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Encapsulation of Extracted Oil from *Mentha piperita* in Alginate Beads

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Abstract

Encapsulating essential oils derived from traditional medicinal flora within alginate beads represents an up-and-coming technique for enhancing their stability, bioavailability, and controlled release properties. In this study, we employed a Clevenger apparatus to extract the essential oil from *Mentha piperita*. The hydrodistillation process of *M. piperita* yielded an essential oil extraction of 0.27 ± 0.05%. The ionic gelation methodology facilitates the cross-linking of alginate with Calcium chloride, forming a gel-like matrix that effectively encapsulates essential oil droplets within stable, gelled beads. The essential oil-loaded beads were quantified spectrophotometrically at a wavelength of 340 nm (λ_{max}). Furthermore, we evaluated and characterized the beads for size, weight analysis, sphericity, swelling behavior, dissolution kinetics, pH stability, drying rate, and accelerated stability studies. The size of alginate beads exhibited a significant increase concomitant with an elevation in sodium alginate concentration. The release profile of the oil content demonstrated a more sustained and regulated release within a phosphate buffer at pH 6.8 as opposed to that observed in 0.1N HCl.

Keywords: *Mentha piperita*; Encapsulation; Alginate beads; Essential Oil; Ionic gelation.

Abbreviations: SA: Sodium Alginate

INTRODUCTION

Nepal is renowned for its rich herbal diversity, housing over 1,624 species of medicinal and aromatic plants, making it a valuable source of bioactive compounds with therapeutic potential (Patel et al., 2021). Ethno-medicine, a traditional medical practice based on indigenous knowledge passed down through generations, has gained international recognition due to its contributions to modern drug discovery (Chhetri et al., 2020). Extracting bioactive compounds from these plants is a growing interest as researchers seek to harness their therapeutic properties and integrate them into modern drug delivery systems (Mirghani et al., 2012). Essential oils, known for their aromatic and medicinal properties, are among the most valuable products derived from plants. However, they are prone to instability and degradation due to environmental factors such as temperature, light, and oxidation, which reduces their effectiveness and shelf life (Maurya et al., 2021). Encapsulation technology has emerged as a promising solution, mainly using alginate beads to encase essential oils (Hong & Park, 1999). Alginate, a biopolymer derived from brown seaweed, forms a gel-like structure when cross-linked with calcium ions, making it an ideal medium for protecting essential oils from environmental degradation (Mumper et al., 1994). This method also allows for the controlled release of the oils, extending their therapeutic benefits. The ionic gelation technique used in this study offers an efficient means of encapsulating essential oils within alginate beads, optimizing their stability and bioavailability (Pisoschi et al., 2018). The primary objective of this research is to prepare alginate beads encapsulating essential oils from medicinal plants. The study aims to enhance the oils stability and effectiveness in medical applications (Turchiuli et al., 2005). The rationale behind this work is rooted in the increasing demand for natural medicines and the growing interest in plant-based treatments, which offer potential therapeutic benefits with fewer side effects compared to synthetic drugs. Despite the promising potential of essential oils, a significant challenge lies in the lack of standardized procedures for encapsulating these oils and the limited understanding of how the oils interact with the alginate matrix. Moreover, the long-term stability of encapsulated oils is uncertain, which poses a barrier to their practical application in medicine.

In this study, we extracted essential oils from *Mentha piperita* using a Clevenger apparatus. The oils were then encapsulated in alginate beads formed through the ionic gelation technique using sodium alginate and calcium chloride. Various factors, such as the beads' size, shape,

and swelling behavior, were evaluated under different pH conditions to simulate the gastrointestinal environment. These experiments aimed to determine how well the encapsulated oils would release in controlled conditions, which is crucial for their potential use in oral drug delivery systems.

Overall, this research demonstrates the potential of alginate beads as a viable method for encapsulating and stabilizing essential oils. The findings could pave the way for developing plant-based drug delivery systems that bridge traditional medicinal practices with modern pharmaceutical technologies, offering new avenues for using natural compounds in therapeutic applications.

MATERIALS AND METHODS

Collection and Identification of plant

M. piperita was collected from different places in Syangja and Kaski districts, Western Nepal, in March 2024 for verification. Furthermore, fresh plants were collected during the extraction process. The plants were identified in the National Herbarium and Plant Laboratories (NHPL), Kathmandu.

Essential oil extraction

The oil extraction process used a Clevenger apparatus. Fresh leaves of *M. piperita* weighing 400 g were collected. The hydro-distillation process involved using a fresh sample at 50°C. In a 1 L flask, 100 g of *M. piperita* leaves, about 2-4 cm long, were mixed with 500 ml of distilled water. The flask was connected to the Clevenger apparatus and heated until it reached boiling point. As the steam formed, it passed through a condenser, which was kept cool by a continuous flow of water. This caused the steam to condense back into liquid form. The essential oil was then separated from the water because the two liquids had different densities.

Essential oil (EOs) loaded beads

The extracted essential oil was first combined with three different concentrations of sodium alginate (SA) solution (Table 1) on a magnetic stirrer to make the core solution, which was then used to create EOs-loaded alginate beads. This mixture ensures uniform distribution of the essential oil within the alginate matrix. The 1% calcium chloride ($CaCl_2$) solution was put into a beaker in the first gelling bath. A timer was set to ensure consistency in the gelation process. The essential oil-containing core solution was fully drawn into a pipette and was held 10 cm above the surface of the calcium chloride solution using a ruler to ensure consistency. Droplets of the core solution were then carefully released into the calcium chloride bath, with the pipette being moved to a new spot after each drop to prevent fusion of the forming beads. After 10 min for the beads to form, they were removed from the calcium chloride bath using a muslin cloth. The beads were then transferred to a 2% calcium chloride solution to harden, separated from the solution with a sieve, and placed on a paper towel. To enhance bead purity, they were gently rinsed with distilled water. Depending on the experiment's needs, the EO-loaded alginate beads were analyzed immediately or air-dried for 24 hours before further evaluation.

Table 1. Preparation of SA and $CaCl_2$ solution.

SA Concentration	SA Powder (g)	Distilled water (ml)
1%	1	100
2%	2	100
3%	3	100
$CaCl_2$ Concentration	$CaCl_2$ Powder (g)	Distilled water (ml)
1%	1	100
2%	2	100

Size, weight, and sphericity analysis of the beads

The diameter of beads before and after drying was determined with a Vernier caliper at three different positions for each bead with an accuracy of ± 0.01 mm. The mean diameter of 12 beads was calculated (Lai et al., 2007). Additionally, a digital balance determined the average weight of twelve beads before and after drying. The mean weight was calculated using three separate determinations to represent the bead mass accurately.

The sphericity factor (SF) (Bayu) was used to indicate the bead where the value zero indicates a perfect sphere and higher values indicate a greater degree of shape distortion (Chan, 2011). SF was calculated as:

$$SF = \frac{Dmax - Dmin}{Dmax + Dmin} \quad (1)$$

Where, D_{max} = maximum diameter and D_{min} = minimum diameter

Swelling test

The beads' swelling rate of the beads was measured at different pH. Several beads (3-4 mg) were placed in 6.8 pH phosphate buffer and 0.1N HCl (1.2 pH) solutions at 37°C. The beads were removed and weighed at different time intervals after drying the excess water using filter papers. Their weight changes were measured during swelling. The percent swelling ratio (ΔD_t) was calculated using Equation 2 (Gallo et al., 2020).

$$\Delta Dt\ (\%) = \frac{Dt - Do}{Do} X100 \quad (2)$$

Where, D_t = diameter after time t, D_0 =initial bead diameter

Oil content release

The release profile of EOs-loaded calcium alginate beads was assessed using a USP Type II dissolution apparatus. The experiment was carried out in 900 ml of phosphate buffer with a pH of 6.8, maintained at 37 ± 0.5°C, and stirred at 50 rpm to mimic basic conditions. It was also conducted in 0.1 N HCl to simulate an acidic

environment. Samples were collected and analyzed at specific intervals using a UV spectrophotometer to measure the oil concentration. The release kinetics were tracked to calculate the percentage of oil released. The oil released into the different pH solutions was measured at each time point, and the release percentage was calculated using Equation 3.

$$\text{Percent Release} = \frac{\text{Amount of oil Released}}{\text{Total Amount of EOs-loaded beads}} \times 100 \quad (3)$$

pH stability

The beads may show reactions undergoing varying pH conditions in our body fluids. That is why, to evaluate the stability of the EOs-loaded alginate beads under varying pH conditions, the beads were subjected to two distinct physiological environments: a phosphate buffer solution at pH 6.8 and a 0.1 N HCl solution (pH 1.2). These conditions were selected to simulate the pH environments encountered in different segments of the GI tract.

The phosphate buffer at pH 6.8 was chosen to mimic the slightly acidic to neutral conditions in the small intestine. At the same time, the 0.1 N HCl solution approximated the highly acidic environment of the stomach.

Drying rate study

The oil-loaded beads were dried using two different methods: freeze drying and oven drying. Freeze drying was chosen for the experiment due to its superior ability to preserve the oil-loaded alginate beads structural integrity and bioactive properties. Unlike conventional drying methods, freeze drying operates by sublimating ice directly into vapor under low temperatures and vacuum conditions, effectively minimizing the thermal degradation of heat-sensitive components. For the freeze-drying process, the beads were arranged on drying trays and subjected to freeze-drying under a vacuum pressure of 1 pa at a condenser temperature of −79.5°C for 1 hour. An oven drying method was chosen for the experiment due to its capability to provide controlled and consistent drying conditions. The initial size of the freshly prepared beads was recorded. Drying rate studies were conducted by exposing the EOs-loaded alginate beads to a constant temperature of 37°C, and changes in structure were analyzed at intervals of 5 minutes using a drying rate curve (Lai et al., 2007).

Stability Studies

To conduct this study, the dried beads were stored in a closed plastic container and borosilicate glass at room temperature and in a refrigerator. We observed the physical appearance and stability regularly throughout the three months. This long-term stability assessment aimed to understand how well the beads retained their encapsulated essential oil.

Statistical Analysis

Data analysis was performed using the Microsoft Excel software package. Results are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Extraction Yield Value

Hydro distillation of *M. piperita* resulted in an essential oil yield of 0.27 ± 0.05% from a 400 g plant sample. The extracted oils were successfully encapsulated within alginate beads. The plant materials intrinsic oil content and composition play a significant role in yield. Additionally, the time and place of collection can impact oil content, as essential oil concentrations fluctuate based on the plant's growth stage, seasonal changes, and environmental conditions specific to the collection location. Although steam distillation does not use solvents, the quality of steam or water can influence extraction efficiency. Variations in the distillation apparatus, such as the type and efficiency of the equipment, as well as differences in the design and precision of the distillation unit, can also affect the yield. Finally, factors such as the duration and temperature of the distillation process are crucial; inadequate distillation time or incorrect temperature settings can lead to incomplete extraction of essential oils.

Morphological and structural characterization

In this study, the shape and size focusing on the sphericity of the formulated beads were determined. It was found that bead size and shape were significantly affected by the sodium alginate concentration. Increasing the concentration of sodium alginate (SA) leads to a larger bead size, as shown in Figure 1.

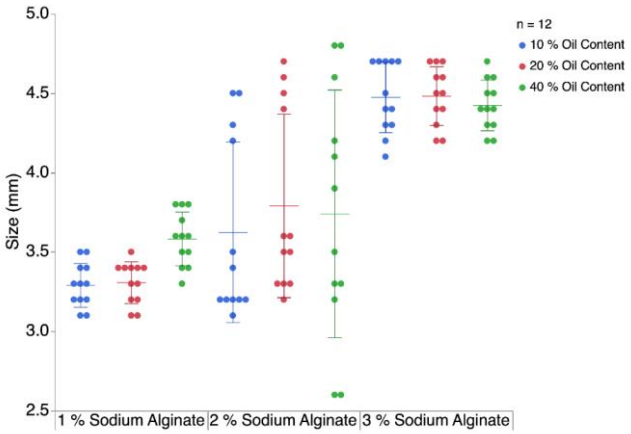

Figure 1. Size analysis of EOs-loaded beads.

The effects of alginate concentration and oil loading on the shape of the ca-alginate beads after oil encapsulation were analyzed, too. Figure 2 shows the typical size and shape of the beads in different sodium alginate concentrations.

Figure 2. Representing alginate beads in different concentration.

The sphericity factor (threshold <0.05) confirmed that beads prepared with 3% alginate were perfectly spherical, even at 40% oil loading, while those with 1–2% alginate were irregular (sphericity >0.05). Higher oil loads (10–40%) in 3% alginate further improved sphericity, with 40% oil yielding the most spherical beads. In contrast, 1–2% alginate produced tear or pear-shaped beads with tails, regardless of oil content. Thus, 3% alginate is optimal for achieving uniform, spherical beads as shown in figure 3.

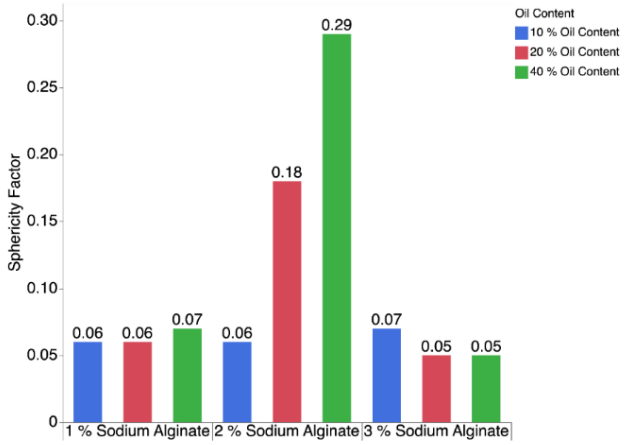

Figure 3. Sphericity Factor of EOs-loaded alginate bead

Swelling behavior

The swelling ratio (ΔD_t), calculated with a basis of the diameter variation using Equation (2), was greatly affected by the time of immersion in the liquid medium simulating a marinating solution, as well as by the production process of the alginate beads. After 24 hr of immersion at average room temperature, the beads showed their distinct swelling nature in the liquid medium.

Table 2. Swelling behavior of *Mentha piperita* EOs loaded alginate beads.

	Oil content					
	10%		20%		40%	
SA Concentration	Initial diameter (mm)	Final diameter (mm)	Initial diameter (mm)	Final diameter (mm)	Initial diameter (mm)	Final diameter (mm)
1%	4.9	-	3.8	-	3.5	3.6
2%	4.2	4.44	4.5	4.6	4.2	4.3
3%	4.77	4.9	4.8	4.9	3.3	4.4

Oil content release

The graph (Figure 4) illustrates the comparative oil release profiles of the formulated drug in two different media: a 6.8 pH phosphate buffer and 0.1N hydrochloric acid (HCl), for 30 min. The y-axis represents the percentage of cumulative oil released, while the x-axis represents the time in minutes. The data reveals that drug release occurs more rapidly in a 0.1N HCl (1.2 pH) environment during the initial time, indicating a higher release rate in acidic conditions. However, after this initial phase, the release rates in both environments become comparable, with the 6.8 pH phosphate buffer showing a slightly more sustained release from 10 min. This suggests that while the oil can be released in both acidic and neutral environments, it appears more controlled and sustained in the 6.8 pH phosphate buffers. This could indicate that the beads formulation is better suited for environments closer to neutral pH, such as the small intestine, where a more controlled and prolonged release might be beneficial for maintaining therapeutic levels over an extended period. To achieve controlled and sustained drug release, it is essential to consider factors such as the choice of a polymer matrix, the degree of cross-linking in bead formulations, and the physical characteristics of the beads (e.g., size and porosity). For future studies, optimizing these parameters can further

improve the drug release profile, ensuring a more targeted and effective delivery in the desired pH environment.

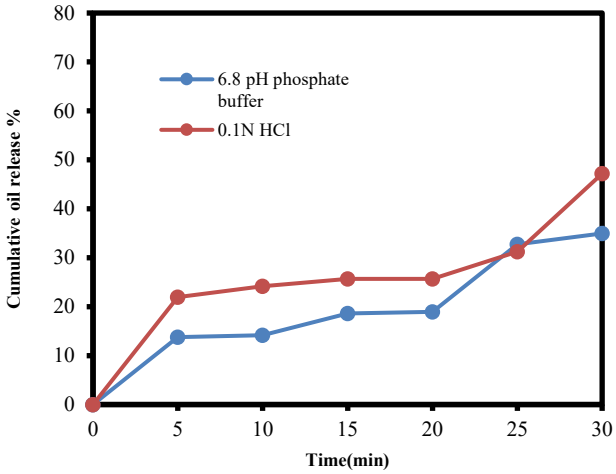

Figure 4. Cumulative oil release % of Ca-alginate beads.

pH stability

The alginate beads loaded with essential oils (EOs) degraded in this acidic environment, suggesting that the beads are unstable for extended periods in conditions simulating the gastric environment. The beads remained stable in this near-neutral pH environment, indicating good stability in conditions simulating the small intestinal environment. The degradation in acidic conditions suggests that the alginate matrix is susceptible to acid hydrolysis, which leads to premature release of the encapsulated EOs in the stomach. The stability at pH 6.8 indicates that the beads could maintain their integrity in the small intestine. This suggests the potential for targeted delivery to the intestinal region.

Drying Rate

The freeze-dried alginate beads felt dry and non-oily to the touch unless squeezed, which released the trapped oil. They were also free-flowing, likely due to their porous structure holding the oil inside. This suggests that there was very little free oil on the surface. We also observed the physical properties of hot air-dried particles, noting and comparing them with those of naturally dried and freeze-dried beads. We found that oven-drying caused the oil to leak from the oil-loaded wet beads during the drying process. As a result, the drying trays and beads were oily and sticky after drying. Freeze-drying tends to cause small ruptures in the beads, leading to many smaller particles.

Table 3. Comparison of drying methods.

Drying method	Oil Loading	Diameter (mm)		Sphericity Factor
		Initial	Final	
Oven Drying	20%	2.8	1.9	0.19
	40%	3.4	2.7	0.11
Freeze Drying	20%	3.1	2.8	0.05
	40%	3.2	2.9	0.04

n=1, nozzle diameter= 0.56 mm

The data presented compares the drying rates of alginate beads subjected to two different drying methods: oven drying and freeze-drying at varying levels of oil loading. It is observed that the drying method and oil loading significantly affect the beads final diameter and sphericity factor. For beads with an initial oil loading of 20%, the diameter decreased from 2.8 mm to 1.9 mm under oven drying, while it reduced from 3.1 mm to 2.8 mm under freeze drying. Similarly, at 40% oil loading, the diameter decreased from 3.4 mm to 2.7 mm with oven drying and from 3.2 mm to 2.9 mm with freeze drying.

The drying method also influences the sphericity factor, which measures how close the shape of the beads is to a perfect sphere. A lower sphericity factor indicates a more deformed shape. For oven drying, the sphericity factor at 20% oil loading is 0.19; at 40% oil loading, it is 0.11, indicating a significant deviation from sphericity. In contrast, freeze-dried beads show much lower sphericity factors of 0.05 and 0.04 for 20% and 40% oil loading, respectively. This suggests that freeze-drying preserves the spherical shape of the beads better than oven-drying.

The differences in drying rate between the two methods can be attributed to the different mechanisms involved. Oven drying, which involves the removal of water by heat, tends to cause more shrinkage and deformation due to the relatively rapid moisture loss and potential overheating. Freeze drying, on the other hand, involves sublimation of water at low temperatures, a gentler process that better preserves the structural integrity of the beads, resulting in less shrinkage and higher sphericity.

In summary, freeze-drying is more effective in maintaining alginate beads shape and size than oven drying, which leads to more significant shrinkage and deformation. Therefore, the choice of drying method has significant implications for the physical properties of alginate beads, particularly in applications where the shape and size are critical.

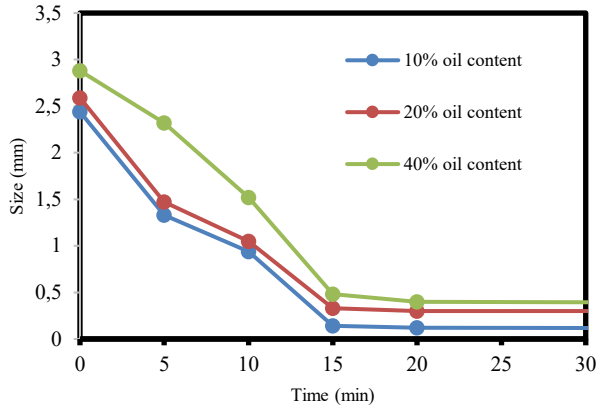

Figure 5. Drying Rate of *Mentha piperita*

The drying rate curve shows how the size of samples with different oil contents (10%, 20%, and 40%) decreases over time during the drying process. Initially, all samples experience a rapid size reduction, indicating a high drying rate as surface moisture is quickly lost. The sample with 10% oil content shows the fastest decrease, suggesting that lower oil content leads to quicker moisture loss and more significant shrinkage. As drying continues, the size reduction rate slows down, particularly for samples with higher oil content, which acts as a barrier to moisture loss. In the final phase, the size of the samples stabilizes, with the 10% oil content sample reaching the smallest final size. Overall, higher oil content slows the drying process and helps retain the sample size better, highlighting the impact of oil content on drying behavior.

Discussion

The research results demonstrate the strong influence of alginate concentration and the encapsulation process on the formation and stability of essential oil-loaded beads. The increased bead size with higher sodium alginate concentration can be attributed to the more extensive cross-linking with calcium ions, forming a denser network that traps the oil more effectively. This interaction explains why beads at higher concentrations maintain their spherical shape and structural integrity compared to lower concentrations. The spherical shape in 3% alginate is likely due to the balance between surface tension and gravitational forces during droplet formation, stabilizing the bead structure upon contact with the calcium chloride solution (Chan, 2011).

The release behavior of the oil-loaded beads also provides insight into the pH-dependent stability of alginate beads. The faster degradation in acidic conditions is caused by acid hydrolysis of the alginate matrix, which weakens the gel structure and results in quicker release of the encapsulated oil. This explains why beads showed more controlled and sustained release in the neutral pH, where the alginate matrix remains intact for extended periods. The role of the encapsulation matrix in regulating oil release is crucial, especially for targeted drug delivery systems designed for the intestinal environment.

The drying methods further clarify the effects on bead morphology and integrity. Freeze drying preserved the bead structure due to its gentle sublimation process, minimizing shrinkage and maintaining the original spherical shape. The structural integrity is consistent with those reported by (Gallo et al., 2020) who observed a similar reduction in particle size and increased surface irregularities, which enhanced the surface area and accelerated the release rate of the encapsulated compound. In contrast, oven drying led to oil leakage and bead deformation, likely because the faster moisture loss from heat caused the matrix to collapse before the oil could stabilize within the beads. Thus, freeze drying's lower impact on bead structure validates its superiority for preserving essential oil-loaded beads.

CONCLUSIONS

Encapsulation of *M. piperita,* an essential oil in Ca-alginate beads, was successfully achieved using varying concentrations of sodium alginate and oil loadings. The alginate-oil emulsion, with oil loadings up to 40% in 3% sodium alginate solution, remained stable, and the oil-loaded, wet beads were spherical and well-formed. After freeze-drying and oven-drying, the oil content remained high, with freeze-dried beads being non-oily, free-flowing, and maintaining their spherical shape. In contrast, the oven-dried beads were oily and deformed, indicating a loss of structural integrity during the drying process, making freeze-drying the preferable method for preserving bead structure and functionality.

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Authors Contributions: This manuscript stems from a bachelor's thesis project. PP designed the study and provided supervision throughout the research. KS, MK, and AK collected the plant samples and prepared the herbarium, with KS and MS verifying the herbarium and conducting the data analysis. KS, MK, and AK performed the laboratory experiments and drafted the manuscript. KS and PP reviewed the draft and made the final revisions. All authors have read and approved the final version of the manuscript.

Competing Interests: The authors declare no conflicts of interest for this study.

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