Palmarosa essential oil encapsulated in chitosan nanoparticles by ionotropic gelation: formulation and characterization

G H Nguyen1,2, X T Le1,2*

1Department of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City, Vietnam
2Vietnam National University Ho Chi Minh City, Ho Chi Minh City, Vietnam
*Corresponding author’s e-mail: tien.le@hcmut.edu.vn

Abstract. In this study, chitosan nanoparticles containing palmarosa essential oil (PEO-CNPs) were formed by ionotropic gelation, consisting of two parts: emulsion preparation followed by ionotropic gelation encapsulation with tripolyphosphate ions (TPP) as a crosslinker. The encapsulation method was optimized by varying three parameters, including chitosan concentration, initial oil loading in the emulsion and TPP concentration. The effects of these parameters on the encapsulation efficiency (EE) and loading capacity (LC) were analyzed. EE had an initial increase followed by a decrease in the range of three parameters. However, LC rose with varying initial oil content while it reduced with changing polymer and TPP concentration. The optimum experiment with the highest EE (10.0 g/L of chitosan, 5.0 g/L of TPP and 30.0 g/L PEO) was chosen to analyze the particle size using Dynamic Light Scattering method (DLS). With DLS measurement, the z-average diameter was 235.3 nm, and the particle size distribution was in the range of 100 – 500 nm.

1. Introduction

Palmarosa essential oil (Cymbopogon martinii) belongs to Poaceae family, which is firstly found in India and Turkey [1]. The chemical composition of palmarosa essential oil depends on harvesting time, seasons, maturation, … [1]. One of the main compositions of palmarosa essential oil is geraniol, which is an acyclic monoterpene alcohol [2]. Possessing geraniol helps this essential oil to have many biological activities, including insecticide and repellent, antioxidant, antimicrobial, … [2].

Essential oils are aromatic and active compounds harvested from a variety of plants. They are interested in many fields of science and industry because of their biological applications, such as antibacterial, antioxidant, antifungal, etc., which can be found in some essential oils like oregano, clove, cinnamon, thyme, … [3], [4]. However, most of them are volatile substances and easily decomposed by external factors, such as oxygen, light, moisture, temperature, …[3]. These disadvantaged properties prevent the direct applications of essential oils in industry, therefore, there needs to be a way to protect these natural compounds in different applications [3].

Encapsulation is a considerable technique, which creates microtechnological or nanotechnological carriers to protect many active compounds and guaranteed slow release over time [3]. In encapsulation, the carrier system can be produced from various wall materials, which can be an individual polymer (sodium alginate [4], pectin [5], chitosan [6], …) or a combination of two polymers (pectin–sodium alginic [7], chitosan–gum arabic [8], …). Chitosan is a polysaccharide derived from chitin, which is obtained by the deacetylation process. Chitosan has been used in many applications like agricultural, wastewater treatment, medical, food industry, … because of its
biodegradability, biocompatible and non-toxic [9]. Chitosan nanoparticles can be obtained by various methods and one suitable method is ionotropic gelation, which is non-solvent, non-toxic, cheap, and high efficiency [10]. This method depends on the cross-link bond between the ionic polymer and oppositely ionic charged ion to produce microparticles or nanoparticles.

Figure 1. The structure of chitosan nanoparticles.

The aim of this study is to develop systems based on chitosan nanoparticles by crosslinking with tripolyphosphate (TPP) ions. The method used in this study is oil-in-water emulsification followed by ionotropic gelation to encapsulate palmarosa essential oil (showed in Figure 1). By changing the amount of polymer, crosslinker, and oil, the effects of all variations on the encapsulation efficiency and loading capacity are reported here.

2. Materials and methods

2.1. Materials

Chitosan (made from King crab) with deacetylation degree of 95% was purchased from Tin-Cay, Vietnam. Glacial acetic acid was obtained from XiLong, China. Tween 80 was purchased from Croda International, England. Hexane was come from Chemsol, Vietnam.

The palmarosa essential oil was steam-distilled from *Cymbopogon martinii* leaves, which were collected in May at the North of Vietnam, with fully mature spikelet stage showing brown inflorescence. From a tone of leaves, the obtained product could gain about 4 kilograms of raw essential oil. The raw essential oil was purified by distillation process with prismatic spiral packing at 60 mmHg and 150 °C. The main compositions of obtained product were 71.35% geraniol and 22.32% geranyl acetate.

2.2. Preparation of palmarosa essential oil encapsulated chitosan nanocapsules

PEO-loaded chitosan particles were prepared by a modified method (showed in Figure 2) from previous reports [6], [11]. Chitosan solution (100 mL) was prepared by dissolving chitosan in aqueous acetic acid solution (1 % v/v) at ambient temperature until the solution was transparent. The palmarosa essential oil was added dropwise to the chitosan solution followed by 500 g of Tween 80 at ambient temperature. The mixture was agitated at constant speed of 1200 rpm for 30 min to obtain an emulsion. After formation of a fine homogenization, TPP solution was added dropwise into the chitosan/oil emulsion by using a Syringe pump KD Scientific 100 with agitation at 800 rpm for 60 min. The cross-linked nanoparticles were collected by centrifugation for 30 min at 3000 rpm and ambient temperature. The supernatant liquid and nanoparticles were stored at 4 °C until use.
2.3. Determination of encapsulation efficiency and loading capacity
The free oil in the supernatant emulsion was collected by liquid-liquid extraction using 50 mL of hexane, 3 times. Then, a rotary evaporation was carried out at 35 °C to obtain the free oil. Then, the amount of free oil was determined by weight. All of particles from the encapsulation process were dried at the ambient temperature for 8 hours and then was also weighted. The encapsulation efficiency and loading capacity were determined by using two equations below:

\[
EE\% = \left( 1 - \frac{\text{mass of free oil}}{\text{mass of initial oil}} \right) \times 100
\]

\[
LC\% = \frac{\text{Mass of initial oil} - \text{mass of free oil}}{\text{Mass of total nanoparticles}} \times 100
\]

Where EE% and LC% were encapsulation efficiency of the process and loading capacity of the particles, respectively.

2.4. Particle size measurement
The particle sizes of the prepared capsules were measured by using the particle analyzer (DLS – Horiba SZ 100). The sizes were determined using water as the dispersing medium.

3. Results and discussion
3.1. The effect of the variation of TPP concentration
Four TPP concentrations (2.5; 5.0; 7.5 and 10 g/L) were carried out while keeping the amount of oil (20 g/L) and chitosan (10 g/L) in the emulsion. The results showed in Figure 3, which indicated that the encapsulation efficiency increased from 15.1% to the maximum of 34.0%. The growth in encapsulation efficiency could be due to a higher level of cross-link bonds, which improved the oil content inside chitosan capsules. Then, the encapsulation efficiency decreased to 29.4% with increasing TPP further. This stage could be attributed to the formation of a denser cohesive structure, which makes pore sizes much smaller [4], [12]. Furthermore, the loading capacity went down from 11.8% to 3.1% with increasing TPP concentration. The main reason is that many cross-link bonds are produced between polymer chains, which leads to
the shrinkage of particles. Hosseini et al. (2013) have observed an initial increase in oregano essential oil loading capacity followed by a decrease with increasing cross-linker concentration (from 0.25% to 10.0% w/v) [12]. Soliman et al. (2013) have also observed this phenomenon in clove oil, cinnamon oil, and thyme oil encapsulation with changing calcium chloride concentration (from 0.125% to 2.0%) [4]. Therefore, the smallest TPP concentration in the experiment might be smaller than 2.5 g/L, which could observe the initial increase in loading capacity.

![Figure 3. The effect of TPP concentration on the encapsulation efficiency and loading capacity.](image)

### 3.2. The effect of the variation of chitosan concentration

Four chitosan concentrations (5.0; 7.5; 10.0 and 12.5 g/L) were carried out with constant amounts of oil (20 g/L) and TPP (5 g/L). The results showed in Figure 4, which indicated that the efficiency encapsulation went up from 22.8% to 34.0% in the range of 5.0 – 10.0 g/L. With increasing polymer concentration, more polymers would be available to encapsulate the oil in the emulsion [13]. Further, higher chitosan concentration reduced the encapsulation efficiency from 34.0% to 26.1% at 12.5 g/L. The reduction in encapsulation efficiency could be due to the higher viscosity of the solution with increasing the chitosan concentration, which leads to the lower efficiency of emulsion formation [11]. Also, the loading capacity went down from 4.1% to 2.3% with changing chitosan concentration. The excess polymers take the place inside the particles, which causes the smaller pore sizes and reduces the entrapped oil content [11], [14]. However, Soliman et al. (2013) have observed an initial growth in loading capacity followed by a decline with varying polymer concentration in three oil encapsulations (from 0.5% to 8.0% w/v) [4]. In their report, they tried the initial polymer concentration, which was lower than in this study. Therefore, they could observe the growth in loading capacity.
Figure 4. The effect of chitosan concentration on the efficiency encapsulation and loading capacity.

3.3. The effect of the variation of oil loading

The effect of variation of oil loading on encapsulation efficiency and loading capacity is shown in Figure 5 with fixed chitosan concentration (10.0 g/L) and TPP concentration (5.0 g/L). The encapsulation efficiency had an initial growth from 19.6% to 36.2% followed by a reduction to 32.9% with increasing initial oil content. The efficiency of emulsion formation was decreased with adding more oil loading, which forming larger and larger oil vehicles, consequently higher encapsulation efficiency at the initial stage [11], [13]. However, encapsulation efficiency reduced at 400% oil content of chitosan due to the encapsulation limitation [6]. In addition, the growth of loading capacity was observed in all experiments (from 1.0% to 5.5%). Perhaps because the number of oil vehicles in chitosan capsules increases to reach their full capacity [6], [11], [13].
3.4. Particle size of chitosan nanocapsules

After optimizing the encapsulation process, the experiment with the highest encapsulation efficiency was chosen to analyze the particle size using DLS method. Three process parameters, the Z-average diameter and PDI value are shown in Table 1. According to DLS measurements, the distribution of particle size was a bell-shaped curve and the most of capsules were in the range of 100 - 500 nm (shown in Figure 6). From Table 1, the overall z-average diameter was 235.3 nm, but the PDI value was too high when comparing to other reports [15], [16]. Consequently, the chitosan nanocapsules were polydispersed.

Table 1. The overall Z-average diameter and PDI value from the experiment with the highest EE

| Chitosan concentration | Oil loading | TPP concentration | Z-average diameter* | PDI* |
|------------------------|-------------|-------------------|--------------------|------|
| 10.0 g/L               | 30.0 g/L    | 5.0 g/L           | 235.3±6.0 nm       | 1.829±0.130 |

*The values represent the mean of three replicates ± SE (n=3)
4. Conclusion

Overall, ionotropic gelation is possible for the formulation of palmarosa essential oil-loaded chitosan capsules. By varying the process parameters, the optimum formulation for the capsules is chitosan polymer 10.0 g/L, oil loading 30.0 g/L and TPP concentration 5.0 g/L (with the optimal weight ratio of chitosan to palmarosa essential oil is 3:1 w/w). The overall Z-average from DLS measurements was 235.3 nm and the particle size distribution was in the range of 100 – 500 nm.

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