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# Studies on Encapsulation of Gas Releasing Agent in Ca-Alginate Beads and Controlled Release Pattern of the Beads

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**Abstract.** Calcium alginate beads have been used to control release gases and volatile compounds for agricultural applications, drug delivery, waste treatment, and food product enhancement. The release rate of the gas and vapor depends on the diffusion of gas and vapor through the Ca-alginate gel matrix. However, the knowledge about the effect of process variables on the diffusion rate of gas and vapor still needs to be improved. Therefore, this study aims to investigate the effect of alginate concentration, gas release agent concentration, and bead size on the concentration of gas released from Ca-alginate beads. In this study, calcium carbonate was used as the model gas release agent, encapsulated in Ca-alginate beads to release carbon dioxide when the beads reacted with an acetic acid solution. The results showed that the concentration of carbon dioxide released by the beads depended on the bead diameter. The gas release rate was increased when the diameter of the beads was increased. The total amount of gas produced by the large beads was observed to be higher than that of small beads. Compared to small beads, large beads take longer to react completely to produce carbon dioxide gas. It was also found that the amount of carbon dioxide produced by the Ca-alginate beads was inversely proportional to alginate concentration but directly proportional to calcium carbonate concentration. The results indicated that the gas diffusion rate of the Ca-alginate beads can be tailored by adjusting the process variables of the Ca-alginate bead encapsulation process.

**Keywords:** Ca-alginate Bead, Encapsulation, Gas Releasing Agent, Gas Release Kinetic

## INTRODUCTION

Sodium alginate is a naturally occurring anionic biopolymer. It is typically extracted from brown seaweed. It is often ionically cross-linked with calcium chloride to produce

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calcium alginate gel beads for various encapsulation purposes (Lee *et al.*, 2013). Encapsulation of active and sensitive materials in Ca-alginate beads is useful to protect them from harsh environments, maintain their stability and, segregate them in a continuous process, as well as control release them at a desired rate and location (Choi *et al.*, 2002 Lee *et al.*, 2013; Magdhut *et al.*, 2023; Paris *et al.*, 2020; Treesinchai *et al.*, 2019). The use of Ca-alginate beads in controlled drug delivery systems is popular because they have been proven effective, non-toxic, biocompatible, and biodegradable in human body (Zhang *et al.*, 2021).

Encapsulation of gas releasing agents in Ca-alginate beads has been getting much attention in recent years. For example, carbon dioxide gas generating agents such as carbonate and bicarbonate salt, which can generate $CO_2$ under acidic conditions to produce low-density floating drug delivery carriers (Choi *et al.*, 2002; Magdhut *et al.*, 2023; Rasel *et al.*, 2012; Russo *et al.*, 2020). Oxygen releasing agents such as calcium peroxide, and magnesium peroxide have been encapsulated in Ca-alginate beads to continuously release oxygen at a desired rate to overcome anoxia problems that occur in aerobic biodegradation of groundwater (Lee *et al.*, 2014). Ethylene gas releasing Ca-alginate gel capsules were developed to ripen bananas at a controlled rate (Liu *et al.*, 2023). Encapsulation of essential oils (i.e. extracted from lemongrass, rosemary, lavender, and cinnamon)(Cha *et al.*, 2021) in Ca-alginate beads has been reported to improve the stability and the controlled-release kinetics of the volatile compounds for antimicrobial against foodborne microorganism and food flavor enhancement (Cha *et al.*, 2021; Paris *et al.*, 2020; Petzold *et al.*, 2014, Soliman *et al.*, 2013). In short, the amount and rate of gas or vapor released from the beads must be controlled at different values required to achieve the desired purpose of an application.

The release of gas or vapor from the encapsulated gas releasing agent or volatile compounds in Ca-alginate beads can be initiated by introducing an acidic or alkaline solution, moisture, or heat to decompose the encapsulated materials (Cha *et al.*, 2021; Lee *et al.*, 2014; Magdhut *et al.*, 2023; Paris *et al.*, 2020; Petzold *et al.*, 2014; Rasel *et al.*, 2012; Russo *et al.*, 2020). The effusion of gas or vapor from Ca-alginate involves the decomposition of the encapsulated gas releasing agent or volatile compounds to release gas or vapor and subsequently the diffusion of the gas or vapor through the Ca-alginate gel matrix before the gas or vapor is released to the surrounding. Therefore, the release rate of gas or vapor is expected to vary according to the bead properties which are affected by the solution formulation and process variables of the encapsulation process. However, limited studies are conducted to investigate the correlation between the encapsulation process variables and the gas release rate of Ca-alginate beads encapsulating a gas releasing agent or volatile compounds.

This study systematically investigates the effect of encapsulation process variables on the gas release rate of Ca-alginate beads encapsulating a gas releasing agent. Calcium carbonate is selected as the model gas releasing agent, which can generate carbon dioxide when it reacts with acetic acid (Choi *et al.*, 2002; Russo *et al.*, 2020). Calcium carbonate loaded Ca-alginate beads with different sizes were produced using different concentrations of alginate and calcium carbonate and diameters of the dripping tip. The concentration of $CO_2$ released from the beads was measured using a $CO_2$ gas sensor, and the gas release pattern was reported.

## MATERIALS AND METHODS

### Preparation of Solutions

An agitator motor (IKA, Malaysia) was used to dissolve sodium alginate powder (KIMICA Corporation, Japan) uniformly in distilled water to prepare an alginate solution with concentrations of 1.5 and 2.5 % w/v. Subsequently, calcium carbonate powder (local supplier, Malaysia) was dispersed into alginate solutions under constant stirring to prepare alginate solutions containing 5.0 % and 10.0 % w/v $CaCO_3$. The sodium alginate and $CaCO_3$ mixtures were left to stand overnight to remove the entrapped air. The acetic acid (Food grade, Cool Chemicals, Malaysia) was dropped into distilled water using a dropper, and the pH of the acetic acid solution was measured using a pH meter (Hanna, USA) after each drop of the acetic acid was added until the desired pH value, i.e., pH 2 was obtained.

### Production of Gas-releasing Ca-alginate Beads

The extrusion dripping method was used to produce calcium alginate gel beads containing $CaCO_3$ as a releasing agent at room temperature, as illustrated in Figure 1. Hypodermic needles with different inner diameters (i.e., 0.6, 0.8, and 1.2 mm) were used to produce beads with different sizes. The sodium alginate and $CaCO_3$ mixtures were extruded through a hypodermic syringe (Terumo, Japan) into a gelation bath made of 1.5% w/v calcium chloride (Bendosen, Norway). The $CaCl_2$ gelation bath was placed on a magnetic stirrer and stirred at 150 rpm. The distance between the dripping tip and the gelation bath was set at 0.10 m. Lastly, the gel beads were left in the bath for 30 minutes to ensure they were uniformly gelled.

### Measurement of Ca-alginate Bead Diameter

After gelation, 30 samples of $CaCO_3$ encapsulated Ca-alginate beads were collected from the gelation bath. A digital camera (Huawei, China) was used to capture the images of the samples. Then, the diameter of the samples was determined by an image analyser (ImageJ, USA) (Chan *et al.*, 2009; Lee *et al.*, 2013).

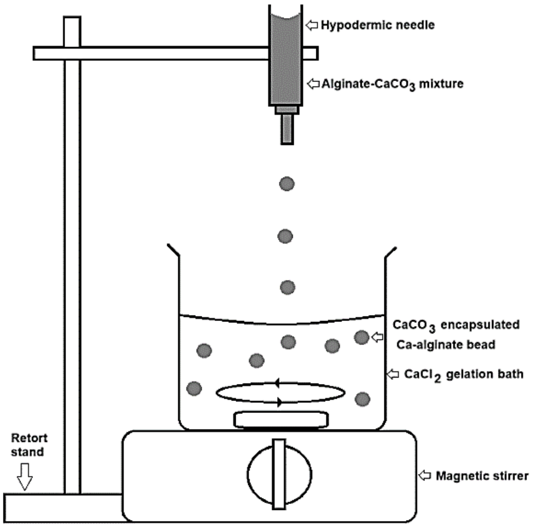

**Fig.1:** Setup of extrusion dripping method to produce calcium carbonate (gas releasing agent) encapsulated Ca-alginate beads

### Measurement of Carbon Dioxide Gas Concentration

A total of 300 Ca-alginate beads were prepared for each experimental run and transferred into a glass universal bottle. Approximately 20 ml of acetic acid solution (pH 2) was poured into the universal glass bottle filled with Ca-alginate gel beads. It was immediately placed inside a desiccator along with a $CO_2$ gas detector (Henan Inte Electrical

Equipment Co. Ltd., China) (Fortunato *et al.*, 2023). The $CO_2$ gas concentration measurement was conducted at room temperature. The reading of the $CO_2$ concentration continued until the value shown on the detector screen was constant.

### Statistical Analysis

The average diameter of $CaCO_3$ encapsulated Ca-alginate beads was determined based on the measurement of 30 gel beads. All standard error bars in the graph represent the standard deviation calculated using a 95% confidence interval using a t-statistic.

## RESULTS AND DISCUSSION

### Effect of Process Variables on Bead Diameter

Figure 2 (a) and (b) respectively show the average diameter of the calcium carbonate encapsulated calcium alginate gel beads that are produced using 5.0% w/v and 10% w/v $CaCO_3$ and different diameters of dripping tip and concentrations of alginate solution.

#### *Effect of Dripping Tip Diameter on Bead Diameter*

The average diameter of the gel beads was increased from 2.37 mm to 2.77 mm and from 2.52 mm to 3.02 mm as the diameter of the dripping tip was increased from 0.6 mm to 1.2 mm, for 1.5% w/v and 2.5% w/v of alginate concentration, as shown Figure 2 (a) and (b), respectively. The results can be explained based on Tate's law. A sodium alginate-$CaCO_3$ mixture droplet detaches from the dripping tip orifice when the gravitational force (its droplet volume or weight) overcomes the surface tension force of the solution (Lee *et al.*, 2009). The surface tension force of the solution is proportionally increased as the dripping tip diameter increases (Lee *et al.*, 2009). Therefore, the droplet volume or weight detached from the dripping tip is also increased to overcome the increased surface tension force as the dripping tip diameter is increased. Consequently, the diameter of the gel beads after gelation is also increased, as observed by previous researchers (Chan *et al.*, 2009; Russo *et al.*, 2020; Takka and Acarturk, 1999).

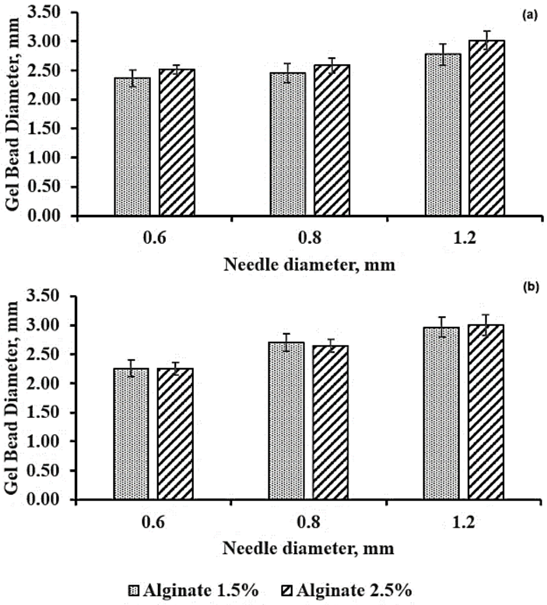

**Fig. 2:** Diameter of calcium carbonate (gas releasing agent) encapsulated Ca-alginate beads produced using different needle diameters and alginate concentrations, where the concentration of encapsulated $CaCO_3$ was (a) 5.0% w/v, and (b) 10.0% w/v

#### *Effect of Alginate Concentration on Bead Diameter*

Figure 2 shows the average diameter of $CaCO_3$ encapsulated Ca-alginate gel beads produced using different dripping tip diameters for alginate concentrations of 1.5% w/v and 2.5% w/v. In general, the average diameter of the gel beads was not significantly affected by the alginate concentration. In this study, the mass ratio of alginate to $CaCO_3$ was low, between 0.15 and 0.50. Therefore, the shrinkage of the gel beads was not apparent, although the gel density was increased during the cross-linking gelation process (Chan *et al.*, 2009). However, the diameter of $CaCO_3$ encapsulated beads was reported to be dependent on alginate concentration after the beads were left for one hour of curing in a gelation bath and overnight air drying in a previous study (Magdhut *et al.*, 2023).

### *Effect of Calcium Carbonate Concentration on Bead Diameter*

As illustrated in Figure 2, the average diameter of Ca-alginate gel beads produced using 5.0% w/v and 10% w/v $CaCO_3$ was not significantly different for a particular dripping tip and alginate concentration. The results did not agree with those of previous studies, where the gel bead diameter was not significantly increased when the concentration of $CaCO_3$ in Ca-alginate gel beads was increased (Choi *et al.*, 2002; Russo *et al.*, 2020). The reason is the mass ratio of $CaCO_3$ to alginate of the gel beads produced in this study is higher than that of previous studies. Hence, the high amount of inert $CaCO_3$ in the alginate solution has no apparent influence on the cross-linking gelation process of the beads.

### Effect of Process Variables on Carbon Dioxide Release Pattern of Gel Bead

Figures 3 and 4, respectively show the carbon dioxide concentration-time profiles of the Ca-alginate gel beads encapsulating 5.0% w/v and 10% w/v $CaCO_3$ that are being produced using different dripping tips and alginate concentrations.

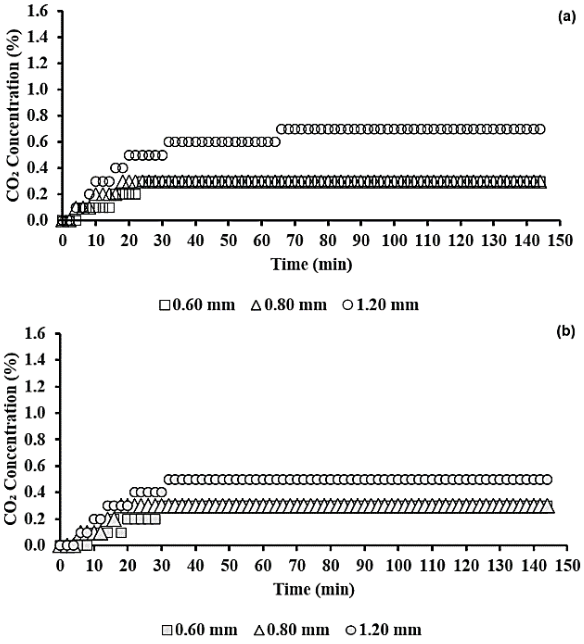

**Fig. 3:** Concentration of carbon dioxide released from calcium carbonate (5.0% w/v) encapsulated Ca-alginate beads produced using different dripping tip diameters and alginate concentrations (a) 1.5% w/v, and (b) 2.5% w/v

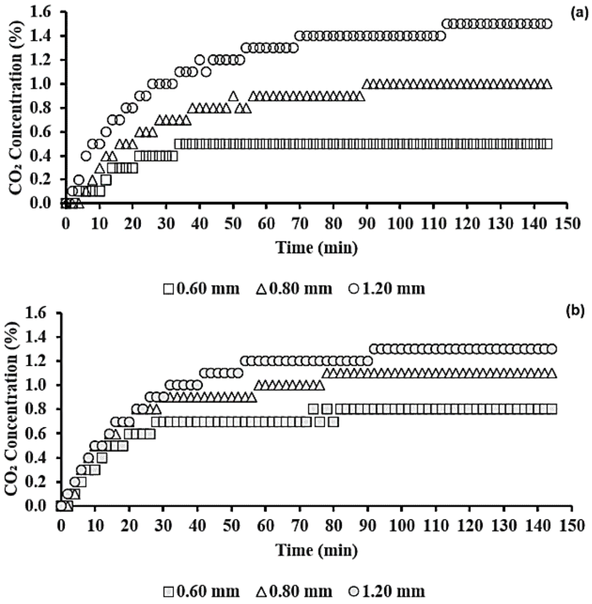

**Fig. 4:** Concentration of carbon dioxide released from calcium carbonate (10.0% w/v) encapsulated Ca-alginate beads produced using different dripping tip diameters and alginate concentrations (a) 1.5% w/v, and (b) 2.5% w/v

### *Effect of Dripping Tip Diameter on Carbon Dioxide Release Rate*

In both cases, the total amount of $CO_2$ effused from the gel beads was increased when the diameter of the dripping tip was increased, as shown in Figures 3 and 4. It is observed that the small Ca-alginate gel beads (i.e., produced from the dripping tip of 0.60 mm diameter) used a shorter time to reach equilibrium $CO_2$ concentration, as compared to that of the larger gel beads. Under the tested conditions, the concentration of $CO_2$ released from the small gel beads was lower than that of the large gel beads. This could be due to the amount of $CaCO_3$ encapsulated in the gel beads being proportional to the bead size; hence the amount and rate of $CO_2$ released from the gel beads are also increased when bead size is increased.

### *Effect of Alginate Concentration on Carbon Dioxide Release Rate*

The concentration of $CO_2$ effused out from $CaCO_3$ encapsulated Ca-alginate gel beads that were produced using 1.5% and 2.5% w/v alginate was presented in Figures 3 and 4. The gel beads formulated using 1.5% w/v alginate concentration released higher concentrations of $CO_2$ than those formulated using 2.5% w/v alginate concentration. The results showed that the amount and rate of $CO_2$ gas released from the gel beads were inversely proportional to the alginate concentration. The decrease in the rate and amount of $CO_2$ gas released from the gel beads was reported in a previous study (Rasel and Hasan, 2012). This is because the increase of polymer concentration in the gel bead has increased the density of the polymer matrix and the diffusional path length of gases through the polymer matrix (Rasel and Hasan, 2012).

### *Effect of Calcium Carbonate Concentration on Carbon Dioxide Release Rate*

Calcium carbonate reacts with acetic acid and releases carbon dioxide gas. Hence, it is expected that the amount of $CO_2$ gas released from the $CaCO_3$ encapsulated increases when the concentration of $CaCO_3$ is increased. This data trend was observed in the study results as shown in Figures 3 and 4. Regardless of dripping tip diameter and alginate concentration, the gel beads formulated using 10.0% w/v $CaCO_3$ concentration generated a higher concentration of $CO_2$ gas than that of the gel beads formulated using 5.0% w/v $CaCO_3$ concentration. Similar results were obtained in the floating gel beads study, where more $CO_2$ gases were generated to prolong the floating time of the gel beads when the $CaCO_3$ concentration was increased (Russo *et al.*, 2020).

## CONCLUSIONS

The extrusion dripping method was developed to encapsulate $CO_2$ releasing agents in Ca-alginate beads. The effect of process variables of the encapsulation setup on the properties and release pattern of the $CO_2$ releasing agent beads was investigated. The diameter of the gel bead was increased when the diameter of the dripping tip was increased regardless of the concentration of alginate and $CaCO_3$. However, the diameter of the gel bead was not significantly affected by the concentration of alginate and $CaCO_3$. On the other hand, the concentration of $CO_2$ gas released from the gel bead produced using different solution formulations and bead sizes was measured. The results showed that the amount and rate of $CO_2$ gas released from Ca-alginate beads were directly proportional to the bead diameter and $CaCO_3$

concentration. However, the effect of the alginate concentration on the amount and rate of $CO_2$ gas released from Ca-alginate beads presented an opposite data trend. In the future, different dissolution models will be applied and analyzed to $CO_2$ gas release data to evaluate the $CO_2$ gas release mechanisms and kinetics. This is because the knowledge of the effusion of $CO_2$ gas through Ca-alginate gel matrix is important in the design of floating drug delivery systems, food packaging films, and anaerobic bioprocesses.

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