

Studies on the Effect of Liquid-Core Capsule Properties on pH Control in Different Medium

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Abstract: Liquid-core capsule composed of calcium carbonate (CaCO₃) and carboxymethylcellulose (CMC) as inner liquid-core surrounded by a cross-linked alginate membrane was prepared in this study. Liquid-core capsules have been widely used in various industrial applications. The performance of fermentation medium is affected by the accumulation of product(s) in the medium which will reduce the pH that may inhibit the growth of the fermenting microbes. The use of liquid-core capsules to control pH of fermentation medium is a new approach. The objective of this research was to study the effect of liquid-core capsule properties (i.e. diameter, membrane thickness) on pH control in different fermentation medium (i.e. lactic acid solution, MRS broth, CMC solution, and starch solution). The results showed that capsule diameter and membrane thickness have significant effect on pH change rate. Among the tested properties, the capsules with diameter of 4.50mm and membrane thickness of 0.81mm have the fastest pH change rate (5.75×10^{-3} pH/min). In short, the pH regulator capsules were capable to control the pH of the tested solutions at the desired level.

Key words: Encapsulation; Sodium-alginate; pH control; Fermentation medium; Starch solution; CMC solution

INTRODUCTION

The cell growth and productivity of a biological system is strongly influenced by the pH of the working medium (Huang *et al.*, 2003). In order to control the pH of the system, several approaches have been introduced in the past. The pH of the biological systems could be controlled using pH controller, separation process to remove the organic acid in the medium, manipulation of fermentation mode, and addition of pH buffer to regulate the medium pH.

The use of pH controller is the simplest approach but it is relatively expensive and requires advanced instrumentation. The separation process requires expensive membrane and the separation is commonly carried out outside the fermenter, which increases the risk of contamination. On the other hand, the continuous and fed batch fermentation modes are reported to be effective to control pH of the medium. Moreover, the risk of contamination is high when continuous mode is used. The use of fed batch mode may be economically not feasible as it requires large volume of fermentation medium to adjust the medium pH (Lee *et al.*, 2007).

Encapsulation is defined as the particles formed such as capsules or beads by which a gaseous, liquid or solid active ingredients, is surrounded by a continuous film or coating. The inner core of the capsules contains the active ingredients, which is protected with the surroundings by the capsule wall or semi-permeable membrane (Lee *et al.*, 2005).

In this research, the pH regulating agent or buffer will be encapsulated in liquid core capsule form. The pH buffer in the capsules will be used to control the pH in the fermentation medium. The use of encapsulation technology to control pH of biological system is a new approach. The performance of the capsules to control pH in the mentioned fermentation medium systems will be evaluated with consideration of the capsule properties such as diameter and membrane thickness.

MATERIALS AND METHODS

Sodium alginate:

Sodium alginate (Alginate I-3) was purchased from Kimitsu-Kimica (Japan) and the specifications were shown in Table 1.

Experimental:

Preparation of liquid-core capsule:

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Liquid-core capsule was prepared by extrusion method as illustrated in Fig. 1. 1.5% (w/v) of alginate (Kimitsu-Kimica, Japan) and 0.15% (w/v) of tween-80 (Merck, Germany) were prepared as the gelation bath. The liquid-core solution was prepared by adding 40% (w/v) of calcium carbonate (CaCO_3) (HmbG Chemical, France), 1.5% (w/v) of calcium chloride (CaCl_2) (R&M Chemical, United Kingdom) and 1.5% (w/v) of carboxymethylcellulose (CMC) (Calbiochem, Germany). The liquid-core solution was extruded through hypodermic needle (Terumo, Japan) into gelation bath. The capsules were then left for curing in 0.5% (w/v) alginate solution for 30 minutes or otherwise. After the curing, the capsules were washed with distilled water and stored in CaCl_2 solution. In this study, the capsules were prepared using different sizes of hypodermic needle (i.e. outer diameter of 0.8mm and 1.2mm) and syringe without needle (i.e. outer diameter of the syringe edge is 2.3mm) for capsule size variation. The capsules were also prepared with different gelation times (i.e. 1 hour, 2 hour and 3 hour) for membrane thickness variation.

Table 1: Sodium-alginate from Kimitsu-Kimica, Japan specification

Test	Specification
M/G ratio	M55:G45
Grade	1-3G
Loss of drying	Max 15.0%
Viscosity (1% in solution)	300-400 cps
pH (1% in solution)	6.0-8.0

(Note: The above information was provided by Kimitsu-Kimica, Japan)

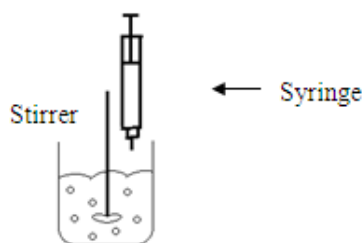


Fig. 1: Encapsulation set-up by extrusion method Characterization of liquid-core capsules

The image of 30 capsules was captured using a digital camera (Coolpix S2600, Nikon, Japan) for capsule properties characterized. The diameter and membrane thickness of capsules was determined using Sigma ScanPro5 software.

Preparation of testing medium:

Several solutions were prepared to simulate the fermentation medium used for food and lactic acid fermentation. The solutions included lactic acid solution, de Man, Rogosa and Sharpe (MRS) broth, starch and CMC solution. Lactic acid 90% purity (Eurochemo-PharmaSdn. Bhd., Malaysia) was added to distilled water to prepare 20 g/L lactic acid solution. Subsequently, the lactic acid solution was used to prepare MRS broth, starch and CMC solution. The MRS powder (Oxoid, UK) was added into the lactic acid solution. The starch (ThyeHuat Chan Sdn. Bhd., Malaysia) was added into the solutions. Starch solutions of 1 % (w/v), 5 % (w/v) and 10 % (w/v) were prepared. The CMC (Calbiochem, Germany) powder was added into the solutions. CMC solutions of 0.5 % (w/v), 1 % (w/v) and 2 % (w/v) were prepared. The starch and CMC solutions were used to test the performance of the capsules in viscous solutions.

pH measurement of testing medium:

All the experiments were conducted under normal room conditions at temperature of about 25°C. The pH of the solutions was measured using pH meter (Mettler Toledo, Switzerland) at different interval of times.

RESULTS AND DISCUSSION

Physical properties of liquid-core capsules:

Table 2 shows that the diameter and membrane thickness of capsules produced using different dripping nozzles and gelation times. As shown in Table 2, the diameter and membrane thickness of the capsules produced were in the range of 4.50 mm to 10.50 mm and 0.81 mm to 2.88 mm, respectively. The diameter and membrane thickness of the capsules were dependent on the dripping nozzle size and gelation time. According to the results, the diameter and membrane thickness of the capsules increased as the dripping nozzle size and gelation time increased. The trend of the results was in agreement with that reported in the previous study (Chai *et al.*, 2004).

Table 2: The physical properties of the capsule and the pH change rate in lactic acid solutions when the capsules were used.

Hypodermic needle size (mm)	Gelation time (minutes)	Diameter (mm)	Membrane thickness (mm)	Exponential phase (min^{-1})
0.80	60	4.50 (± 0.33)	0.81 (± 0.13)	5.75×10^{-3}
	120	4.61 (± 0.42)	0.94 (± 0.11)	5.50×10^{-3}
	180	5.12 (± 1.53)	1.05 (± 0.13)	5.42×10^{-3}
1.20	60	4.57 (± 0.43)	0.83 (± 0.16)	5.50×10^{-3}
	120	4.59 (± 0.51)	0.85 (± 0.16)	5.25×10^{-3}
	180	4.62 (± 0.87)	0.91 (± 0.16)	5.00×10^{-3}
2.30	60	8.35 (± 0.95)	1.99 (± 0.22)	3.81×10^{-3}
	120	9.38 (± 0.77)	2.39 (± 0.27)	3.46×10^{-3}
	180	10.50 (± 1.14)	2.88 (± 0.23)	3.10×10^{-3}

Experimental conditions:

The capsules were prepared with 1.5% (w/v) of sodium-alginate, 1.5% (w/v) of CMC and 1.5% (w/v) of CaCl_2 concentration. The standard deviation of the measurement was presented in parentheses.

The extrusion method employed was simple and having advantages over controlling membrane thickness of hydrogel membrane by varying gelation time of capsule formation (Chai *et al.*, 2004). Based on (Somesht *et al.*, 1988), the thickness of capsules membrane (varying needles diameter) increased with the increasing of gelation time. This is because longer gelation times would indirectly boost up the residence time of the capsules in alginate solution, causing the outward diffusion of calcium ion which functioned as gelling inducer. The Calcium layer around the core would progressively build up and increased the membrane thickness. The layer was proportional to the gelation time. Hence, gelation times tend to increase the membrane thickness of capsule (Burgos-Rubio *et al.*, 2000).

Effect of capsules properties on pH control in LA solutions:

Fig. 2 shows the pH values of LA solutions against time for the capsules formed with different dripping nozzles and gelation times. As shown in Fig.2, the capsules, regardless of the size, were capable to increase the pH in the LA solutions from 2.0 to 6.0. In which, the smallest and thinnest capsule (i.e. diameter of 4.50 mm; membrane thickness of 0.81 mm) gave the fastest rate in controlling the pH in the LA solution. The pH of the solution reached 5.82 after 12 hours with the rate of 5.75×10^{-3} pH/min (see Table 2). The biggest and thickest capsule (i.e. diameter of 10.50 mm; membrane thickness of 2.88 mm) gave the slowest pH change rate, 3.10×10^{-3} pH/min (see Table 2). After 12 hours, the pH of the solution only reached 5.33.

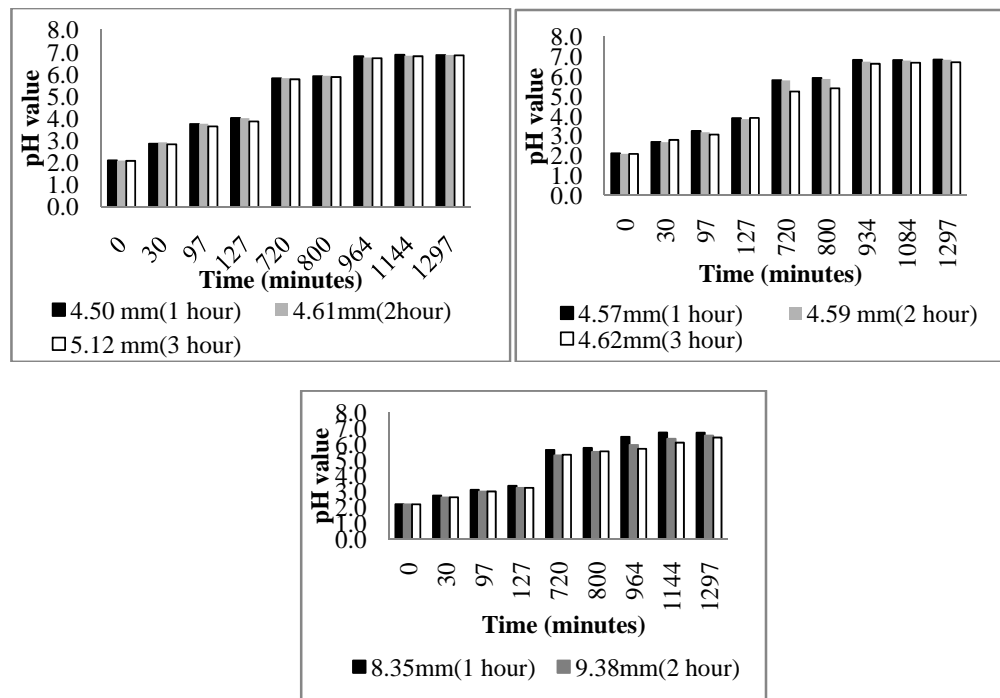


Fig. 2: The pH of 20 g/L lactic acid solution versus time (minutes) for capsules produced from (a) 0.8mm, (b) 1.2mm and (c) 2.3mm of dripping nozzle at varied gelation time. The capsules were prepared with 1.5% (w/v) of sodium-alginate, 1.5% (w/v) of CMC and 1.5% (w/v) of CaCl_2 concentration. The capsule diameter and gelation time (in the parentheses) were indicated in the legend.

Based on the results shown in Table 2 and Fig.2, the diameter and membrane thickness of the capsules have significant effect on the pH change rate. The results were in good agreement with the results reported by (Chai *et al.*, 2004; Wyss *et al.*, 2004). In which the drug release and absorption characteristics of the capsules were inversely proportional to the capsule diameter and membrane (Chai *et al.*, 2004; Wyss *et al.*, 2004).

Performance of liquid-core capsule to control pH in fermentation medium (MRS broth):

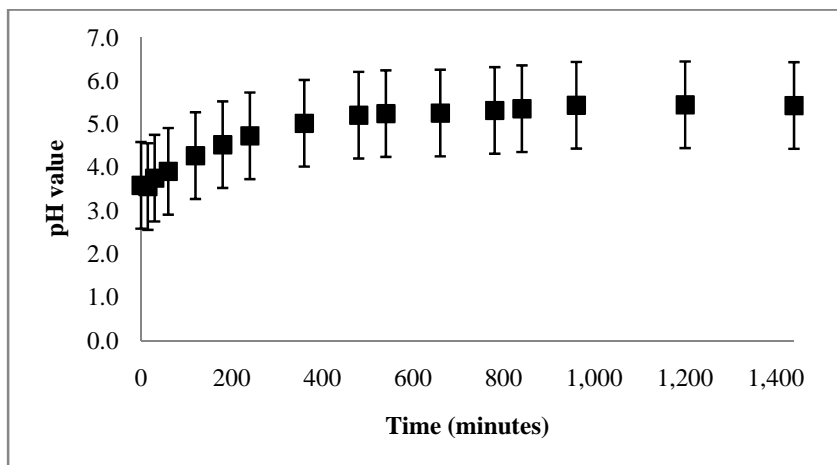


Fig. 3: pH of MRS broth at different time. The MRS medium was prepared with 20 g/L lactic acid. The capsules were prepared with 1.5% (w/v) of sodium-alginate, 1.5% (w/v) of CMC and 1.5% (w/v) of CaCl_2 concentration. The capsule diameter and membrane thickness of the capsules were 4.50 mm and 0.81 mm, respectively.

As illustrated in Fig. 3, the pH of MRS broth which acidified by 20 g/L lactic acid at different time. The results showed that the pH of MRS broth was increased from 3.58 to 5.42 by the capsule within 24 hours. MRS broth is commonly used for lactic acid fermentation and the desired pH in the medium is above pH 5.0 (Wenge and Mathews, 1999). Therefore, the results indicated that the capsules gave good pH control for the medium. Moreover, at the end of the experiment (24 hours), the capsules were still mechanically stable in the medium (Dembezynski and Jankowski, 2002).

Performance of liquid-core capsule to control pH of viscous solution:

Fig. 4 shows the pH values of starch solution (i.e. 1%, 5%, and 10% w/v) after acidified by 20 g/L lactic acid against time. The pH of the starch solution was generally increased by the capsules. The pH of the 1% w/v and 5% w/v starch solutions was raised by the capsules to 6.0 after 20 hours. However, the pH of the viscous 10% w/v solution only reached 3.50 after 20 hours.

In addition to starch, the performance of the capsule in 0.5 % w/v, 1 % w/v, and 2 % w/v CMC solutions was evaluated, as shown in Fig. 5. The pH of 0.5 % w/v CMC solution was well controlled to above 5.5 after 24 hours. Similar to starch solutions, the viscous CMC solutions (i.e. 1.0 % w/v and 2 % w/v) also failed to achieve pH above 5; they were regulated to around pH 3.0 only after 24 hours (see Fig. 5). The results suggested that the capsules were only capable to control the pH of viscous solution to certain extent. In viscous solutions, the movement of the capsules and the diffusivity of the solutes in the solution were restricted. Therefore, the performance of the capsules was affected in viscous solutions.

Conclusion:

In this study, a simple design of encapsulation system was developed to produce calcium carbonate loaded liquid core capsules. The effect of the capsule physical properties on pH control in different medium (i.e. lactic acid solution, MRS broth, starch solution and CMC solution) has been studied. The pH change in the tested medium was inversely proportional to the diameter and membrane thickness of the capsules. The greatest pH change rate achieved was 5.75×10^{-3} pH/min when the smallest capsules (diameter of 4.50 mm and membrane thickness of 0.81 mm) were used. The performance of the capsules in MRS broth was good and the capsules were capable to regulate pH to above 5.0 as desired for lactic acid bacteria fermentation. Moreover, the capsules could be used to regulate pH of starch and CMC solutions. However, the performance of the capsules was limited by the viscosity of the solutions.

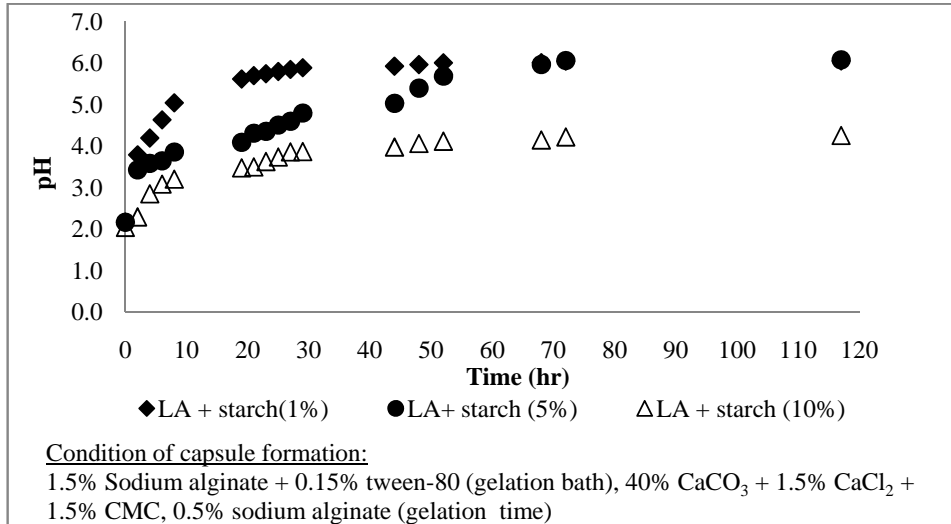


Fig. 4: pH of the starch solutions against time. The starch solutions were prepared with 20 g/L lactic acid. The capsules were prepared with 1.5% (w/v) of sodium-alginate, 1.5% (w/v) of CMC and 1.5% (w/v) of CaCl₂ concentration. The capsule diameter and membrane thickness of the capsules were 4.5 mm and 0.8 mm, respectively.

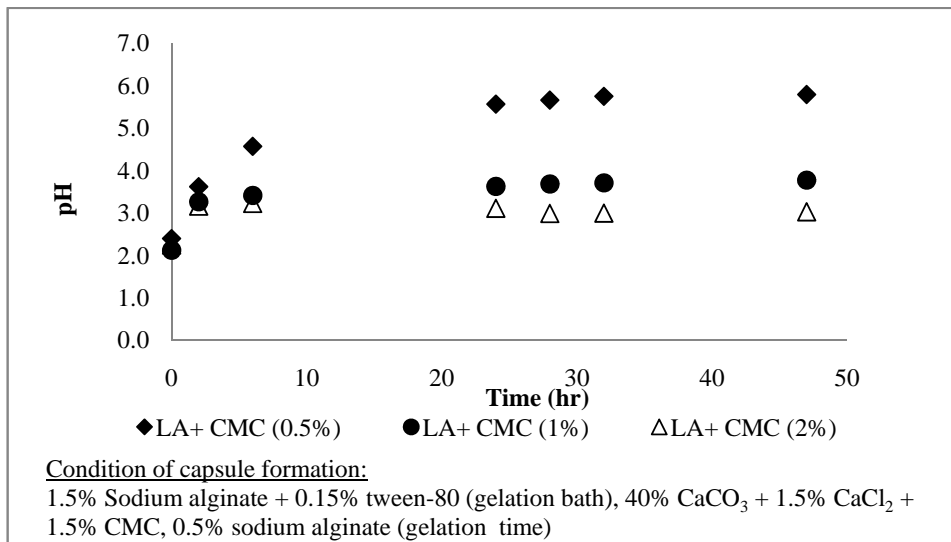


Fig. 5: pH of the CMC solutions against time. The CMC solutions were prepared with 20 g/L lactic acid. The capsules were prepared with 1.5% (w/v) of sodium-alginate, 1.5% (w/v) of CMC and 1.5% (w/v) of CaCl₂ concentration. The capsule diameter and membrane thickness of the capsules were 4.5 mm and 0.8 mm, respectively.

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