaine, 2013; Schmitt et al., 2010) and other applications of protein microgel particles in general have recently been reviewed by Dickinson (2015).

2. Materials and methods

2.1. Materials

Sodium alginate (Kelgin*LV) was supplied by Kelco International Ltd (London, UK). Extensive drying of the alginate powder in an oven at 110 °C revealed it had a residual moisture content of 11 ± 0.5 wt.%, but solutions were made up on basis of the weight of the powder as received. Calcium chloride dihydrate (99.5%, MW 147, Lot 81K0252), sodium azide (99.5%), fluorescein isothiocyanate-dextran (average molecular weight 2,000,000 Da, Lot SLBB6384V) and lysozyme with protein content greater than 90% (from chicken egg white, product code L6876, Lot 111H7010) were all from Sigma Chemicals, St. Louis, MO, USA. The xanthan gum (Keltrol®) used was obtained from CPKelco (Surrey, UK). Bovine lactoferrin (Bioferrin 2000, bioactive whey peptide, Lot #2783491) was kindly donated by Glanbia Nutritionals (Middlesbrough, UK). The lactoferrin and iron content were specified as greater than 95% total protein and 150 mg per 100 g of protein, respectively. In protein-containing samples, 0.05 mol dm^{-3} sodium bicarbonate (Fisher Scientific, Loughborough, UK) was used as the buffer solution. The pH was adjusted with 0.5 mol dm^{-3} HCl solution (Convol, BDH Chemicals Poole, Dorset, UK) and 1 mol dm^{-3} NaOH (Fisher Scientific, Loughborough, UK) using Jenway pH meter (Guildford, UK) to reach pH 6, 8 and 10 after adjustment. All chemicals were used without further purification. Water purified by a MilliQ apparatus (Millipore, Bedford, UK), with a resistivity not less than 18.2 MΩ cm, was used for the preparation of all solutions.

2.2. Methods

2.2.1. Preparation of microgel particles

Solutions containing a range of concentrations of calcium ions [Ca^{2+}] varying from 1 to 70 mM were prepared by dissolving the $CaCl_2.2H_2O$ solid in water. Sodium alginate solutions at concentrations of 0.5, 1, and 2 wt.% alginate were prepared by dispersing the alginate powder in water while heating at 50 °C, under magnetic stirring for at least 2 h. No further procedures were performed to purify the alginate. The alginate solutions were cooled down to room temperature (20 ± 3 °C) before use to prepare microgel particles. Sodium azide was also dissolved in the sodium alginate solutions at 0.01 wt.% to act as a preservative.

Fig. 1 shows the essential features of the Jet Homogenizer. Two cylindrical chambers of different volumes are connected via fixed thin capillary tubing to an outlet via very small hole (0.5 mm diameter) drilled into a stainless steel disk. A compressed air driven ram pushes on pistons placed in the top of each chamber to force the liquids out of the chambers and through the hole. The fluid velocities generated through the hole can be extremely high (>300 m s^{-1}), creating highly turbulent flow with Reynolds number > 10^5 (Burgaud et al., 1990; Casanova & Higuita, 2011), depending on the pressure applied (typically 100–400 bar). The duration of the whole process is very short (<<1 s). When oil and appropriate surfactant solutions are placed in the separate