Preparation of monodisperse aqueous microspheres containing high concentration of L-ascorbic acid by microchannel emulsification

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Abstract

Monodisperse aqueous microspheres containing high concentrations of L-ascorbic acid with different concentrations of sodium alginate (Na-ALG) and magnesium sulfate ($MgSO_4$) were prepared by using microchannel emulsification (MCE). The continuous phase was water-saturated decane containing a 5% (w/w) hydrophobic emulsifier. The flow rate of the continuous phase was maintained at 10 mL h^{-1}, whereas the pressure applied to the disperse phase was varied between 3 and 25 kPa. The disperse phase optimized for successfully generating aqueous microspheres included 2% (w/w) Na-ALG and 1% (w/w) $MgSO_4$. At a higher $MgSO_4$ concentration, the generated microspheres resulted in coalescence and subsequent bursting. At a lower $MgSO_4$ concentration, unstable and polydisperse microspheres were obtained. The aqueous microspheres generated from the MCs under optimized conditions had a mean particle diameter (d_{av}) of 14 to 16 µm and a coefficient of variation (CV) of less than 8% at the disperse phase pressures of 5 to 15 kPa.

Keywords: L-ascorbic acid, microencapsulation, microchannel emulsification, monodispersity, sodium alginate, magnesium sulfate

Introduction

Encapsulation technology has attracted interest in fields including food and pharmaceutical industries, and its advancement will stimulate the development of novel drugs and become a driving force for drug therapy and baby food development (Reis et al., 2006). The food industry has utilized controlled-release technology for food additives, sweeteners, colors, nutrients, essential oils, antioxidants, and antimicrobial agents (Desai and Park, 2005). Controlled release helps overcome both the ineffective utilization and the loss of food additives during processing and storage (Pothakamury and Barbosa-Cánovas, 1995).

Techniques frequently used for microencapsulation include spray-drying, coating, extrusion, liposome entrapment, coacervation, and freeze drying (Desai et al., 2006). All of these techniques have numerous advantages and disadvantages. Recently, microfluidic devices have surpassed these conventional techniques to produce microencapsulated products with more monodispersity (Vladisavljević et al., 2012). Bioactive substances can be encapsulated using carbohydrates; gums; lipids; proteins; polymers such as polylactides, polyglycolides, and poly(lactide-co-glycolides); and copolymers such as poly(DL-lactide-co-glycolide). A few suitable polymers have been approved for use in foods, so certain food materials can be modified to increase their porosity and to alter other characteristics, thus enabling their use as coating materials in microencapsulation (Stevanovic and Uskokovic, 2009).

Anionic polysaccharide gels like alginate (ALG) particles have numerous applications for encapsulation and delivery systems. Potential applications include encapsulation of drugs (Caballero et al., 2013), probiotics (Jiang et al., 2013), control flavor release (King Alan, 1988), enzyme protection, and guided delivery of drugs to their target organs (Anal et al., 2003). ALGs have an inert nature, high porosity, superior coverage, superior penetration rate, mild

encapsulation temperature, and biocompatibility with numerous bioactive substances (Capone et al., 2013). Sodium alginate (Na-ALG) is a water-soluble compound that gels in the presence of divalent cations (Aslani and Kennedy, 1996). Such gels can be heat-treated without melting, although they may eventually degrade. Gelling depends on ion binding (Ca^{+2}< Zn^{+2}< Sr^{+2}< Ba^{+2}), with the control of cation addition being important for producing homogeneous gels (Reis et al., 2006).

ALG composition is an important parameter in ALG particle formation. At a Na-ALG concentration below 1.0%, almost no spherical particles were formed, probably due to the lack of enough carboxyl groups for gelation. Increasing Na-ALG concentration causes higher viscosity of an aqueous phase, resulting in larger droplets with a wide distribution (Liu et al., 2003; Reis et al., 2006). Thus, for a given application, the Na-ALG concentration must be controlled in particle size, shape, and size distribution.

Different emulsification techniques have been adapted to produce emulsions using conventional and microfluidic devices. Traditional emulsification devices include rotor-stator homogenizers (e.g., colloid mills, toothed-disk dispersing machines, and stirred vessels) and ultrasonic and high-pressure homogenizers (McClements, 2004). These devices incorporate intense energy in the system because of vigorous external forces, resulting in a broader droplet size distribution with polydispersity (Herrera, 2012). Over the last two decades, membrane emulsification (ME), microchannel emulsification (MCE), and microfluidic emulsification (MFE) using different types of geometries (Vladisavljević et al., 2012) have been developed to produce monodisperse emulsions with narrow size distributions. The major advantages of these emulsification techniques include the generation of uniform droplets, the precise control of

droplet size and shape, and *in situ* microscopic monitoring (unusual for ME) that enables fine tuning of the process parameters during droplet generation (Vladisavljević et al., 2012).

Kawakatsu et al. (1997) introduced MCE, a promising technique that is capable of generating uniformly sized droplets with average diameters of 1 to 500 μm and coefficients of variation (CVs) of <5% (Kobayashi et al., 2007; Kobayashi et al., 2012). MCE works on the mechanism of spontaneous transformation of the liquid-liquid interface on the terrace and is driven by interfacial tension (Sugiura et al., 2001). Microchannel (MC) arrays fabricated for MCE are classified as grooved MC arrays (each consisting of parallel MCs with slit-like terraces outside them) (Kawakatsu et al., 1997) and straight-through MC arrays (each consisting of two-dimensionally positioned through-holes) (Kobayashi et al., 2005b). Droplet generation via each grooved MC array can be easily judged using direct microscopic observation, whereas straight-through MC arrays are advantageous for producing monodisperse emulsions at higher droplet productivity. Kobayashi et al. (2012) recently produced monodisperse O/W emulsions at a maximum droplet productivity of 1.4 L h^{-1}.

Various food-grade materials (e.g., refined vegetable oils, a medium-chain triglyceride oil, hydrophilic and hydrophilic emulsifiers, proteins, and hydrocolloids) have been examined for producing monodisperse O/W, W/O, and W/O/W emulsions using MCE (Vladisavljević et al., 2012). MCE has promising potential for producing uniformly sized oil droplets containing functional lipids such as β-carotene (Neves et al., 2008b), γ-oryzanol (Neves et al., 2008a), and hydrophilic compounds like oleuropein (Souilem et al., 2013) and L-ascorbic acid (Khalid et al., 2014). L-ascorbic acid is a powerful antioxidant because of its capacity to neutralize free radicals. The chemistry, functions, metabolism, bioavailability, and effect of processing have been comprehensively reviewed in a recent publication (Abbas et al., 2012). L-ascorbic acid is

113 also important in minimizing the risk of serious diseases (e.g., heart disease, cataracts, and
114 cancer) and improving the immune system. L-ascorbic acid exposed to high temperature during
115 cooking and processing, moisture, oxygen, pH, and light has decreased antioxidant activity, thus
116 resulting in the formation of toxic compounds (Gallarate et al., 1999).

117 The objective of this study was to develop monodisperse aqueous microspheres
118 containing high concentration of hydrophilic bioactive compound using MCE. We encapsulated
119 L-ascorbic acid in liquid microspheres at high concentrations (up to 30% (w/w)) along with
120 varying concentrations of Na-ALG. We also investigated optimization of the formation
121 conditions and effects of osmotic pressures and varying concentrations of L-ascorbic acid on
122 MCE.

123

124 **Experimental**

125 **Materials**

126 Na-ALG (viscosity 80 to 120 mPa s), sorbitan trioleate (Span 85), magnesium sulfate ($MgSO_4$),
127 *n*-hexane, L-ascorbic acid (purity 99.9%), and *n*-decane were purchased from Wako Pure
128 Chemical Industries, Ltd. (Osaka, Japan). Tetra glycerin condensed ricinoleic acid ester (TGCR,
129 CR-310) was kindly supplied by Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan). 1,1,1,3,3,3-
130 Hexamethyldisilazane (LS-7150), purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan),
131 was used for surface hydrophobization of the silicon MC array plate and the glass plate. Milli-Q
132 water with a resistivity of 18 MΩ cm^{-1} was used for preparing all aqueous solutions.

133 **Emulsification Setup**

Fig. 1a depicts a simplified schematic diagram of the experiment setup used for MCE. A hydrophobized silicon MC array plate is tightly attached to a hydrophobized glass plate in the emulsification module initially filled with the continuous phase. A syringe pump (Model 11, Harvard Apparatus, Inc., Holliston, USA) was used to supply the continuous phase. The heating system provides temperature-controlled water circulation inside the module and outside the reservoir. A microscope video system was used to monitor and record droplet formation by MCE (Kobayashi et al., 2009). Fig. 1b and c depict a dead-end silicon MC array plate (Model MS407, EP Tech., Co. Ltd., Hitachi, Japan) consisting of 400MCs fabricated on four MC arrays. Each channel has terraces outside its inlet and outlet, and wells are fabricated outside the terraces. Channel and terrace dimensions are presented in Fig. 1(d), except for channel and terrace depth (7 μm).

The glass and silicon MC array plates were treated with LS-7150 to make their surfaces hydrophobic, so that they became suitable for preparing aqueous liquid microspheres in the continuous oil phase. This hydrophobic treatment was performed using slight modification of the procedure by Kawakatsu et al. (2001a). Briefly, these plates were surface-oxidized in a plasma reactor (PR500, Yamato Scientific Co., Ltd., Tokyo, Japan) for 15 min. The plates were then dipped in LS-7150 for the MC array plate or in a mixture of LS-7150 (20% (w/w)) and hexane (80% (w/w)) for the glass plate for two nights at room temperature. Finally, the unreacted materials were washed away.

Emulsification Procedure

Na-ALG solutions at different concentrations of 0.5 to 4.0% (w/w) were prepared by dissolving Na-ALG powder in Milli-Q water for at least 2 h with constant stirring using a magnetic stirrer at

156 room temperature. The solutions at 4±1 ^{o}C were stored overnight to ensure complete hydration.
157 They were then maintained at 45±1 ^{o}C prior to MCE. The disperse aqueous phase used for MCE
158 contains 0.5 to 4.0% (w/w) Na-ALG, 0 to 2% (w/w) $MgSO_4$, and 5 to 30% (w/w) L-ascorbic
159 acid. The continuous phase is a solution of water-saturated decane containing 5% (w/w) Span 85
160 or TGCR. To prevent water diffusion from the surface of Na-ALG droplets, decane was pre-
161 treated prior to preparing the continuous phase. Decane was saturated with water by mixing at a
162 volume ratio of 9:1 (decane:water) for 30 min, after which they were separated by centrifugation
163 at 1500 × *g* for 15 min using a table centrifuge (KN-70, Kubota Co., Tokyo, Japan). The decane
164 supernatant part was used as the continuous phase (Sugiura et al., 2008).

165 The disperse phase in a reservoir was introduced into a module filled with the continuous
166 phase by applying pressure using a pumping device (Fig. 1a). The module temperature was kept
167 at 45±1 ^{o}C during MCE. Liquid microsphere generation occurred when the disperse phase was
168 forced through the MCs into the continuous phase. The resulting microspheres were then swept
169 away by the cross-flow of the continuous phase, which was set at 10 to 15 mL h^{-1}. The flows of
170 the disperse and continuous phases were controlled in real time by monitoring liquid
171 microsphere generation via MC arrays.

172 After the MCE experiments, the MC array plate was cleaned using an ultrasonic bath
173 (VS-100III, As One Co., Osaka, Japan) at a frequency of 45 kHz in the following sequence: the
174 MC plate was cleaned in Milli-Q water for the first 20 min followed by Milli-Q water containing
175 a non-ionic detergent for another 20 min, Milli-Q water containing ethanol (1:1 v/v proportion)
176 for the next 20 min, and another round of cleaning with Milli-Q water for the final 20 min. The
177 cleaned MC array plate was left to dry in an oven at 60 ^{o}C and subsequently stored in air at room
178 temperature until the next use.

Determination of particle size and distribution

The particle diameter of the microspheres was determined by measuring the diameter of the captured images of over 200 particles using image-processing software (Winroof, Mitani Co., Fukui, Japan). The CV was calculated using the following equation:

$$\mathrm{CV} = \left(\frac{\delta}{d_a}\right) \times 100, \qquad (1)$$

where σ is the standard deviation and d_{av} is the average particle diameter.

Determination of viscosity and interfacial tension

The viscosities of the dispersed and continuous phases were measured using a vibrational viscometer (SV-10, A &D Company Ltd., Tokyo, Japan) at 25 ^{o}C. The vibrational viscometer measures fluid viscosity by detecting the electric current necessary to resonate two sensor plates (immersed in the fluid sample whose viscosity is to be determined) at a constant frequency and amplitude. The driving electric current is detected as the magnitude of viscosity produced between the sensor plates and the fluid sample. The fluid viscosity measured was then calculated to obtain the absolute viscosity (η) using the following formula:

$$\eta = \eta_{mea}/\rho, \qquad (2)$$

where η_{mea} is measured fluid viscosity and ρ is fluid density.

The interfacial tension between disperse and continuous phases was determined using the pendant drop method; the density of each phase was measured using a digital density meter (DA-130 N, Kyoto Electronics Manufacturing, Kyoto, Japan). The profile of the disperse-phase drop formed in the continuous phase was measured using a fully automatic interfacial tensiometer

(PD-W, Kyowa Interface Science Co., Ltd., Saitama, Japan). Each measurement was repeated at least 20 times, and the calculated mean values were used.

Results and discussion

Effect of $MgSO_4$ concentration on preparation of liquid microspheres

Salt content is a key factor affecting the interfacial properties of emulsions and their stability (Binks and Rodrigues, 2005; Binks et al., 2006), so we investigated the influence of salt content on the preparation of liquid microspheres using MCE and their stability. MCE experiments were conducted using varying concentrations of $MgSO_4$ (0 to 2% (w/w)) in the disperse phase containing 2% (w/w) Na-ALG and 5% (w/w) L-ascorbic acid. The disperse phase was fed into the module at 15 kPa, while the flow rate of the continuous phase was set at 10 to 15 mL h^{-1}. Stable microspheres were generated using the MC array at a $MgSO_4$ concentration of 1% (w/w). The generated microspheres detached smoothly from the MC arrays and had a d_{av} of 15.5 µm and a CV of 5.0%, demonstrating their monodispersity (Figs. 2a and b). Higher $MgSO_4$ concentrations produced polydisperse microspheres, and at 2% (w/w) $MgSO_4$ the microspheres first exhibited a high degree of coalescence and then disappeared (burst). This phenomenon leads to unstable microsphere generation. The microspheres obtained at 1% (w/w) $MgSO_4$ remained stable for more than 2 h inside the module without coalescence. In contrast, the microspheres generated without adding $MgSO_4$ (Fig. 2c) exhibited little coalescence after 30 min in the MC module, indicating that a certain osmotic pressure is needed to generate microspheres by MCE. The d_{av} of microspheres containing 2% (w/w) $MgSO_4$ increased to 21 µm with a CV of 16%.

The disperse phase osmotic pressure (Π_d) for the two-phase systems used in this section can be calculated using the van't Hoff equation (Strathmann, 1990):

221 $$\Pi_d = iMRT \quad , \qquad (3)$$

222 where *i* is the van't Hoff factor with a value of 1.51 for $MgSO_4$ and 1 for L-ascorbic acid; *M* is
223 the molar concentration of $MgSO_4$ (kmol m^{-3}), L-ascorbic acid, and Na-ALG; *R* is a constant
224 with a value of 8.31 kPa m^3 K^{-1} mol^{-1}; and *T* is the thermodynamic temperature (K). Π_d in the
225 absence of $MgSO_4$ was 0.17 MPa, and Π_d in the presence of $MgSO_4$ ranged from 0.39 to 0.50
226 MPa. Shimizu et al. (2002) and Cheng et al. (2006) reported that the use of disperse phases with
227 Π_d over a threshold value is needed to stably produce W/O emulsions with narrow droplet size
228 distributions by ME using surface-modified Shirasu Porous Glass (SPG) membranes. Similar to
229 ME, MCE also has a certain threshold Π_d necessary to generate droplets from MCs. Kobayashi
230 et al. (2009) demonstrated that Π_d exceeding a certain threshold level stably produces
231 monodisperse W/O emulsions with a CV of less than 3%. Similarly, at a higher Π_d, the transport
232 of water molecules via the water–oil interface is suppressed because of weak interaction between
233 charged hydrophilic groups and emulsifiers at the interface of W/O emulsions (Opawale and
234 Burgess, 1998). The generation stability of liquid microspheres via MC arrays in this study might
235 correspond to this mechanism.

236 The aqueous microspheres generated without adding $MgSO_4$ exhibited coalescence just
237 after formation, and there *in situ* stability was quite low. In contrast, the *in situ* stability of
238 microspheres generated in the presence of 0.5 to 1% (w/w) $MgSO_4$ was very high. The generated
239 microspheres remained stable for more than 2 h without any coalescence. It has been reported
240 that microgel particles containing ionizable groups become deswollen in an aqueous phase
241 containing salt because increased ionic strength decreases the Debye screening length on the
242 particle surface and reduces the repulsive electrostatic forces between charged groups on the

neighboring particles (Kratz et al., 2000; Kim and Vincent, 2005). In our study, the electrostatic interactions at low $MgSO_4$ concentrations between the charged groups ($MgSO_4$, water, and L-ascorbic acid) in the liquid microspheres are in the stable range, leading to microsphere stability and monodispersity. In contrast, the electrostatic repulsive force in the liquid microspheres at high $MgSO_4$ concentrations increases due to higher osmotic pressure, which could burst the microspheres and result in less monodispersity.

Effect of Na-ALG concentration on preparation of liquid microspheres

Fig. 3a illustrates the effect of Na-ALG concentration on the preparation of liquid microspheres using MCE. The generated aqueous microspheres contained 0.5 to 4% (w/w) Na-ALG, 1% (w/w) $MgSO_4$, and 5% (w/w) L-ascorbic acid. The disperse phase was supplied into the MC module at 15 kPa with the flow rate of the continuous phase was maintained at 10 mL h^{-1}. A decrease in d_{av} of microspheres was observed with increased concentration of Na-ALG up to a certain concentration (2% (w/w)), whereas further increase in the Na-ALG concentration increased the d_{av} of microspheres. Microspheres with the largest d_{av} of 24.6 µm and a CV of 10% were observed at a Na-ALG concentration of 4% (w/w). At a low Na-ALG concentration of 0.5% (w/w), a relatively broader particle size distribution occurred with a d_{av} of 20 µm and a CV of 12% (Fig. 3b). The optimum condition for successful microsphere production was 2% (w/w) Na-ALG, since a narrow size distribution was observed with a d_{av} of 15 µm and the smallest CV of 5% at this Na-ALG concentration (Fig. 3b). As presented in Table 1, the viscosity of the disperse phase increased sharply at Na-ALG concentrations exceeding 2% (w/w). Such high viscosities of the disperse phase (>100 mPa s) impeded crossing the narrow MCs, and only a few MCs made microspheres in the whole MC array plate. The viscosity of the disperse phase

containing Na-ALG increased in the presence of $MgSO_4$ (Table 1), which plays a key role in the stability of microspheres as well as in microsphere generation.

ALG microspheres have traditionally been produced by extruding Na-ALG solution from a needle into a divalent cationic solution (Poncelet et al., 1992; Kuo and Ma, 2001). These cationic solutions then induce gelation in microspheres. Gelling depends on ion binding (Ca^{2+}< Zn^{2+}< Sr^{2+}< Ba^{2+}). Mg^{2+} salt is also divalent but does not completely gelatinize the solution. This soft gel-like structure could modify the structure of ALG, as indicated by reduction of the viscosity of the disperse phase in the presence of $MgSO_4$ (Table 1). Furthermore, this soft gel-like structure creates weak linkage of Mg^{+2} ions with the ALG structure (Fig. 4), giving microspheres better stability. Improved stability in the presence of $MgSO_4$ was observed in our study.

Factors controlling microsphere production include the MC geometry, the composition of two liquid phases, and the type of emulsifiers (Tong et al., 2000; Saito et al., 2005). The viscosity ratio of the dispersed phase to the continuous phase was indicated as an important factor affecting the size of emulsion droplets generated by MCE (Kawakatsu et al., 2001b). In our study, the viscosity of the disperse phase containing Na-ALG increased with increased Na-ALG concentration, while interfacial tension remained almost unchanged at the Na-ALG concentrations applied (Table 1). In our study, d_{av} decreased with increasing Na-ALG concentrations (0.5 to 2% (w/w)) and increased with increasing Na-ALG concentrations (2 to 5% (w/w)). These results at higher Na-ALG concentrations deviate from the previous study of Chuah et al. (2009), who found size reduction in the resultant emulsion droplets with increasing Na-ALG concentration. However, these emulsion droplets do not contain any hydrophilic bioactive substance. The result presented in Fig. 3a correlated well with the previous MCE study of

Kobayashi et al. (2005a), who reported that a decrease in the d_{av} of oil-in-water (O/W) emulsion droplets stabilized using sodium dodecyl sulfate is influenced by increased viscosity of the disperse phase (silicone oil).

Effect of pressure of the dispersed phase on preparation of liquid microspheres

In order to investigate the effect of the hydraulic pressure of the disperse phase on the size and size distribution of the resultant microspheres, the disperse phase pressure was varied from 3 to 25 kPa at a fixed continuous flow rate of 10 mL h^{-1}. It should be noted that the flow rate of the continuous phase hardly affected the d_{av} and CV of the generated microspheres (Fig. 5a), which is advantageous for stable preparation of monodisperse liquid microspheres. The disperse phase used here contained 1 to 4% (w/w) Na-ALG, 1% (w/w) $MgSO_4$, and 5% (w/w) L-ascorbic acid in Milli-Q water.

Fig. 5b illustrates the effect of the disperse phase pressure on the d_{av} and CV of the microspheres prepared at different Na-ALG concentrations. The breakthrough pressure ranged from 3 to 7 kPa with increasing concentration of Na-ALG. The d_{av} of the microsphere decreased with increasing disperse phase pressure. A higher disperse phase pressure produced more active MCs generating microspheres. Monodisperse liquid microspheres with d_{av} of 15 to 18 μm and CV of 4.5 to 9.5% were prepared at disperse phase pressures of 10 to 15 kPa, regardless of Na-ALG concentration. There was a slight effect on their d_{av} and CV at disperse phase pressures exceeding 15 kPa at Na-ALG concentrations of 2 and 3% (w/w). At a certain disperse phase pressure, d_{av} depended on the Na-ALG concentration. The d_{av} of microspheres increased with increasing concentration of Na-ALG. The microspheres prepared with 4% (w/w) Na-ALG and generated at a disperse phase pressure of 25 kPa had a d_{av} of 26 μm and a CV of 10%. The

results obtained from this part of the study confirmed the existence of a range of optimum disperse phase pressures for successfully preparing monodisperse liquid microspheres.

Effect of L-ascorbic acid concentration on preparation of liquid microspheres

Fig. 6 illustrates the effect of L-ascorbic acid concentration on the d_{av} and CV of liquid microspheres prepared using MCE. Different concentrations (5 to 30% (w/w)) of L-ascorbic acid were dissolved in Milli-Q water solution containing 2% (w/w) Na-ALG and 1% (w/w) $MgSO_4$. CV and d_{av} of the resultant microspheres increased slightly with increasing L-ascorbic acid concentration. Smooth and stable generation of microspheres was observed with increasing L-ascorbic acid concentration. The d_{av} ranged from 14.4 to 15.5 µm, and the CV ranged from 6 to 10%. In our previous studies using a rotor-stator homogenizer, L-ascorbic acid of a high concentration (up to 30% (w/w)) was encapsulated in W/O and W/O/W emulsions with similar compositions in the absence of Na-ALG (Khalid et al., 2013a; Khalid et al., 2013b; Khalid et al., 2014). The encapsulation efficiency of freshly prepared microspheres encapsulating 20% (w/w) L-ascorbic acid was determined using straight-through MCE. The freshly prepared aqueous microspheres had an initial concentration of 2.7 mg mL^{-1} (total emulsion volume) and exhibited encapsulation efficiency exceeding 70% (data not shown) during the 10 days of storage at 40°C (Khalid et al., 2015). These results indicate that MCE has the ability to encapsulate a high concentration of L-ascorbic acid and other bioactive compounds into liquid microspheres with more monodispersity than conventional emulsification devices.

L-ascorbic acid encapsulated within a polymeric matrix such as poly(DL-lactide-*co*-glycolide) and D,L-lactide-*co*-glycolide has significantly higher efficiency (above 90%) than traditional simple conventional emulsions (Stevanović et al., 2007; Stevanović et al., 2007). In

order to overcome chemical instability of L-ascorbic acid, a considerable amount of research has focused on its encapsulation or immobilization (Stevanović et al., 2007; Feczkó et al., 2008). Stevanović et al. (2007) prepared poly(lactide-*co*-glycolide) (PLGA) particles using physicochemical methods and centrifugal processing. L-ascorbic acid was encapsulated in the polymer matrix using homogenization of aqueous and organic phases. The mean size of nanoparticles containing PLGA/L-ascorbic acid ranged from 130 to 200 nm. Desai et al. (2006) encapsulated L-ascorbic acid in tripolyphosphate–chitosan microspheres. The obtained microspheres were relatively polydisperse with d_{av} of 3 to 6 µm. The present methodology encapsulated 30% (w/w) L-ascorbic acid in aqueous microspheres without any significant increase in particle size diameter and is more stable than other formulation techniques.

Conclusions

The findings obtained from this study provide the foundation for preparing monodisperse aqueous microspheres loaded with L-ascorbic acid using MCE, which is an extremely mild emulsification technique. The methodology presented in this study enables producing encapsulated products containing high concentrations of L-ascorbic acid with potential applications in food, pharmaceutical and cosmetic industries. Appropriate control of Na-ALG and $MgSO_4$ concentrations, compositions of the dispersed and continuous phases, and operating conditions are needed to prepare stable monodisperse aqueous microspheres containing high concentrations of L-ascorbic acid via MC arrays. The successful composition includes 1% (w/w) $MgSO_4$, 2% (w/w) Na-ALG, and a maximum L-ascorbic acid concentration of 30% (w/w). The results also indicated that partial linkage of Mg^{2+} ions with Na-ALG could develop a soft gel-like structure, resulting in smooth detachment and generation of microspheres from MC arrays. Uniformly sized aqueous microspheres generated under mild processing conditions with MCE

could increase the encapsulation efficiency and storage stability of L-ascorbic acid in different food and pharmaceutical products.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the article

Figures and Table caption

Fig. 1. (a) Simplified schematic of microchannel emulsification (MCE) setup**.** (b) Top and cross-section views of the MC array plate (model MS407). (c) Schematic diagram of part of an MC array. (d) Optical micrograph and dimensions of part of an MC array.

Fig. 2. (a) Effect of the $MgSO_4$ concentration on the average particle diameter (d_{av}) (—●—) and CV (—○—) of the resultant liquid microspheres. (b, c) Optical micrographs of monodisperse liquid microspheres of different $MgSO_4$ concentrations.

Fig. 3. (a) Effect of Na-ALG concentration on d_{av} (—●—) and CV (—○—) of the resultant liquid microspheres. (b) Droplet size distributions of the liquid microspheres of different Na-ALG concentrations.

Fig. 4. Potential mechanism representing the soft gel-like structure of Na-ALG in the presence of $MgSO_4$.

Fig. 5. (a) Effect of the flow rate of the continuous phase (decane containing 5% (w/w) TCGR) on d_{av} (—●—) and (—○—) of the resultant liquid microspheres containing 2% (w/w) Na-ALG. (b) Effect of the hydraulic pressure of the disperse phase on the d_{av} and CV of the liquid microspheres of different Na-ALG concentrations. Na-ALG concentrations are denoted as (—●—) for 1% (w/w), (—▲—) for 2% (w/w), (—■—) for 3% (w/w), and (—◆—) for 4% (w/w), while similar open keys represents CVs of microspheres at different Na-ALG concentrations.

Fig. 6. Effect of L-ascorbic acid concentration on d_{av} (—●—) and CV (—○—) of the resultant liquid microspheres of different L-ascorbic acid concentrations.

Table 1. Viscosity and interfacial tension data for the liquid phases used for this study.

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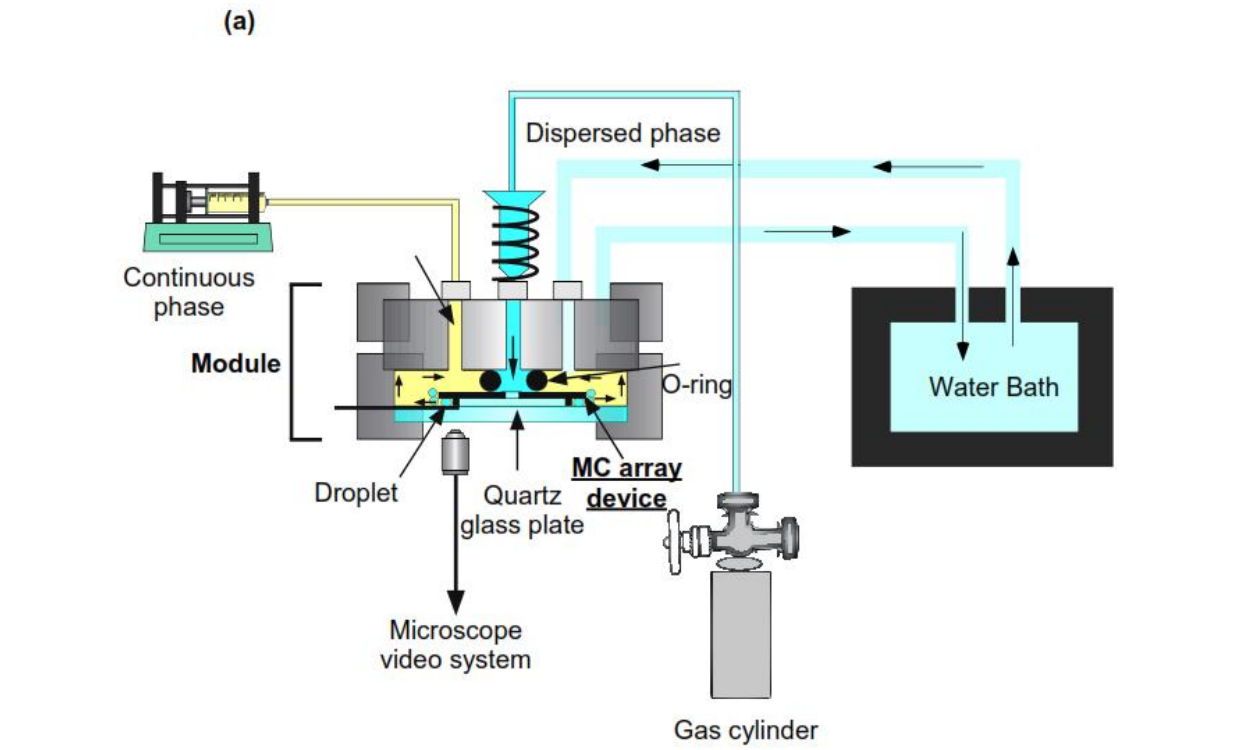
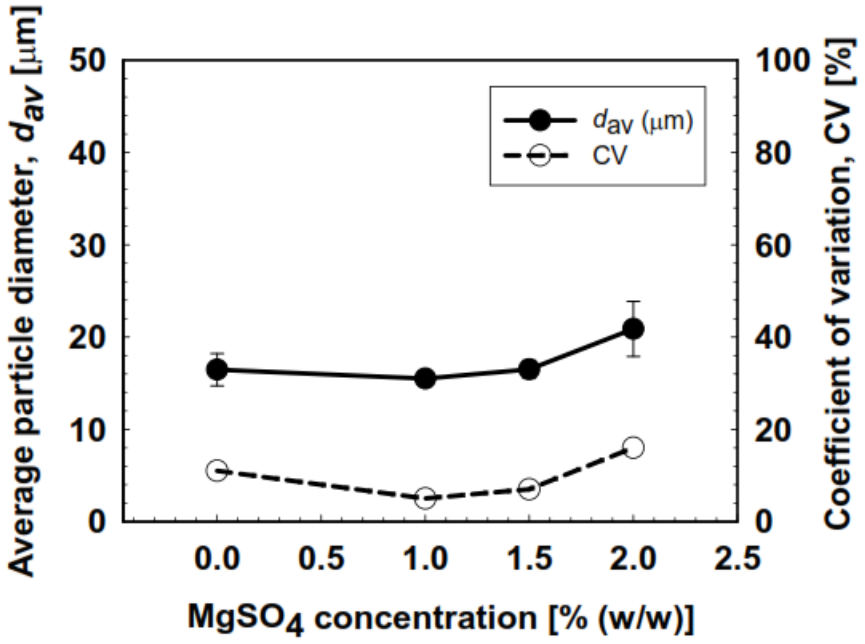

(a)
Dispersed phase
Continuous phase
Module
O-ring
Water Bath
Droplet
Quartz glass plate
MC array device
Microscope video system
Gas cylinder
(b)
Top view of MC plate
15mm
15mm
Cross section view of MC plate
100mm
0.5mm
(c)
Channel length
channel
channel width (Average)
Terrace
well
Terrace length
(d)
10 μm
7 μm
43.3 μm
61.7 μm
65.5 μm

Figure 1

(a)



(b) 1% (w/w) **(c) 0% (w/w)**

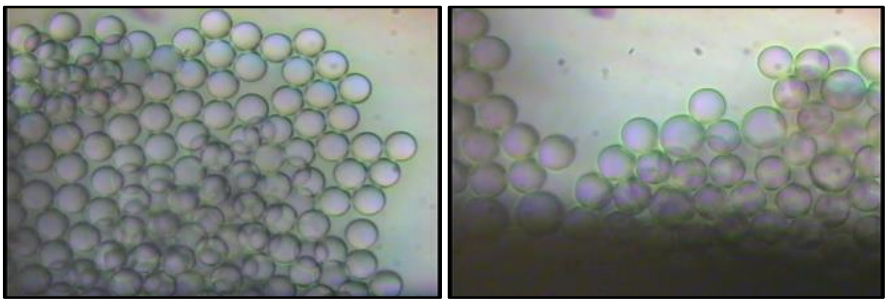
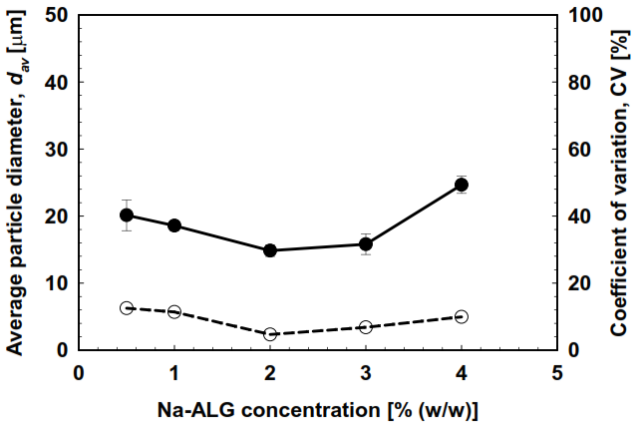


Figure 2

(a)


Average particle diameter, d_{av} [μm]
Coefficient of variation, CV [%]
Na-ALG concentration [% (w/w)]

(b)

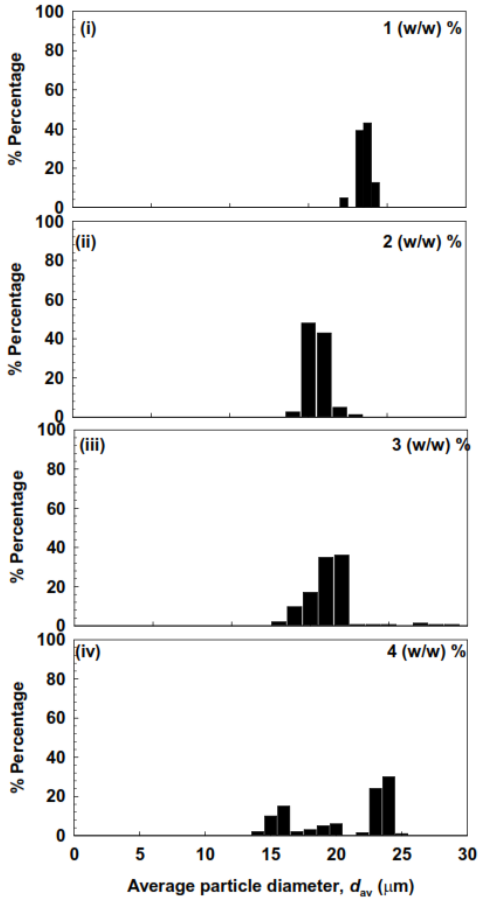

(i) 1 (w/w) %
(ii) 2 (w/w) %
(iii) 3 (w/w) %
(iv) 4 (w/w) %
% Percentage
Average particle diameter, d_{av} (μm)

Figure 3

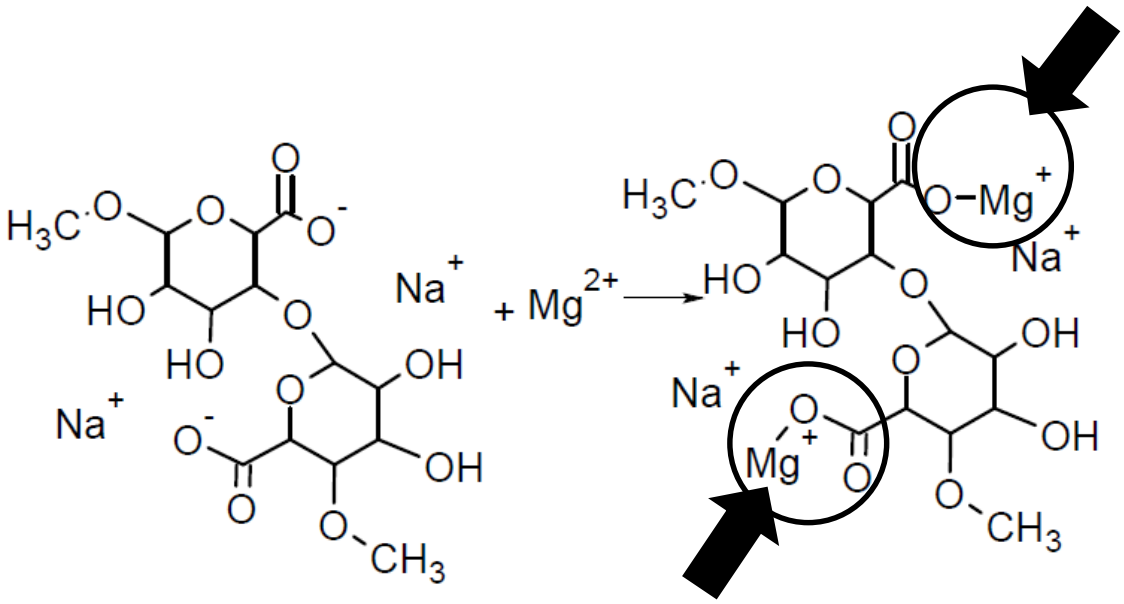
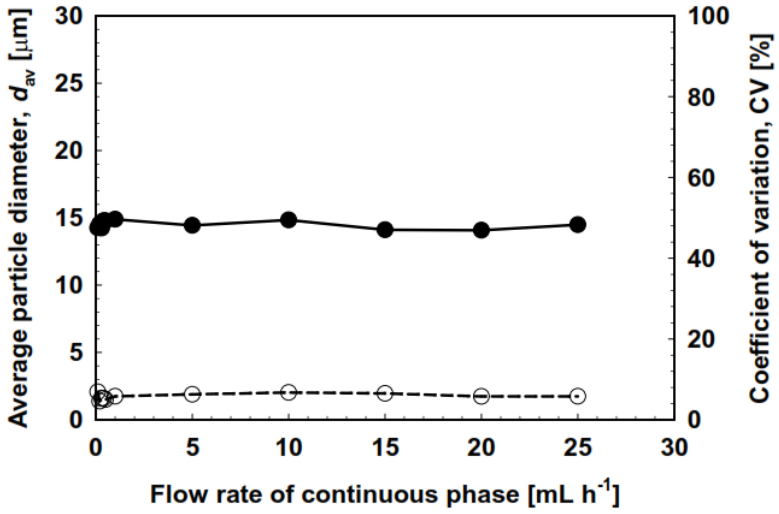


Figure 4

(a)


Average particle diameter, d_{av} [μm]
Coefficient of variation, CV [%]
Flow rate of continuous phase [mL h^{-1}]
0
5
10
15
20
25
30
0
20
40
60
80
100

(b)

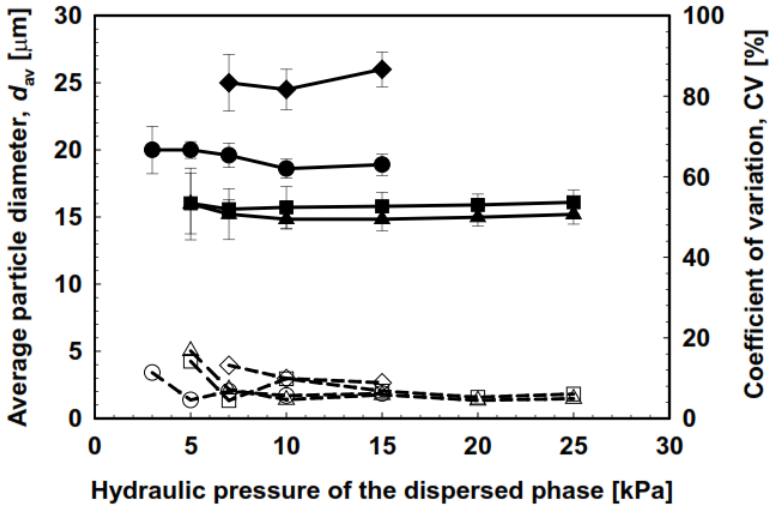

Average particle diameter, d_{av} [μm]
Coefficient of variation, CV [%]
Hydraulic pressure of the dispersed phase [kPa]
0
5
10
15
20
25
30
0
20
40
60
80
100

Figure 5

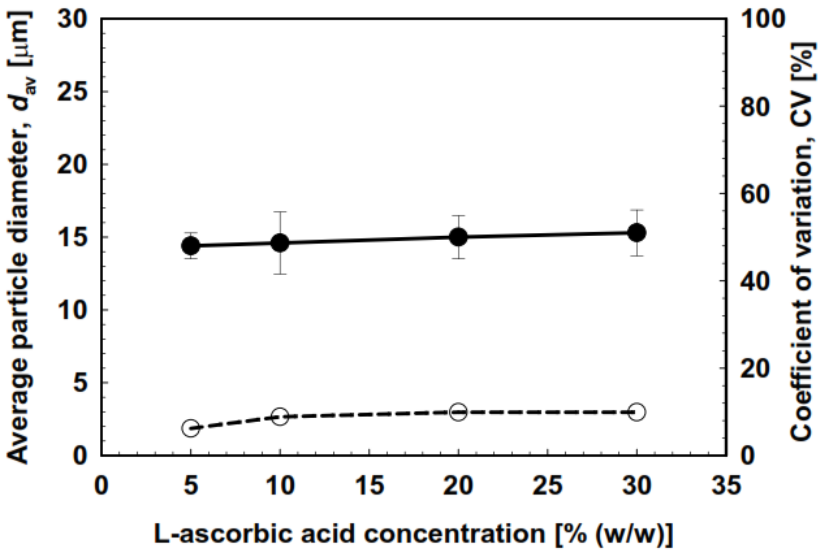

Average particle diameter, d_{av} [μm]
Coefficient of variation, CV [%]
L-ascorbic acid concentration [% (w/w)]
0
5
10
15
20
25
30
35
0
20
40
60
80
100

Figure 6

Table 1

concentration % (w/w)	Viscosity, η^a (mPas)	Viscosity, η^b (mPas)	Interfacial tension, γ^c (mN/m)	Interfacial tension, γ^d (mN/m)
DP composition*				
0.5	1.67±0.02	4.11±0.01	6.5±0.1	6.9±0.2
1	3.75±0.05	8.85±0.05	6.7±0.5	6.6±0.4
2	34.9±3.51	59.4±1.80	6.9±0.1	6.7±0.1
3	112±2.11	334±3.51	7.0±0.5	6.8±0.3
4	731±6.50	777±8.50	6.2±0.7	6.4±0.6
DP with L-AA**				
(5-30% (w/w))				
10	40.2±0.40	62.50±1.50	6.9±0.3	6.7±0.3
20	45.7±0.88	69.21±4.01	7.0±0.2	6.9±0.1
30	50.6±3.06	73.72±6.32	7.0±0.4	6.8±0.3
CP composition***				
Decane+Span 85	0.80±0.005	-	-	-
Decane+TGCR	0.84±0.004	-	-	-

*Dispersed phase composition

**L-ascorbic acid

**Continuous phase composition

[a] Viscosity of dispersed phase in presence of 1% (w/w) $MgSO_4$

[b] Viscosity of dispersed phase in presence of 0% (w/w) $MgSO_4$

[c] Interfacial tension in presence of 1% (w/w) $MgSO_4$

[d] Interfacial tension in presence of 0% (w/w) $MgSO_4$