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Short communication

Fabrication of solid lipid microcapsules containing ascorbic acid using a microfluidic technique

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ABSTRACT

The importance of ascorbic acid (AA) in the human diet has motivated food researchers to develop AA-fortified food products. However, this compound is very unstable. The aim of this work was to produce solid lipid microcapsules (SLMs) loaded with AA using microfluidic technology. The morphology of the SLMs was analysed by optical, scanning electron and confocal microscopy. We determined the encapsulation efficiency, particle size and stability of the encapsulated material. Two different means of enhancing the encapsulation efficiency and stability of AA were demonstrated: a pore blocking method and a micromolecule-chelating agent within the core. The results indicated the enormous potential of the designed vehicle to prevent AA degradation in a food product; additionally, this vehicle could mask the acidic taste of AA.

1. Introduction

AA is an ingredient/additive commonly used in foods by virtue of its vitamin and antioxidant properties. However, its application is limited due to its instability, which is influenced by several factors. Furthermore, due to its acidic nature, AA can interact with other food components and thus negatively affect the sensory properties and shelf life of AA-fortified foods. Therefore, microencapsulation is widely used as an alternative to remedy such problems.

AA was microencapsulated by several techniques, including spray-drying (Trindade & Grosso, 2000), double emulsion followed by complex coacervation (Comunian et al., 2013), ionic gelation (Desai, Liu, & Park, 2005), extrusion (Chang et al., 2010) and fluid-bed (Knezevic, Gosak, Hraste, & Jalsenjak, 1998). However, encapsulation of AA using microfluidic techniques has not been investigated yet, although use of this technique is expected to enhance stability during storage and mask the acidic taste of AA.

Microfluidics is a promising technology for the fabrication of microcapsules, with excellent flow and particle size control (Sun, Shum, Holtze, & Weitz, 2010; Utada, Chu, Link, Holtze, & Weitz, 2007; Zhao et al., 2011). This technique has been used to encapsulate a variety of actives, and it is widely used in the medical and pharmaceutical field (Tan, Hettiarachchi, Siu, Pan, & Lee, 2006).

The aim of this work was to encapsulate AA using microfluidic techniques, characterise the structure of the microcapsules as well as their encapsulation efficiency and to determine the stability of free and encapsulated AA.

2. Materials & methods

2.1. Materials

Pure AA was used as an active material (mp: 190–194 °C; Sigma–Aldrich, St. Louis, Missouri, United States) and distilled water (18.2 MΩ cm^{-1}, Millipore Milli-Q system) was used to prepare the inner and continuous phase. Palm fat (mp: 37 °C; Agropalma Co., Belém, Pará, Brazil) was employed as the oil phase and polyvinyl alcohol (PVA; MW: 13,000–23,000 g/mol, 87–89% hydrolysed) (Sigma–Aldrich, St. Louis, Missouri, United States) was used as an emulsifier in the external aqueous phase.

2.2. Methods

2.2.1. Encapsulation

The experiments were carried out using a glass microfluidic device as described in previous reports (Sun et al., 2010; Zhao et al., 2011). Cylindrical capillaries (World Precision Instruments, Inc., Sarasota, Florida, United States) with inner and outer diameters of 0.58 and 1.0 mm, respectively, were used to fabricate the devices. The tapered cylindrical capillaries were inserted into a square capillary (Atlantic International Technology, Inc., Rockaway,

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New Jersey, United States) with an inner dimension slightly larger than that of the cylindrical capillaries.

The internal phase consisted of AA solution with or without the presence of salt and chitosan, and the continuous phase consisted of an aqueous solution of polyvinyl alcohol (PVA) with a concentration of 10% (w/w). The different formulations are shown in Table 1. The flow rates used for experiments M1, M2 and M3 were 3000, 2500 and 14,000 μL/h for the internal aqueous, oily and external aqueous phase, respectively; for experiments M4 and M5, these flow rates were 1000, 3000 and 12,000 μL/h. The flow rates were determined in preliminary tests. All fluids were pumped into the microfluidic device using a syringe pump (Harvard PHD 2000 series). A thermal jacket was used to heat the palm oil to 66 °C within the glass syringe prior to injection and melt it down for injection into the capillary device.

The double emulsion was formed when the three flows met in the collection tube (Zhao et al., 2011). The resultant emulsion was collected in an ice bath to rapidly solidify the double emulsion and convert it to capsules with a consolidated shell; these capsules are also referred to as solid lipid microcapsules (SLMs).

2.2.2. Morphology of the microcapsules

The double emulsion was monitored during their production using an inverted microscope (DM-IRB; Leica, New York, USA) connected to a fast camera (Phantom 9; Vision Research, Wayne, New Jersey, United States). After microcapsule preparation, images were obtained using an inverted microscope (TE2000-E; Melville, New York, USA). Scanning electron microscope (SEM) images were obtained using an Ultra55 Field Emission Scanning Electron Microscope (FESEM Ultra55; Carl Zeiss, New York, USA).

Confocal images were obtained at room temperature using an inverted fluorescence microscope (Leica, DMIRBE; New York, USA).

2.2.3. Particle size distribution

Images were obtained using an inverted microscope (DM-IRB; Leica, New York, United States), and particle size analyses were carried out by examining 100 microcapsules of each formulation using the ImageJ program.

2.2.4. Encapsulation efficiency

The encapsulation efficiency was determined by quantifying total AA present in the microcapsule and in the supernatant, using Eq. (1). The SLMs were melted to release the AA encapsulated.

$$\text{Encapsulation efficiency} = [(AA_{total} - AA_{supernatant})/(AA_{total})] \times 100 \quad (1)$$

2.2.5. Stability of the encapsulated material

The stability of the microencapsulated AA in comparison to the free AA in solution was monitored according to the spectrophotometric method described by Farajzadeh and Nagizadeh (2003), at 0, 7, 15, 21 and 30 days after encapsulation. To compare the effect of temperature the samples were placed in glass containers protected from light and stored in the presence of O_2 at 4 and 20 °C.

2.2.6. Statistical analyses

All experiments were performed in duplicate. The data were analysed by ANOVA and Tukey test at the 5% significance level using the SAS statistical program (SAS, 1995).

3. Results and discussion

To obtain SLM, melted palm fat was used as the middle phase and microcapsules were collected in an ice bath to solidify the middle layer.

The decreased encapsulation efficiency observed in experiment M1 (Table 2) was attributed to the presence of pores in the lipid phase that can be seen in Fig. 2c. Therefore, AA might have leaked out of the capsules through these pores and become exposed to O_2, which would make it more susceptible to oxidation. To minimise these problems, Na_2CO_3 was incorporated in the internal aqueous phase along with AA, and $CaCl_2$ was added to the solution in which the microcapsules were collected and stored; this methodology was used based on the results reported by Zhao et al. (2011). These salts should diffuse through the lipid phase faster than the AA, and due to the low K_{sp} (Solubility Product Constant), they should form a precipitate composed of calcium carbonate, as shown in the schematic (Fig. 1) (Zhao et al., 2011). The precipitate would then clog the pores of the lipid phase, resulting in microcapsule structures (Experiments 2 and 3) with higher encapsulation efficiency (Table 2) and improved AA stability (Tables 3 and 4).

Two concentrations (1% and 2%) of Na_2CO_3 and $CaCl_2$ were used at a ratio of 1:1 (Table 1). In experiment M3, drastic improvement in encapsulation efficiency was observed due to the higher concentration of salts with consequent improved clogging of the pores, resulting in higher encapsulation efficiency and AA stability. It is observed that the experiment M1 showed the lowest encapsulation efficiency and higher mean particle size among the experiments M1, M2 and M3, indicating that the best encapsulation efficiency is attributed to the formation of the precipitate and not to the mean particle size.

To further improve the encapsulation efficiency and enhance the stability of AA, chitosan was added to the inner aqueous phase. Chitosan contains amino groups in a polymeric backbone and can form strong hydrogen bonds with AA. This strong hydrogen bonding allows AA molecules to be captured and retained on the high molecular weight chitosan polymer. Additionally, according to

Table 2
Particle size and encapsulation efficiency of the microcapsules.

Experiment	Particle size (μm)	Encapsulation efficiency (%)
M1	195.0 ± 4.6[c]	73.4 ± 2.8[d]
M2	186.1 ± 7.8[d]	91.7 ± 2.8[c]
M3	170.2 ± 3.3[e]	92.1 ± 1.5[b,c]
M4	299.5 ± 14.9[b]	96.6 ± 0.5[a]
M5	342.5 ± 28.4[a]	95.1 ± 0.9[a,b]

* There were no significant differences among the samples with the same letters in the same column ($p < 0.05$).

Table 1
Composition of each experiment. Concentrations are provided as (w/w).

Experiment	Internal aqueous phase			Oil phase	External aqueous phase	Solutions of collection
	AA (%)	Na_2CO_3 (%)	Chitosan (%)	Oil	PVA (%)	$CaCl_2$ (%)
M1	20	–	–	Palm fat	10	–
M2	20	1	–	Palm fat	10	1
M3	20	2	–	Palm fat	10	2
M4	3	–	0.25	Palm fat	10	–
M5	3	1	0.25	Palm fat	10	1

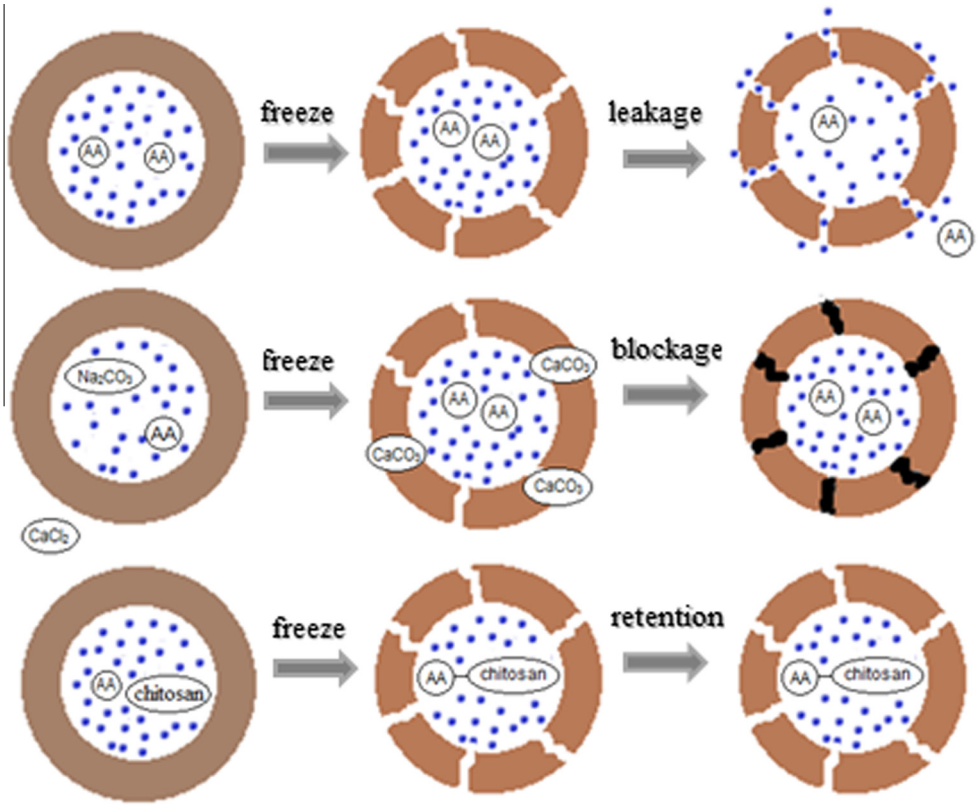

Fig. 1. Schematic representation of the presence and absence of precipitated calcium carbonate (adaptation of Zhao et al., 2011) and presence of chitosan.

Tian, Tian, Wang, and Mo (2009), the AA–chitosan complex has high singlet oxygen scavenging ability; thus, it can maintain the antioxidant activity of AA. Due to its high molecular weight, chitosan cannot diffuse out of the microcapsule membrane; thus, AA is retained along with chitosan within the core. The concentration of AA in the experiments M4 and M5 was lower than in the experiments M1, M2 and M3 due to the presence of chitosan (Table 1). The greater the amount of AA added, the more the solution becomes viscous, making it difficult to use it within the microfluidic. Thus, for purposes of comparison, the final result was expressed in %.

The formation of double emulsions within the glass capillary device and the microcapsules are shown in the optical micrograph in Fig. 2a and b, confirming that this method is feasible for encapsulating active materials. The round shape of the microcapsules was confirmed by SEM for all experiments (Fig. 2c). Moreover, the surface of the microcapsule membrane was rough, which was attributed to the rapid crystallisation of a heterogeneous lipid during collection of double emulsions in an ice bath.

Two sets of experiments that differed based on the internal phase were designed: (1) a 5% (w/w) solution of fluorescein sodium salt and 1% (w/w) of Na_2CO_3 and (2) a 5% (w/w) solution of fluorescein sodium salt. Fig. 2d shows that the green colour was predominantly present in the internal aqueous phase. This result illustrated the efficiency of the precipitate that competed with AA during diffusion through the oil phase. This experiment indicated that after 24 h, microcapsules containing salts (1% Na_2CO_3) demonstrated higher encapsulation efficiency due to retention of the encapsulated green fluorescent dye, whereas the microcapsules prepared without incorporation of salt did not show a fluorescent signal in the core after the same period of time. Fig. 2e shows that the green colour was predominantly outside the microcapsule, which indicated diffusion of the dye through the middle phase. This result can be explained by the absence of the precipitate in the middle phase, resulting in a higher rate of cargo diffusion out of the SLM.

The mean diameter of the particles varied from 170 to 342 μm (Table 2). These values can be considered satisfactory for food applications in which the particles must be notable, such as in AA-fortified food for children consumers or in food in which the AA must be released during the heating process (due to the melting of the fat) to exert its antioxidant properties during food processing and storage. However, large particle sizes may be undesirable in most AA-fortified foods because it could affect food texture.

The difference in the particle size among the experiments with and without chitosan is due to the necessary change of the flow rate because of the viscosity of the inner aqueous solution. When using the microfluidic technique, the microcapsule size can vary due to the use of capillaries with different injection and collection diameters as well as alterations in the flow rates of each fluid phase.

Regarding the encapsulation efficiency, there was a significant difference among the experiments (M1 to M5), indicating that different compositions in the inner aqueous phase resulted in different amounts of encapsulated active material. Comparing the experiments, the use of salts (and mainly chitosan) resulted in higher encapsulation efficiency. Indeed, the formation of precipitates and AA-chitosan complexes limited the diffusion of the AA or pro-oxidant compounds. There was no difference between experiments M4 and M5; therefore, these data suggest that using chitosan could be as efficient as using a salt to ensure good encapsulation efficiency in the studied system.

The values for AA stability are presented in Tables 3 and 4. The instability observed in the control solution is likely due to AA susceptibility to oxidation. The AA concentrations in all experiments were significantly higher than that in the control, which contained

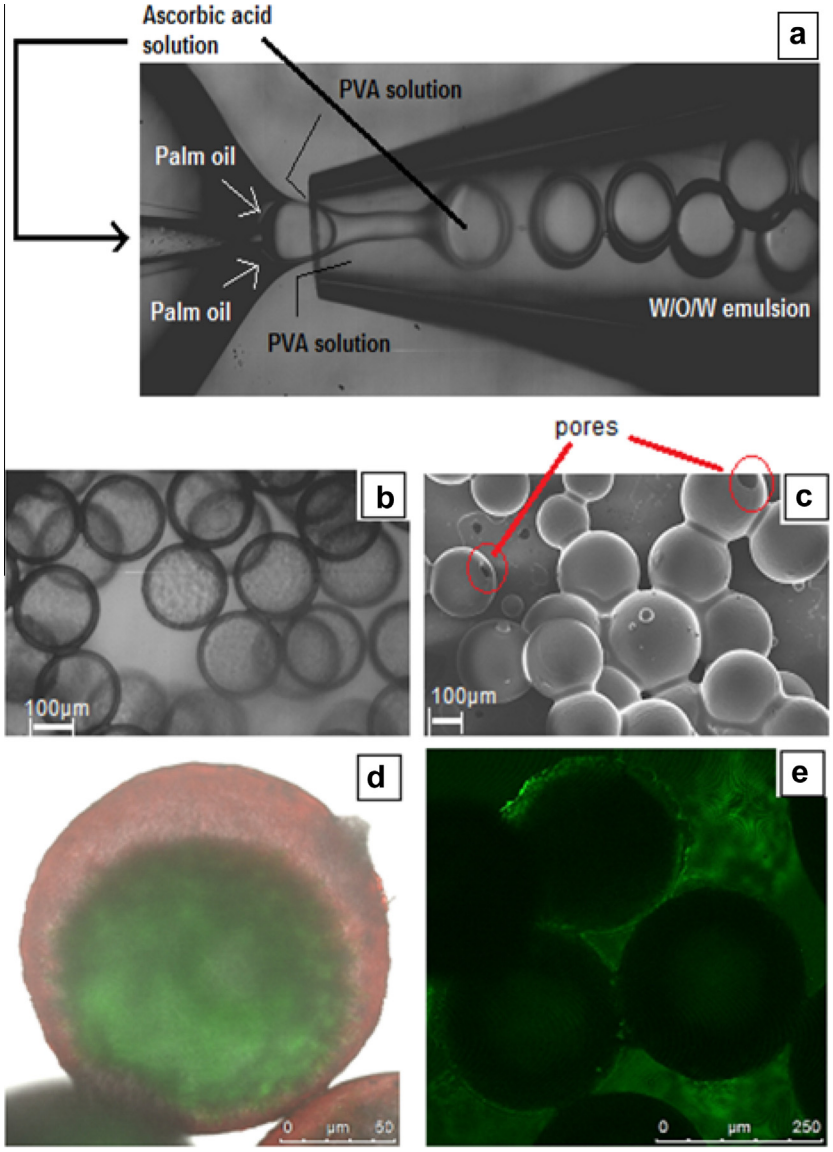

Fig. 2. (a) Diagram showing microcapsule production; (b) optical micrograph (100×) obtained from experiment M2; (c) scanning electron micrograph obtained from experiment M5; (d) confocal micrograph (300×) obtained from the experiment using 1% salt; (e) confocal micrograph (300×) obtained from the experiment without salt.

Table 3
Stability of the control solution of ascorbic acid and the experimental solutions for 30 days at 4 °C.

Experiment	Concentration of AA (%)				
	1 day	7 days	15 days	21 days	30 days
M1	$100 \pm 0.0^{a,b,A}$	$102.57 \pm 3.4^{a,A}$	$98.96 \pm 2.0^{a,b,A}$	$94.89 \pm 1.8^{b,A}$	$55.89 \pm 5.8^{c,C}$
M2	$100 \pm 0.0^{a,A}$	$98.97 \pm 1.5^{a,A}$	$98.37 \pm 2.0^{a,b,A}$	$97.23 \pm 5.0^{a,b,A}$	$94.33 \pm 2.0^{b,A}$
M3	$100 \pm 0.0^{a,A}$	$96.42 \pm 1.1^{a,A}$	$91.35 \pm 2.3^{b,B}$	$85.94 \pm 4.8^{c,B}$	$86.11 \pm 2.2^{c,B}$
M4	$100 \pm 0.0^{a,A}$	$98.09 \pm 0.5^{b,A}$	$97.28 \pm 0.5^{b,c,A,B}$	$95.88 \pm 0.6^{d,A}$	$96.40 \pm 0.7^{c,d,A}$
M5	$100 \pm 0.0^{a,A}$	$99.41 \pm 0.7^{a,b,A}$	$99.80 \pm 0.7^{a,b,A}$	$97.84 \pm 0.9^{c,A}$	$98.58 \pm 0.9^{b,c,A}$
**Control	$100 \pm 0.0^{a,A}$	$96.24 \pm 10.9^{a,A}$	$76.16 \pm 2.2^{b,C}$	$23.46 \pm 7.8^{c,C}$	$4.64 \pm 2.2^{d,D}$

* Small letters in the same row and capital letters in the same column are not significantly different by Tukey's test, where $\alpha = 0.05$.
** Control = pure AA in solution.

free AA in solution. After 30 days, the control retained only approximately 3.4 wt% of its initial AA, whereas the experiments retained 55.89–98.58% AA at 4 °C and 46.06–97.62% AA at 20 °C.

Experiments M4 and M5 showed the most effective AA protection; in both experiments, approximately 97% of the initial AA concentration was retained after 30 days of storage at 4 °C. This result may be due to the formation of the AA-chitosan complex, which has a large molecular weight and thus a very low diffusivity through the membrane. Therefore, the efficiency of encapsulation was also high in these experiments. There was a difference in performance between experiments M4 and M5 only on the last day of analysis for the samples maintained at 20 °C. Experiment M5, which contained salts and chitosan, demonstrated better AA protection than M4, which contained only chitosan. However, the

Table 4
Stability of control solution of ascorbic acid and the experimental solutions for 30 days at 20 °C.

Experiment	Concentration of AA (%)				
	1 day	7 days	15 days	21 days	30 days
M1	$100 \pm 0.0^{a,A}$	$75.94 \pm 2.4^{b,C}$	$49.32 \pm 2.6^{c,C}$	$43.73 \pm 3.5^{d,D}$	$46.06 \pm 1.4^{c,d,E}$
M2	$100 \pm 0.0^{a,A}$	$94.48 \pm 1.5^{b,A}$	$76.46 \pm 0.6^{c,B}$	$78.17 \pm 2.9^{c,B}$	$76.60 \pm 0.6^{c,C}$
M3	$100 \pm 0.0^{a,A}$	$89.61 \pm 2.3^{b,A}$	$91.00 \pm 2.4^{b,A}$	$64.99 \pm 1.3^{c,C}$	$64.46 \pm 1.2^{c,D}$
M4	$100 \pm 0.0^{a,A}$	$97.36 \pm 0.5^{b,A}$	$96.92 \pm 0.6^{b,c,A}$	$96.18 \pm 0.6^{c,A}$	$92.65 \pm 0.7^{d,B}$
M5	$100 \pm 0.0^{a,A}$	$96.72 \pm 0.4^{c,A}$	$97.99 \pm 0.9^{b,A}$	$97.54 \pm 0.9^{b,c,A}$	$97.62 \pm 0.8^{b,c,A}$
**Control	$100 \pm 0.0^{a,A}$	$84.32 \pm 1.9^{b,B}$	$74.91 \pm 3.8^{c,B}$	$14.68 \pm 3.8^{d,E}$	$3.38 \pm 0.0^{d,F}$

* Small letters in the same row and capital letters in the same column are not significantly different by Tukey's test, where $\alpha = 0.05$.
** Control = pure AA in solution.

difference between these experiments was too small to justify the use of salt in conjunction with chitosan.

Thus, the results obtained in this study can be considered quite satisfactory when compared to other AA encapsulation studies. Rozman and Gasperlin (2007) encapsulated AA via W/O microemulsions and reported that 60% of the AA was retained in the samples stored at room temperature. Comunian et al. (2013) encapsulated AA by the double emulsion followed by complex coacervation and obtained retention in the range of 36–73% and 15-40% for samples stored at 20 and 37 °C, respectively, for 60 days. Farhang, Kakuda, and Corredig (2012) encapsulated AA in liposomes and showed that after 7 weeks at 4 °C, liposomes retained 67% of the AA.

4. Conclusions

Encapsulation of AA using a microfluidic and palm fat as the capsule membrane was feasible for obtaining SLMs. The SLMs showed high encapsulation efficiency and excellent performance with respect to AA protection during storage, even at room temperature.

The experiment in which chitosan was used inside the core was considered to be the most suitable, according to the results and the simplicity of the formula. However, the using of the salts could also be an alternative for a high performance encapsulation and preservation of AA.

For future studies, the use of capillaries with smaller diameters and different flow rates may be useful for obtaining SLMs with smaller average sizes, which extends the range of its applications.

Microfluidic device technology should be further studied for use in encapsulation of food ingredients and bioactive compounds. These compounds are generally sensitive to many factors, such as high temperature and organic solvents, which are dispensable when microfluidic device technology is used.

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References

Chang, D., Abbas, S., Hayat, K., Xia, S., Zhang, X., Xie, M., et al. (2010). Encapsulation of ascorbic acid in amorphous maltodextrin employing extrusion as affected by matrix/core ratio and water content. *International Journal of Food Science and Technology, 45*(9), 1895–1901.

Comunian, T. A., Thomazini, M., Alves, A. J. G., Matos, F. E., Balieiro, J. C. C., & Favaro-Trindade, C. S. (2013). Microencapsulation of ascorbic acid by complex coacervation: Protection and controlled release. *Food Research International, 52*(1), 373–379.

Desai, K. G. H., Liu, C., & Park, H. J. (2005). Characteristics of vitamin C immobilized particles and sodium alginate beads containing immobilized particles. *Journal of Microencapsulation, 22*(4), 363–376.

Farajzadeh, M. A., & Nagizadeh, S. (2003). A simple and reliable spectrophotometric method for the determination of ascorbic acid in pharmaceutical preparation. *Journal of Analytical Chemistry, 58*(10), 927–932.

Farhang, B., Kakuda, Y., & Corredig, M. (2012). Encapsulation of ascorbic acid in liposomes prepared with milk fat globule membrane-derived phospholipids. *Dairy Science and Technology, 92*, 353–366.

Knezevic, Z., Gosak, D., Hraste, M., & Jalsenjak, I. (1998). Fluid-bed microencapsulation of ascorbic acid. *Journal of Microencapsulation, 15*(2), 237–252.

Rozman, B., & Gasperlin, M. (2007). Stability of vitamins C and E in topical microemulsions for combined antioxidant therapy. *Journal of Drug Delivery, 14*, 235–245.

SAS (1995). *SAS User's Guide: Basic and statistic*. Cary, North Carolina, USA. Patent.

Sun, B. J., Shum, H. C., Holtze, C., & Weitz, D. A. (2010). Microfluidic melt emulsification for encapsulation and release of actives. *Applied Materials & Interfaces, 2*(12), 3411–3416.

Tan, Y., Hettiarachchi, K., Siu, M., Pan, Y., & Lee, A. P. (2006). Controlled microfluidic encapsulation of cells, proteins, and microbeads in lipid vesicles. *Journal of American Chemical Society, 128*, 5656–5658.

Tian, X. L., Tian, D. F., Wang, Z. Y., & Mo, F. K. (2009). Synthesis and evaluation of chitosan-vitamin C complex. *Indian Journal of Pharmaceutical Sciences, 71*(4), 371–376.

Trindade, M. A., & Grosso, C. R. F. (2000). The stability of ascorbic acid microencapsulated in granules of rice starch and in gum Arabic. *Journal of Microencapsulation, 17*(2), 169–176.

Utada, A. S., Chu, L., Link, D. R., Holtze, C., & Weitz, D. A. (2007). Dripping, jetting, drops, and wetting: The magic of microfluidics. *MRS Bulletin, 32*, 702–708.

Zhao, Y., Shum, H. C., Adams, L. L. A., Sun, B., Holtze, C., Gu, Z., et al. (2011). Enhanced encapsulation of actives in self-sealing microcapsules by precipitation in capsule shells. *Langmuir, 27*, 13988–13991.