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Project title Polymeric Beads Encapsulated with Natural Product Extracts for Oral Care Applications

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Polymeric Beads Encapsulated with Natural Product Extract for Oral Care Applications

by

Miss Pattarawimon Phoonsiri

In Partial Fulfillment for the Degree of Bachelor of Science

Program in Applied Chemistry (International Program)

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By Miss Pattarawimon Phoonsiri

Accepted by Department of Chemistry, Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science Program in Applied Chemistry (International Program)

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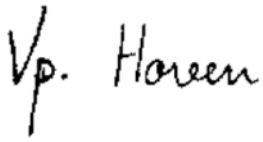

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Date December 25, 2020

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Abstract

In this research, calcium alginate polymeric beads with oral hydrating natural product extracts introduces the new oral care alternative that aims to be used for the aging society. Upon ingestion, breakage of alginate shell capsules should release natural product extracts that increase saliva production as a curing approach for xerostomia. Capsaicin extract and olive oil were selected for encapsulation since their chemical properties contribute to relieving xerostomia symptoms. The preparative method of polymeric beads from alginate and calcium lactate using cryogelation in combination with inverse gelation produced double gelation polymeric layer and became the most effective approach as it yielded monodispersed core-shell polymeric hydrogel. Preparation under low temperature condition enhanced the sphericity of beads and the contact time of bead submersion in the alginate bath increase shell thickness which strengthened mechanical property of the beads. The polymeric beads were characterized for their mechanical strength, shell thickness, aspect ratio, as well as weight.

Keywords: Polymeric beads, Natural product extracts, Spherification, Double Gelation, Cryogelation, Hydrogel

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Chapter 1

Introduction

1.1 Introduction to the research problem and significance

Oral health is one of the most significant matter for the aging society. A malfunction in oral cavity could consequently lead to various symptoms that affect physiological health in long terms. Especially in developing countries, lack of knowledge and awareness for oral hygiene are the main reasons that contribute to the increasing periodontal and dental diseases *(1).* Xerostomia or dry mouth is a common problem of aging society. The symptom includes dryness of mouth and discomfort due to insufficient saliva flow and viscosity as well as decreasing contents of saliva proteins. Thus, without sufficient saliva flow that protects teeth cavities and periodontal tissues, patients with xerostomia experience the consequences of tooth decay due to bacterial infections (*1*). Another cause that has an effect on reducing saliva content is the intake of xerogenic medications. Thus, these factors may contribute to the increasing causes of xerostomia among the elderly *(2).*

Typically, xerogenic management relies on reduction of symptom and/or increase salivary flow. In term of medication, mucosal lubricants, saliva substitutes and saliva stimulants are applied to stimulate salivatory gland and increase salivatory flow *(3).* However, the oral lubricants are quite costly. The patients with limited budget cannot reach the treatment. Mouthwash is an alternative oral care product which is widely used, besides technical toothpastes. Generally, mouthwash contains antiseptic chemical active ingredients that can eliminate bacteria. Furthermore, it is claimed to diminish xerostomia side effects. Fluoride-containing mouthwash has been claimed to treat patients with xerostomia as saliva replacements *(4).* On the other hands, a side effect of using fluoride is tongue staining *(5).* Although bioactive compounds are effective in oral care as well as reducing symptoms of xerostomia, there are still side effects that could interfere with a patient's standard of living and give rise to other health issues.

Natural products are alternative active ingredients to be added in the mouthwash for oral care as well as curing xerostomia symptoms because of antioxidant and anti-inflammatory characteristics and the ability to increase salivation *(5,7,8).* However, most of natural products are poorly water soluble *(8).* To overcome this limitation, this research proposes to develop polymeric beads as carriers for natural product extracts to be used as oral care product in order to cure xerostomia symptoms.

1.2 Research Objective

To develop a method to prepare edible polymeric beads that can encapsulate natural product extracts to be used for oral care applications.

1.3 Literature search

1.3.1 Preparation of calcium alginate

Nowadays, polymeric beads are widely used in many applications including food, pharmaceutics, and biomedicine. Recently, the production of edible polymeric bead is commercialized by Notpla Co, Ltd. The water capsules called "*Ooho"* could be ingested as a result of edible shell made of calcium-crosslinked alginate gel. The gel-like capsule consists of a double membrane at which the outer membrane acts as a food packaging whereas the inner membrane act as the shell to contain core solution which is water *(9).*

Alginate is a naturally derived polymer which can be extracted from cell walls of brown algae. The application of alginate biopolymer as capsule wall material is widely used due to its non-toxicity, solubility, high water retention capacity, and the capacity to form gel at low concentration *(10).* The structure of alginate polymer is made up from random sequence of glycosidic linkages between two monomers of (1-4)-linked- α-L-guluronic acid (G blocks) and β -D-mannuronic acid (M blocks) residues. (**Figure 1.1, top**)

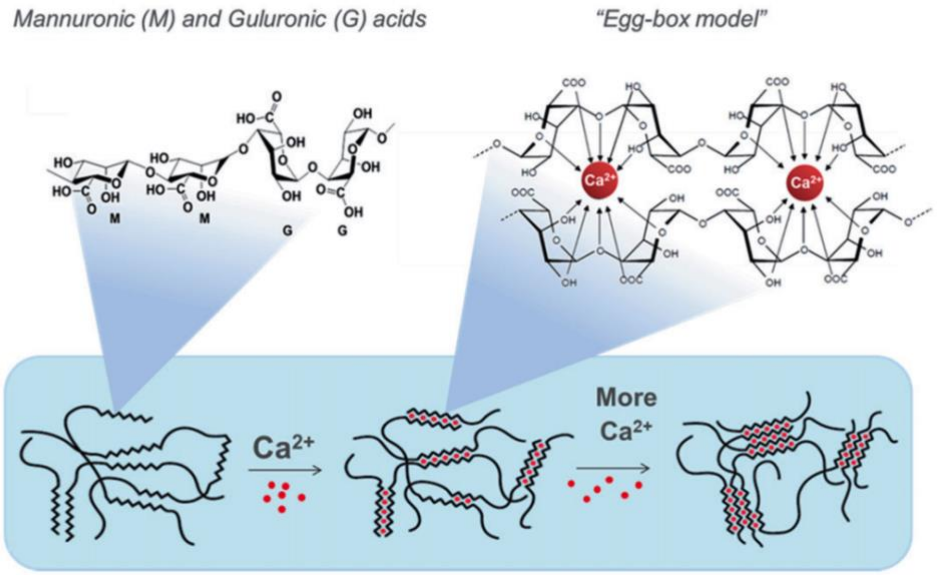

Figure 1.1 Schematic representation of polymeric structure of alginate (top) and ionic crosslinking of alginate with calcium ions (bottom).

The polymeric solution of alginate remains as liquid but rigid and viscous due to electrostatic repulsion between hydroxyl and carboxylate groups in G and M blocks. However, guluronic block residues possess higher tendency for attractive force due to its non-linear configuration, serving as the main coordination for binding sites.

When divalent ions are presented in sodium alginate polymeric solution, divalents will form coordination with 2 oxygen atoms of the carboxylate and hydroxyl groups in guluronic block residue, creating an "Egg-box model" where divalent ions situate between guluronic cavities, thus formulating hydrogel structure (**Figure 1.1, bottom**) *(11).* However, without sufficient divalents in alginate medium, cations can chelate with several carboxylate groups at once, which results in a metastable egg-box structure *(11).* The affinity of divalents towards alginate polymer ranges from Cd^{2+}>Ba^{2+}>Cu^{2+}>Ca^{2+}>Ni^{2+}>Co^{2+}>Mn^{2+} (10). In correspond to molecular gastronomy application, calcium source is used as a divalent gelling agent for alginate crosslinking due to being edible, nontoxic and flavorless in property.

1.3.2 Calcium ions as cross-linking agent

The rate of calcium diffusion for ion-exchange reaction also depends on the source of calcium ions. Lee, P. and Rogers, M. A. (2013) studied the influence of calcium ions source by using three different calcium solutions to evaluate the rate of ion-exchange with sodium ions which was connected to alginate polymer. The procedure was experimented through extruding sodium alginate into three separate calcium salts consisting of 1 %wt of calcium chloride, calcium gluconate, and calcium lactate. Upon timing the rate of hydrogel formation from the beginning of calcium diffusion to reaching maximum gel hardness, calcium chloride-induced gelation reaches maximum hardness under ~100 s, followed by ~500 s from calcium lactate, and ~3000 s from calcium gluconate. However, it was discovered that alginate polymer was exposed to different calcium concentrations varying by calcium sources upon weighing the anion molar mass. By percent weight, the concentration of Ca^{2+} was 0.36 for calcium chloride, 0.09 for calcium gluconate and 0.18 for calcium lactate *(12).*

Through previous experiment, calcium chloride salt presents as the best option for conducting alginate crosslinking by the most rapid diffusion rate. Yet, it possesses limitations for molecular gastronomy due to salty and bitter taste it imparts on food flavor (13). Hence, calcium lactate is an alternative calcium ion source for alginate gelation due to diffusion rate. Possessing versatility in molecular gastronomy synthesis, calcium lactate is compatible as gelling agent for alginate crosslinking in direct spherification, internal and external gelation, as well as inverse gelation due to its flavorless taste *(10,12).* In addition, calcium lactate was found to reduce teeth erosion as it is involved in the process of teeth remineralization (14).

1.3.3 Preparation of polymeric beads

There are two methods commonly used for productions of polymeric beads; namely direct and inverse gelation. Both techniques can be applied for encapsulating natural product extracts, via alginate crosslinking using calcium lactate as gelling agent.

1.3.3.1 Direct spherification

Into a bath containing calcium ion source as the cross-linking agent, sodium alginate is added to the bath at fixed volume. Upon exposing to alginate chains, calcium ions will migrate and diffuse between the chains and replace sodium ions, forming hydrogel which initiates cross-linking at the interface of alginate and calcium ion. Hydrogel formation by inward crosslinking yields polymeric beads with high rigidity and hardness since alginate at the surface is continuously exposed with calcium divalents, promoting high crosslinking outside and low crosslinking inside. **Figure 1.2A** illustrates oil-encapsulated polymer synthesis by direct spherification via external gelation. In this case, oil or natural product is mixed with alginate solution prior to exposure with calcium gelling bath *(10).*

1.3.3.2 Inverse Gelation

In opposite to direct spherification, inverse gelation is initiated by dropping calcium source solution into the alginate bath. In this case, hydrogel formation will happen outwardly, thus making the inner part of polymeric beads highly cross-linked whereas cross-linking at the surface of the beads would be less. **Figure 1.2B** illustrates the formation of polymeric beads under inverse gelation where oil is emulsified in calcium solution prior to dropping in the alginate bath. *(10).*

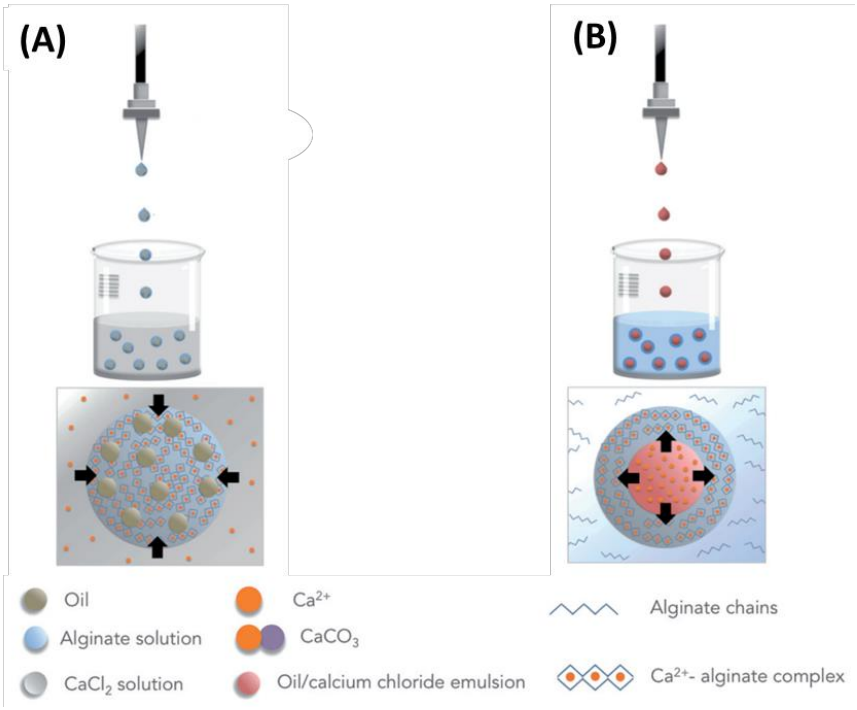

Figure 1.2 Illustrations representing encapsulation techniques: (A) direct and (B) inverse gelation

Cryogelation is another encapsulation technique which relies on the inverse gelation. In this technique, natural product is mixed with cross-linking agent then frozen in the mold prior to immersed in sodium alginate solution. The frozen cross-linking agent is slowly melted and exchanged ion with sodium ions to form a thin layer of calcium alginate which is a boundary of the polymeric beads. The advantage of this technique is to create well-defined polymeric beads and control volume of solution inside the encapsulated beads *(15)*.

1.3.4 Selection of natural product to be encapsulated in polymeric beads

Natural products for encapsulation are required to stimulate saliva gland or maintain moisture in oral cavity. In addition, natural product should be preventive against bacteria growth and adhesion in oral cavities, and acquisition of the functionality to diminish side effects of xerostomia. In targeting elders, the active ingredients were selected for their mild flavors which

will provide comfort after oral usage. Hence, capsaicin and olive oil are selected for this research. **Figure 1.3** illustrates the chemical structure of capsaicin **(Figure 1.3A)** and olive oil **(Figure 1.3B)**.

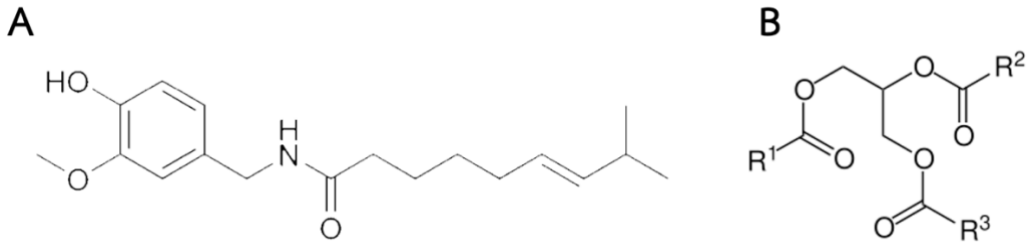

Figure 1.3 Chemical structure of (A) capsaicin and (B) olive oil extracts

Capsaicin (trans-8-methyl-*N*-vanilyl-6-nonenamide) is a naturally-occurring alkaloid constituent mainly found in hot chili peppers from the capsicum family. The application of capsaicin for physiological usages has been implemented for body treatment as it has alleviating effects. In terms of oral care applications, capsaicin promotes saliva flow as the chemical directly induces salivary glands for release. In addition, capsaicin also contains antioxidant and anti-inflammation properties *(15).* With usage in dilute concentration as encapsulated natural product extracts for compensation in taste and sensation, capsaicin can be suitable for oral health enhancements.

Olive oil has been appended in toothpaste formulations due to reduction in bacterial growth. Comprising of oleuropin and phenolic oleocanthal enhances anti-inflammatory and antibacterial effects for olive oil. Math, M. V (2013) conducted an experiment to determine the effectiveness of olive oil-containing oral rinse on patients with xerostamia. Results suggest that olive oil in oral rinse increases the production of saliva, reduction of dental plaque and preventing bacteria growth on teeth and gum cavity through oil coating *(1).*

Chapter 2

Experimental

2.1 Materials

Calcium lactate, *Capsicum oleoresin* extract, sodium alginate, and xanthan gum were purchased from Krungthepchemi Co., Ltd. Naturel olive oil was purchased from Lamsoon (Thailand) Public Company Limited. Apple cinnamon tea was purchased from Celestial Seasonings, Inc. (USA). All chemicals were used as received without further purification.

2.2 Instruments

Encapsulator (B-390, Switzerland), Magnetic stirrer (MR 3001 K, Germany), Orbital shaker (KS 130 Basic, Germany), Syringe Pump (Kd Scientific LEGATO100, USA), Ultrasonic bath (Elma E30H, Germany), Vernier caliper (Hashi, Japan), and Vortex mixer (Vortex-Genie 2, USA) were employed for experiments.

2.3 Polymeric bead preparation

2.3.1 Direct spherification

2.3.1.1 Direct extrusion using syringe pump

Studying the influence of solution and working parameters on formation of polymeric beads

Sodium alginate solution (2 % (w/v)) was filled in a 5-mL syringe that connected with 20G gauge needle and placed in the syringe pump. One milliliter of sodium alginate solution was continued to pump into calcium lactate solution (0.5 and 1 %(w/v)) with a flow rate of 0.5 or 1 mL/min under stirring with a magnetic stirrer. The droplet of sodium alginate solution was sat in calcium lactate solution for 2 min to allow the formation of calcium alginate beads. Then, the beads were removed and rinsed with DI water. The beads were kept in DI water for further characterization.

Preparation of capsicum extract-encapsulated beads using direct extrusion

Capsicum extract (0.8 mL) was mixed with 30 mL of sodium alginate solution (2 %(w/v)) using the magnetic stirrer until the mixture became homogeneous. The solution was filled in the 5-mL syringe and continued to pump through a syringe which was connected with 20G gauge needle into 0.5 or 1 %(w/v) calcium lactate solution. The capsicum-encapsulated beads were left in calcium lactate for 2 min. Then, the beads were removed and rinsed with DI water. The beads were kept in DI water for further characterization.

2.3.1.2 Direct extrusion using BUCHI encapsulator

Preparation of polymeric solution and optimization of instrument parameters

To prepare core-shell polymeric beads, olive oil was used as a core and 1 %(w/v) of sodium alginate solution was used as a shell. Olive oil and sodium alginate solution was pump through concentric nozzles of which the size for core (inner nozzle) and shell (outer nozzle) was fixed at 450 μm and 900 μm respectively. The solution was pumped into 1 %(w/v) of calcium lactate bath and left for 5 min. Working parameters including electrode tension, pressure, and vibration frequency were constant at 2500 V, 546 mbar, and 420 Hz, respectively. The flow rate of the core and shell solution was varied among three trials as shown in **Table 2.1**. The resulting beads were collected and rinsed with in DI water.

Table 2.1 The condition for optimization of the flow rate to form the polymeric beads.

Trial	Inner flow rate (mL/min)	Outer flow rate (mL/min)
1	6.2	8.5
2	9.9	~3

3	~11	4.1

2.3.2 Inverse gelation

2.3.2.1 Inverse gelation by syringe pump

In terms of inverse gelation, calcium lactate solution (0.5 and 4%(w/v)) was filled in the 5 mL syringe which was connected with 20G needle and placed on a syringe pump. Calcium lactate solution was pumped dropwise into 1.5 %(w/v) of sodium alginate bath with the flow rate of 0.1 mL/min under continuous stirring. The beads were collected after 5 min and rinsed with DI water.

2.3.2.2 Inverse gelation by dropwise addition of calcium lactate solution using syringe

Xanthan gum (0.5 g and 0.6 g) was dissolved in 1 %(w/v) of calcium lactate solution and stirred until mixture became homogeneous. Blue or green food color was mixed with xanthan gum solution in a ratio of 5:10,000 by volume. Then, xanthan gum solution was filled in the 5-mL syringe and extruded into 2 %(w/v) of sodium alginate bath and left for 15 min (with and without magnetic stirring). Next, the beads were removed from the sodium alginate bath, rinsed with DI water and submerged into 1 %(w/v) calcium lactate solution for 10 min. Lastly, the beads were taken and washed with DI water.

2.3.2.3 Inverse gelation using cryogelation

The optimization of xanthan gum concentration

Various amount of xanthan gum from 0.4 to 0.8 g was dissolved in 2%(w/v) of calcium lactate. Then, 500 µL of xanthan gum solution was pipetted into 1×1×1 cm^3 mold and frozen in -20°C freezer until it sets. The ice cube of xanthan gum was removed from the mold and placed in a 1 %(w/v) of sodium alginate bath for 6 min to allow a formation of calcium-alginate bead. After that, the beads were removed from the bath and washed with DI water. The beads were further sat in 2 %(w/v) of calcium lactate bath for 5 min. Then, the beads were removed and

washed with DI water. The beads were characterized by determination of weight using balance and size using a vernier caliper.

The optimization of sodium alginate concentration

Frozen mixture of 0.6 %(w/v) of xanthan gum and 2 %(w/v) calcium lactate was submerged in various sodium alginate concentration from 0.5 to 2 %(w/v) of sodium alginate for 6 min. After removed and washed with DI water, the beads were submerged in 1%(w/v) of calcium lactate bath for 5 min. Finally, the beads were collected and washed with DI water. Weight and size of the beads were evaluated.

The optimization of contact time

The ice cube of 0.6 %(w/v) of xanthan gum solution was submerged in 1 %(w/v) of sodium alginate bath, left to stabilize for 1 min and shook for 5, 10, 15, 20, 30, and 60 min. Then, the beads were washed and submerged again in 2 %(w/v) calcium lactate bath for 5 min. The beads were collected and washed with DI water before further characterization. To study the influence of contact time on the bead formation, the weight and shell thickness of the beads were evaluated.

The optimization of calcium lactate concentration

To study the influence of calcium lactate solution to the bead formation, various concentration of calcium lactate solution was prepared at 0.5, 1, 2, 4 (%(w/v)). Primary, the beads were prepared by dissolving 0.6 g xanthan gum in 2 %(w/v) calcium lactate solution. Then, the mixture was submerged in 1 %(w/v) sodium alginate solution for 6 min and followed by placing the beads in different calcium lactate concentration for 5 min. The beads were collected and measured for weight and size.

2.3.3 Natural extract encapsulation in polymeric beads via cryogelation method

In this study, three natural extracts were chosen to be encapsulated in polymeric beads. The solutions were prepared as followed: 1) Dissolving 0.6 g of xanthan gum in olive oil and then mixing with 2 %(w/v) of calcium lactate in 5:15 volume ratio. 2) placing a tea bag of apple-cinnamon tea bag in 200 mL of boiled water for 10 min. Then, 2 g of calcium lactate and 0.6 g of xanthan gum were dissolved in 100 mL of tea. 3) Capsicum extract was mixed with 0.6 %(w/v) of xanthan gum solution in 0.2:20 volume ratio. Each mixing solution (500 µL) was pipetted into the ice mold which was put in a freezer until the mixture became completely frozen.

The ice cubes of frozen mixture were submerged in 1 %(w/v) of sodium alginate bath (1%(w/v) and left to stabilize for 1 min. Gelation mixture was placed on a microplate shaker for 5 min and rinsed with DI water. Next, the beads were dropped in calcium lactate bath (2%(w/v) and left for 5 min. Finally, the encapsulated beads were collected and washed with DI water.

Chapter 3

Result and Discussion

3.1 Spherification by direct extrusion

3.1.1 Direct extrusion using syringe pump

Direct extrusion is a simple technique for the preparation of polymeric beads. The droplet of sodium alginate solution was dropped in calcium lactate solution to allow ion exchange of sodium and calcium ion in solution. With this reaction, the droplet was cross-linked and form calcium-lactate polymeric beads *(10).* To study the formation of the polymeric beads using direct extrusion, the concentration of sodium alginate and calcium lactate solution was fixed at 2 and 1 %(w/v), respectively. The concentration of working solution was fixed according to Pumpho and Puechkamutr (*16*) that this concentration was sufficient for homogeneous spherical bead forming

(16). Herein, the syringe pump was applied to control volume and flow rate of sodium alginate droplet into calcium lactate solution. Resulting polymeric beads extruded by a syringe pump showed that the polymeric beads were in a spherical shape and almost uniform in size (**Figure 3.1A**). However, the beads were rigid and difficult to break that might be the difficulty of releasing the natural extract inside the beads. Hence, the concentration of calcium lactate was diluted from 1 %(w/v) to 0.5 %(w/v) which is expected to reduce polymer linkage. After the concentration of calcium lactate solution was reduced, the polymeric beads were still rigid due to the formation of the beads occurring from outside to inside. When the droplets of sodium alginate contacted to calcium lactate solution, sodium lactate was cross-linked and formed a boundary around the droplets. Then, calcium ions were permeated inside the droplets which caused the cross-linking inside. The cross-linked process continued during the contact time and the beads were more rigid (*10*).

Due to the success of spherical and uniform polymeric beads formation, capsicum extract was mixed with sodium alginate solution to determination for the possibility for encapsulation. After leaving the droplets of mixing solution in calcium alginate bath for approximately 5 min, the spherical shape with homogeneous size of the encapsulated beads were formed (**Figure 3.1B**). Although the beads were formed, they were rigid and cannot release capsicum when broken. (**Figure 3.1C**). Due to the extract was embedded in the alginate polymer, the beads lacked of controlled-release mechanism (*10*). According to the aim of this work, the encapsulated beads must be capable of carrying natural extract and releasing the encapsulated content when being chewed. So, the encapsulated beads prepared by this technique cannot serve the purpose.

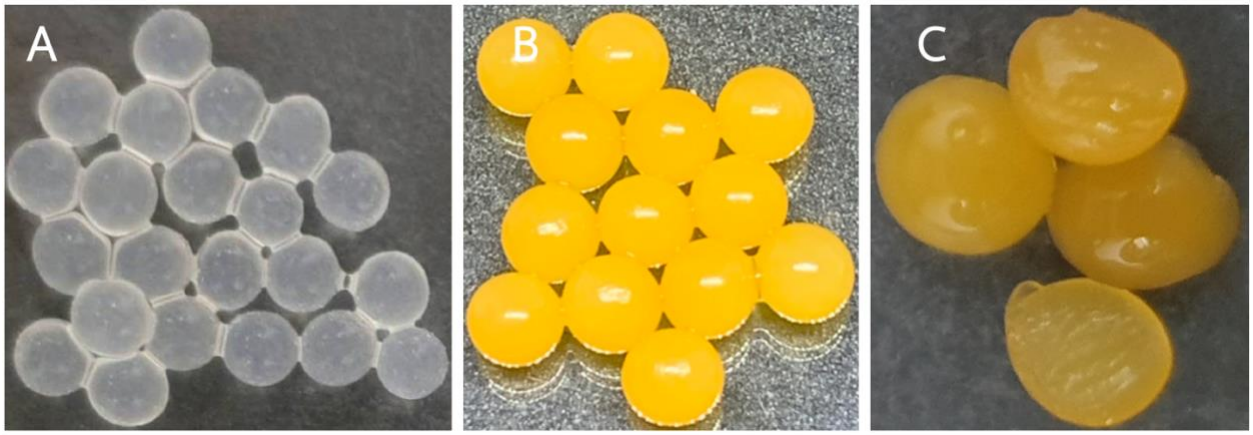

Figure 3.1 Polymeric beads prepared by direct spherification with (A) 1 %(w/v) sodium alginate and 2 %(w/v) calcium lactate, (B) capsicum extract in 2%(w/v) sodium alginate solution, and (C) capsicum extracted-encapsulated beads after being compressed.

3.1.2 Direct extrusion using BUCHI encapsulator

The encapsulator was utilized for the production of monodisperse microbeads. It is believed that core-shell polymeric beads could be formed and the problem due to the hardness of the beads can be overcome. These experiments were set-up using co-extrusion technique which consisted of two nozzle systems, or called concentric nozzles, to extrude core and shell solutions simultaneously (*10*). The inner (core) and outer (shell) nozzles can be adjusted in order to get beads with desired size. Unlike the syringe pump, the encapsulator having numerous parameters that can be adjusted, including flow rate, vibration frequency, electrode, and heating of the extruded solution, as well as the air pressure (BUCHI manual).

Among all working parameters, flow rate was the first parameter to be optimized because it influenced the size of the polymeric beads, the productivity and the thickness of the shell. From the theory, applying high flow rates for both core and shell nozzles would maximize the capacity of core solution and sufficient thickness of the shell. During the three experimental trials under concentric nozzle system, the inner- and outer-nozzle size was fixed at 450 μm and 900 μm respectively. The concentration of sodium alginate and calcium lactate were also fixed at 1 %(w/v) for both solutions as it was sufficient to form the spherical beads.

In the first trial, the flow rate was adjusted to 6.2 mL/min and 8.5 mL/min for the inner and outer feed, respectively. The core-shell beads cannot form in this condition. The olive oil was separated onto the upper layer after the process (**Figure 3.2A**). For the second trial, the outer flow rate was decreased to 3 mL/min and the inner flow rate was increased to 9.9 mL/min. With this condition, the core-shell polymeric beads were formed. Olive oil was encapsulated inside the thin layer of calcium alginate beads (**Figure 3.2D**) with small traces of olive oil leaking (**Figure 3.2B**). For the last trial, the inner flow rate was increased to ~11 mL/min while minimizing the outer flow rate to 4.1 mL/min. The obtained beads can encapsulate olive oil inside the shell without leaking as shown in **Figure 3.2C** and 3.**2E**. From the results, the difference of flow rates between core and shell possesses a strong impact on the ability of the polymeric beads to encapsulate olive oil extract. In the case when the inner flow rate was fixed at a lower value and the outer flow rate is fixed at a higher value, the synthesized polymeric beads had a thicker shell that prevents the encapsulated ingredient to come out.

Although the encapsulator was a high-throughput machine to produce core-shell polymeric beads, there were numerous parameters to be varied. Furthermore, the size of the core-shell beads was limited by the nozzle size which was 2-fold of the nozzle size. The largest core nozzle was 1 mm that was not sufficient for this research.

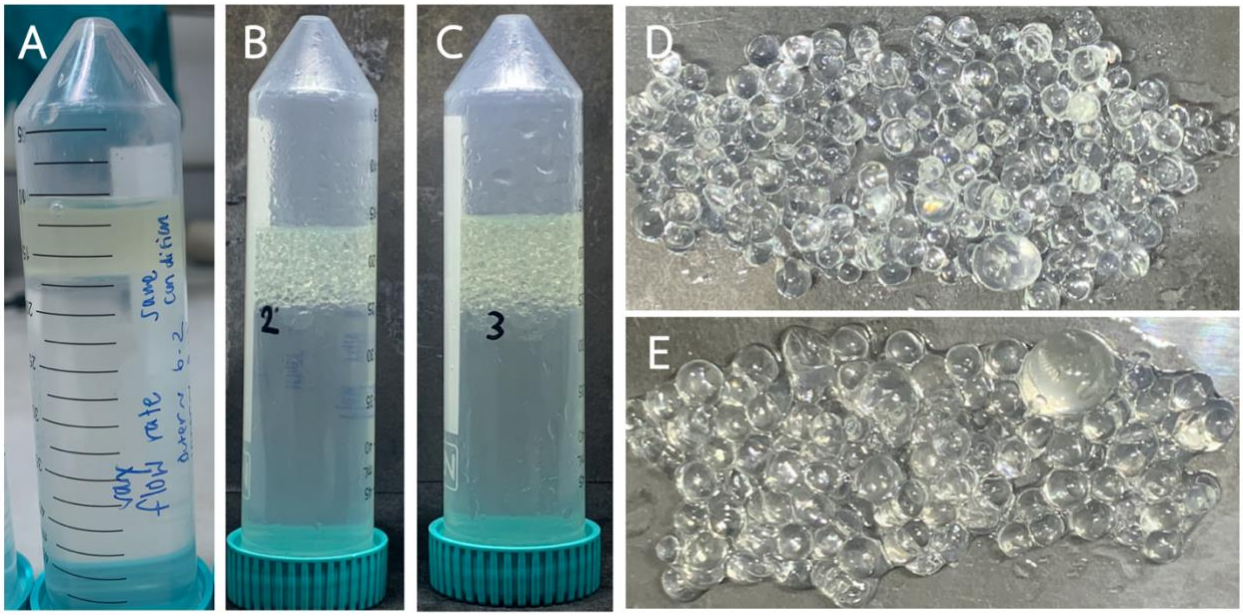

Figure 3.2 Core-shell polymeric beads contained olive oil which prepared by different flow rate: (A) 8.5 mL/min (core) and 6.2 mL/min (shell), (B) 9.9 mL/min (core) and ~3 mL/min (shell), and (C) ~11 mL/min (core) and 4.1 mL/min (shell), respectively.

3.2 Encapsulation via inverse gelation

3.2.1 Inverse gelation by syringe pump

To decrease the hardness of the polymeric beads, inverse gelation was an alternative strategy for the bead preparation. Calcium lactate solution which was a cross-linking agent was dropped in sodium alginate solution to allow the formation of thinner layer of the shell around the droplet. In this work, the concentration of sodium alginate was fixed at 1.5%(w/v), whereas calcium lactate concentration, in dyed blue color, was fixed at 0.5 %(w/v). Following the inverse gelation procedure, calcium lactate was filled in a syringe and dropped into the sodium alginate bath using the syringe pump (0.1 mL/min). As shown in **Figure 3.3**, the calcium lactate droplets formed beads with a blue round shape in sodium alginate solution. At the contact surface, sodium alginate was crosslinked. However, the whole droplets did not sink into the sodium alginate solution. Therefore, only a hydrogel piece was formed.

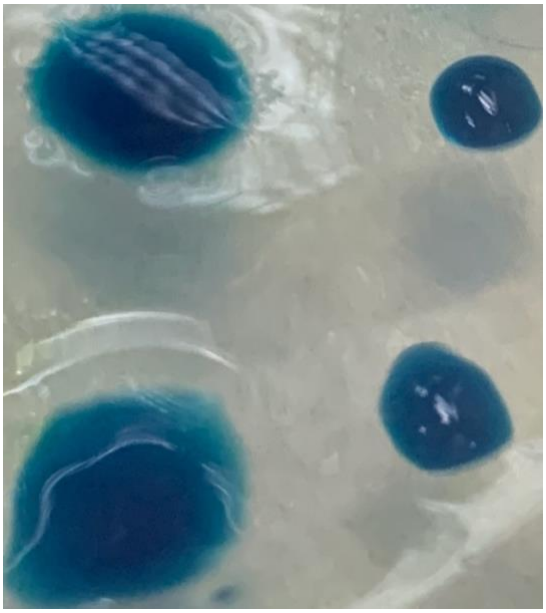

Figure 3.3 Dyed blue hydrogel formed in sodium alginate solution when dropping of calcium lactate (0.5 %(w/v)) in of sodium alginate solution (1.5 %(w/v)) using a syringe pump.

The concentration of calcium lactate solution was increased to 4 %(w/v) which was expected to increase crosslinking while sodium alginate concentration remained at 1.5%(w/v). By using the syringe pump (0.1 mL/min), calcium lactate solution was dyed green to observe the change in crosslinking and was ejected from the syringe into the sodium alginate bath under stirring. Because the contact time was not enough to form separated beads, the droplets of calcium lactate coalesced resulting in bulk hydrogel formation (**Figure 3.4**). Besides, calcium lactate droplets cannot sink in the sodium alginate solution. It was anticipated that an addition of xanthan gum as the thickening agent into the solution should make calcium lactate maintaining the spherical shape and sinking in sodium alginate solution.

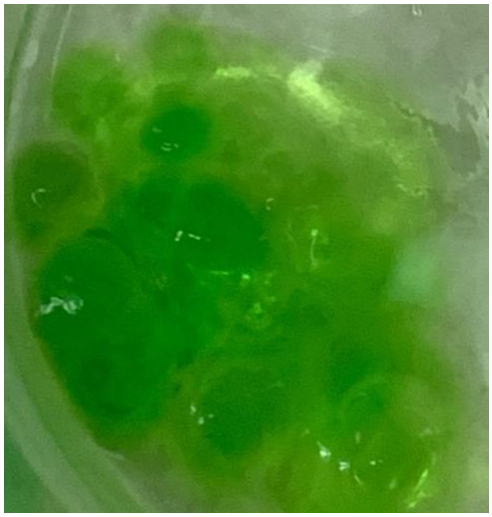

Figure 3.4 The coalescence of dyed green calcium alginate hydrogel which was obtained from the 4 %(w/v) of calcium lactate extrusion in 1.5 %(w/v) of sodium alginate solution.

3.2.2 Inverse gelation by dropwise of calcium lactate solution using syringe

As reported above, the formation of polymeric beads may require a thickening agent to hold the spherical shape of the droplets and have the droplets sunk into the sodium alginate bath. In this study, 0.5 and 0.6 %(w/v) of xanthan gum was prepared in 1 %(w/v) of calcium lactate as reported by de Farias and Zapata Norenã (*17*) as the concentration allowed the bead formation. Then, the xanthan gum solution was dropped in 2 %(w/v) sodium alginate solution. The droplet of xanthan gum was placed in sodium alginate bath for 10 min to allow the polymeric bead formation. Since the droplets can sink into the sodium alginate solution, only the contact surface was cross-linked and formed a bowl-shape like hydrogel with a thick shell of calcium alginate (**Figure 3.5A** and 3.**5B**).

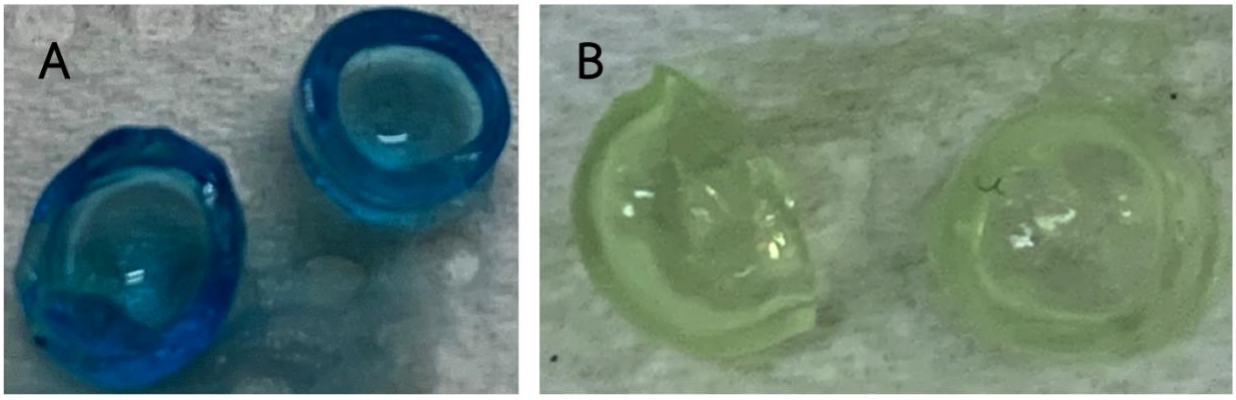

Figure 3.5 A bowl-shape like hydrogel obtained by dropping (A) 0.5 and (B) 0.6 %(w/v) of xanthan gum/calcium lactate solution in sodium alginate solution and placed for 10 min.

To study the influence of stirring on the beads formation, xanthan gum/calcium lactate mixture was dropped in the sodium alginate solution with and without stirring. Herein, the concentration of xanthan gum/calcium lactate was varied as 0.5 and 0.6 %(w/v) and the concentration of sodium alginate was fixed at 2 %(w/v). After dropping the xanthan gum/calcium lactate solution in sodium alginate solution, the droplets of xanthan gum/calcium lactate were placed in sodium alginate bath for 15 min. **Figure 3.6A** showed the droplets of calcium lactate in sodium alginate solution. The addition of xanthan gum in calcium lactate solution can hold the spherical shape of the droplets but the whole droplets cannot sink into the sodium alginate solution resulting in incomplete bead formation. When using 0.5 %(w/v) of xanthan gum, the droplets of xanthan gum cannot sink into the sodium alginate solution, resulting in a bowl-shape formation (**Figure 3.6B**). When the concentration of xanthan gum was increased to 0.6 %(w/v), it was possible to form a spherical bead with rough surface and some beads contained a tail (**Figure 3.6C**). From this experiment, it is obvious that the formation of polymeric beads was influenced by stirring.

Although the polymeric beads were formed as a result of the addition of xanthan gum, the formation of the beads using this technique was limited due to the inability to control the

droplet size which affects the size distribution. Furthermore, the xanthan gum/calcium droplet was able to merge together when stirring, resulting in the polymeric bead having irregular shape.

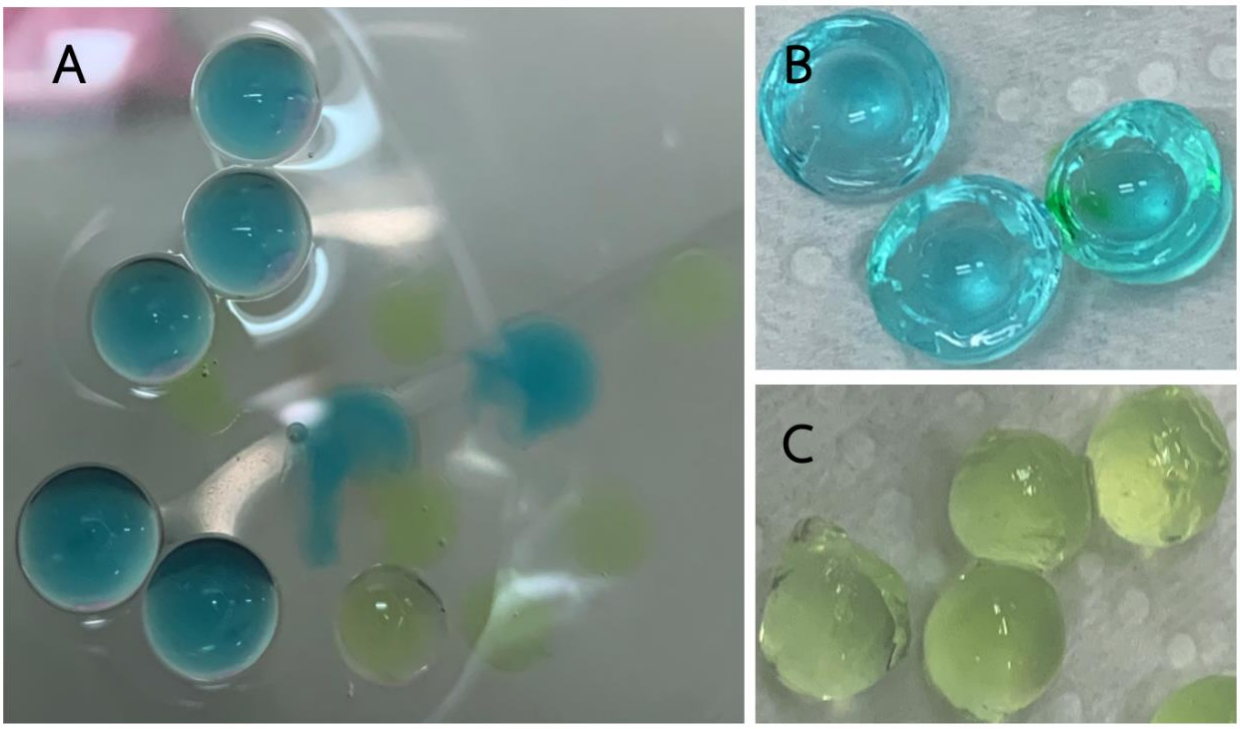

Figure 3.6 (A) droplets of xanthan gum in sodium alginate solution after applied by magnetic stirring. (B) The bowl-shape hydrogel was formed when using 0.5 %(w/v) xanthan gum. (C) Polymeric beads were formed with the rough surface when using 0.6 %(w/v) of xanthan gum.

3.2.3 Inverse gelation using cryogelation

Cryogelation was one of the effective method for the polymeric bead preparation. Cryogelation relied on inverse gelation in which the mixture of active agent and cross-linked agent was frozen prior to immersion in sodium alginate bath. Concentration of sodium alginate was diluted from 2%(w/v) to 1%(w/v) to reduce the solution viscosity so that the extruded solution can be easily immersed into the bath and allows for complete spherification of the droplets. For the pre-liminary test, 0.6 %(w/v) of xanthan gum was dissolved in 2 %(w/v) of calcium lactate solution. The mixture was filled in an ice mold and stored in -20 °C to freeze the mixture. The

ice cube of mixture was immersed in 1 %(w/v) of sodium alginate for 6 min under shaken with plate stirrer (240 rpm). Then, the beads were removed and rinsed with DI water. After that, the beads were submerged in 2 %(w/v) of calcium lactate solution in order to crosslink the outer layer of the beads. The frozen xanthan gum mixture can completely sink in the sodium alginate bath (**Figure 3.7A**). After cryogelation, the polymeric beads having regular spherical shape were obtained (**Figure 3.7B**).

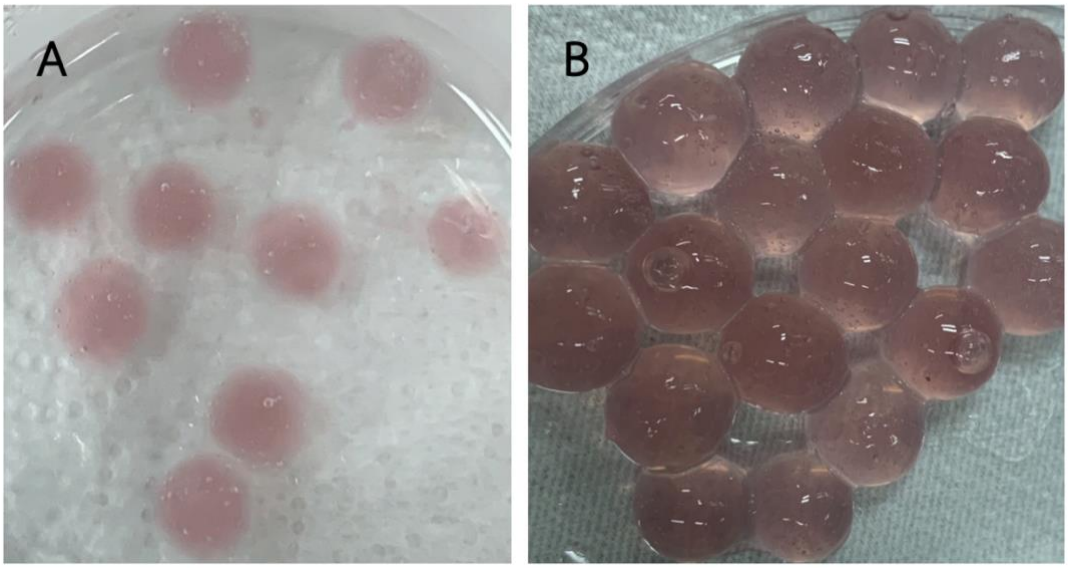

Figure 3.7 Polymeric beads prepared via cryogelation (A) before and (B) after removed from the bath

3.2.3.1 The optimization of xanthan gum concentration

Since xanthan gum influenced the shape and sphericity of polymeric beads, the concentration of xanthan gum was optimized by preparing various concentration of xanthan gum having concentration in a range of 0.4 - 0.8 % (w/v) in 2 %(w/v) of calcium lactate solution. After cryogelation, the polymeric beads with a spherical shape were obtained from all concentration (**Figure 3.8**). By naked eye observation, the polymeric beads were not different. Hence, the aspect ratio of the beads was calculated from the ratio of width and length. If the aspect ratio was close

to 1, the spherical shape of the beads was regular. From the calculation, the aspect ratio of all conditions was closed to 1. However, the concentration of xanthan gum was optimized at 0.6 %(w/v) due to *%RSD* value was lower than 10. Furthermore, 0.6 %(w/v) was the lowest concentration that yielded spherical beads with less defect. The result was similar to the report given by (*17*) in that the best condition for preparation was using 0.6 %(w/v) of xanthan gum.

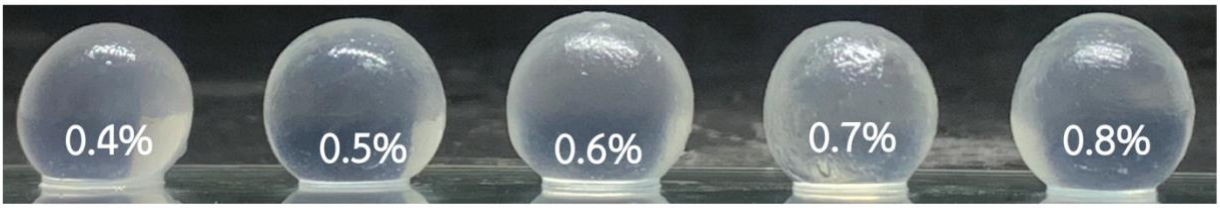

Figure 3.8 Polymeric beads obtained from using different xanthan gum concentration of 0.4, 0.5, 0.6, 0.7, and 0.8 %(w/v)

Table 3.1 Aspect ratio and *%RSD* of the polymeric beads as a function of xanthan gum concentration.

Xanthan Gum conc. (%)	MEAN of Aspect Ratio (mm)	%RSD of Aspect Ratio
0.4	1.02±0.16	15.93
0.5	1.10±0.11	10.46
0.6	1.03±0.09	8.84
0.7	1.03±0.11	10.46
0.8	0.96±0.08	8.10

3.2.3.2 The optimization of sodium alginate concentration

The concentration of sodium alginate was optimized to study the influence of viscosity on the bead formation and the shell thickness. Sodium alginate controlled the boundary of the beads because the frozen xanthan gum/calcium lactate slowly melt and exchanged ions with

sodium alginate. With this process, a thin polymeric layer was formed and the xanthan gum/calcium lactate solution were kept inside. When the concentration of sodium alginate was increased, it was expected that calcium lactate could diffuse through the sodium alginate solution and increased ion exchange reaction, resulting in the increasing of wall thickness.

In this work, the concentration of sodium alginate was varied from 0.5 to 1 %(w/v). The polymeric bead formation from each concentration is presented in **Figure 3.9**. The increasing alginate viscosity due to high concentration of calcium diffusion diminished droplet submersion and caused incomplete gelation. Thus, polymeric beads tended to change into irregular shape when the concentration of sodium alginate increased. However, when polymeric beads were able to immerse into alginate bath, gelation occurred around the beads continuously. In concentrated sodium alginate solution such as 1.5 and 2%(w/v), ion-exchange reaction became faster, resulting in thicker polymeric shells due to high crosslinking level (**Table 3.2**).

Figure 3.9 Polymeric beads formed by using various concentration of sodium alginate.

Table 3.2 Shell thickness and %RSD of polymeric beads prepared from varied concentration of sodium alginate

Sodium Alginate Conc. (%)	MEAN of shell thickness (mm)	%RSD of shell thickness
0.5	0.17±0.05	28.41
1.0	0.25±0.05	21.08
1.5	0.35±0.09	26.35

2.0	0.39±0.06	16.54

3.2.3.3 The optimization of contact time

To optimize the contact time in sodium alginate solution, the frozen xanthan gum/calcium lactate solution was sat in sodium alginate without disturbing for 1 min to allow the effective formation of polymeric layer. After that, the sodium alginate bath was placed on the plate stirrer with various time to study the effect of contact time to the bead formation and the thickness of the shell. As shown in **Figure 3.10**, the bead size was increased when the contact time increased. Calcium ion which was trapped inside the bead can diffuse through the polymeric shell and cross-linked with sodium alginate solution. When the contact time was increased, weight and thickness of the polymeric beads were increased. From this experiment, the optimized condition for contact time was 5 min. The shell thickness must be high enough to carry the natural extract inside the bead but not too rigid to be broken by chewing.

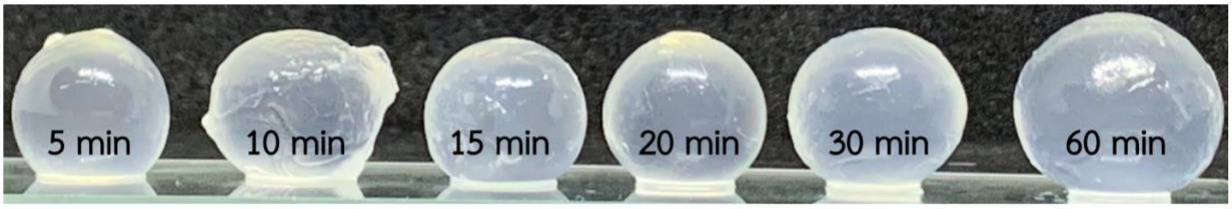

Figure 3.10 Polymeric beads formed under different contact time from 5 to 60 min in sodium alginate solution.

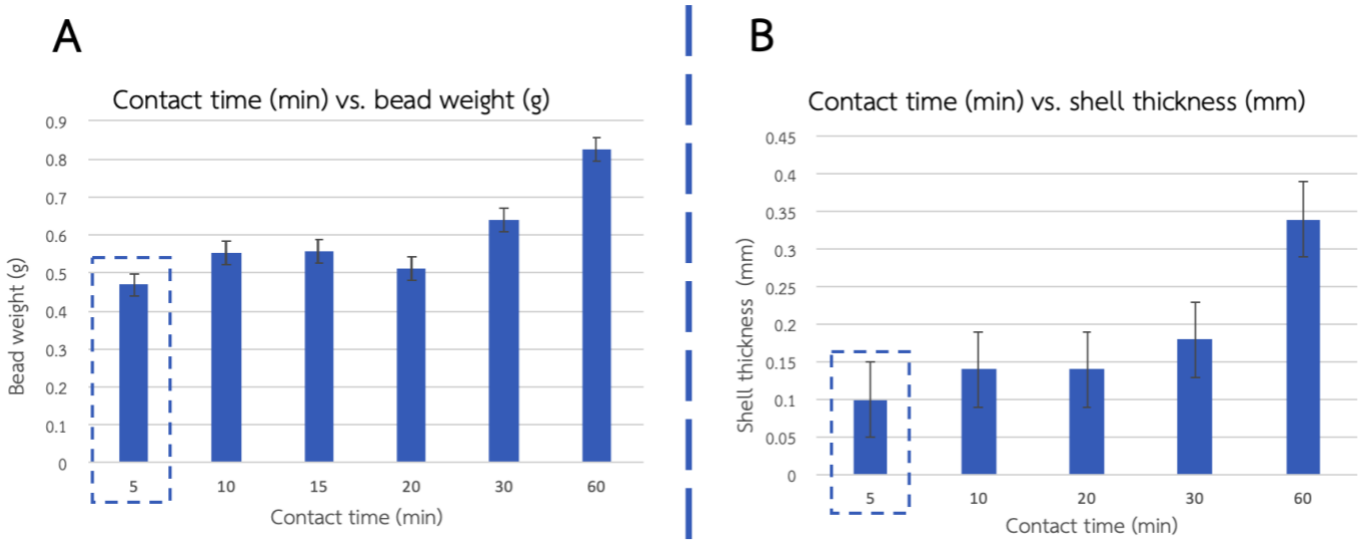

Figure 3.11 The relationship between of contact time and (A) bead weight and (B) shell thickness of polymeric beads.

3.2.3.4 The optimization of calcium lactate concentration

Calcium lactate act as a cross-linking agent which dictates the rate of calcium ion diffusion to initiate polymeric bead gelation (12). In cryogelation technique, calcium lactate was contacted with sodium alginate twice. Firstly, the frozen calcium lactate was immersed in sodium solution. In this process, the frozen calcium lactate was slowly melted and exchanged ions with sodium alginate to form calcium alginate polymeric layer. Secondly, the beads were removed from sodium alginate solution and submerged in calcium lactate solution to allow the crosslinking on the outer layer. This process was applied to increase the strength of the shell. In this work, the concentration of calcium lactate in the second process was optimized using various concentration of calcium lactate between 0.5 to 4 %(w/v). When the concentration of the calcium lactate solution was increased, the bead tended to form an irregular shape (**Figure 3.12**).

Figure 3.12 The morphology of the polymeric bead which prepared by various concentration of calcium lactate from 0.5 to 4 %(w/v).

3.2.4 Natural extract encapsulation in polymeric beads via cryogelation method

Due to successful bead formation using cryogelation method, natural product extracts including capsicum and olive oil were attempted for encapsulation as they provided oral hydration. The volume of natural product extract as core solution was determined on its solubility and the stability provided by the polymeric carrier. In addition, apple cinnamon extract was used for encapsulation to investigate the stability of hydrophilic solution in polymeric carrier. The polymeric solution concentration and contact time used in this synthesis was set prior to synthesis parameter optimization. Preparation of encapsulated bead using cryogelation provided soft shell layer which holds the solution of natural extract inside (**Figure 3.13**). When breaking the beads, the solution inside was released. The preparation of the encapsulated beads by cryogelation possessed the potential for further natural product encapsulation.

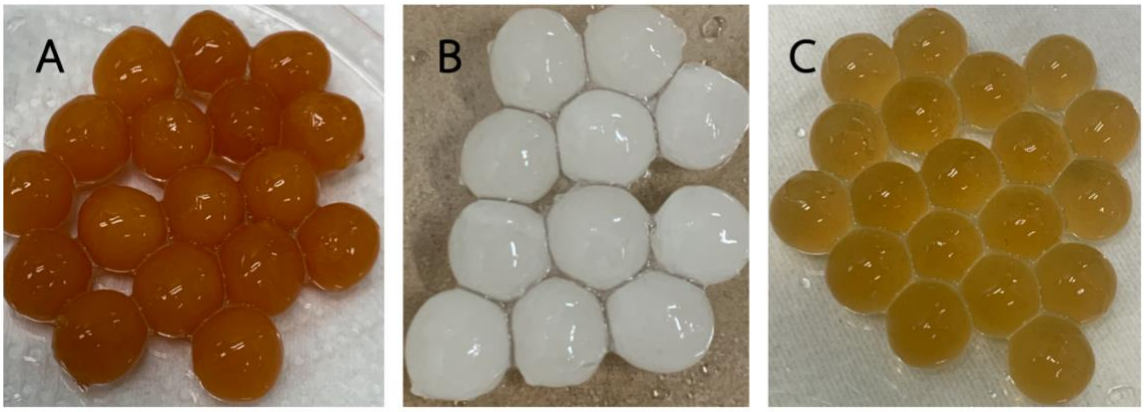

Figure 3.13 Polymeric beads prepared via cryogelation encapsulated with (A) capsicum, (B) olive oil, and (C) apple cinnamon extracts

Chapter 4
Conclusion

In this work, polymeric beads capable of encapsulating natural product extracts were prepared. Two polymer spherification techniques consisting of direct spherification and inverse gelation were investigated. Cryogelation by inverse gelation was the most suitable method as full polymeric spherification was completed, due to presence of double gelation layer, and demonstrated control pressure-releasing encapsulated beads. This study also optimizes the condition using cryogelation and demonstrates the potential to encapsulate natural product extracts.

The encapsulated beads would be further used as natural extracts carrier, so they must be ingestible and have control pressure-releasing mechanism for active ingredients to reach the target organ. In addition, the encapsulated beads needed to be reproducible with monodispersed distribution to represent the consistency of containing fixed volume of active ingredient solution in each bead. Therefore, cryogelation technique presented as the most suitable polymeric synthesis method as it gave the most reproducible results with controllable synthesis parameters. During research, four synthesis parameters were optimized including sodium alginate (% w/v), calcium lactate (% w/v), xanthan gum (% w/v), and contact time (min). From optimization trials, xanthan gum (% w/v) and contact time (min) impacted the formation of polymeric beads in terms of shape and strength.

Herein, the possibility of using encapsulated beads as carriers was demonstrated by the encapsulation of capsicum extract, olive oil and cinnamon tea. The obtained beads were in a

uniform shape and size. Moreover, natural extract solution was released out when the shell was broken upon pressing.

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Biography

Miss Pattarawimon Phoonsiri was born on January 5, 1999 in Bangkok, Thailand. She spent 9 years living abroad in Indonesia and Oman. She earned her college diploma from International Community School (ICS), Thailand, in 2017. Currently, she is studying in her senior year of the Bachelor of Science in Applied Chemistry majoring in Material Chemistry (International program), Faculty of Science, Chulalongkorn University. She will graduate in June 2021. Her current address is 54 moo 2, Sukhumvit 107 road, Bangna, Bangkok, Thailand, 10260