Encapsulation of bioactive compound from extracted jasmine flower using β-Cyclodextrin via electrospray

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Abstract. The ability of electrospray to encapsulate the bioactive compound extracted from Jasmine flower with β-Cyclodextrin (β-CD) without any thermal-assisted processing was demonstrated in this study. The extraction of Jasmine compound were conducted using sonicator at 70 000 Hz, for 10 minutes and followed by mixing of the filtered compound with β-CD. Then, the mixture was electrosprayed under a stable Taylor cone jet mode at the voltage of 4–5 kV, with flow rate of 0.2 ml/hour. The aluminum substrate that used for collecting the deposit was placed at 30 cm from the needle’s tip to allow the occurrence of evaporation and droplet fission until the droplet transform to solid particles. Characteristics of solidified bioactive compound from Jasmine flower (non-encapsulated compound) and solidified bioactive compound with β-CD (encapsulated compound) were studied in this work. From SEM images, it can be observed that the particles size distribution of encapsulated compound deposits have better deposition array and did not aggregate with each other compared to the non-encapsulated compound. FE-SEM images of encapsulated compound deposits indicate more solid crystal looks while non-encapsulated compound was obtained in the porous form. The electrospray process in this work has successfully encapsulated the Jasmine compound with β-CD without any thermal-assisted process. The encapsulation occurrence was determined using FTIR analysis. Identical peaks that referred to the β-CD were found on the encapsulated compound demonstrated that most deposits were encapsulated with β-CD.

1. Introduction
Natural fragrance compound extracted from plants have been widely used in many areas such as in pharmaceutical, aromatherapy, cosmetics, as condiment in several food and drinks, and for adding scents to household cleaning products [1]. It can be obtained naturally from fruits, leaves, and flowers such as lavender, mint, citrus, rosemary, lemon [2], Jasmine, and many more. Jasmine flower for example, is a fun-loving fragrance, provides a unique and enthusiastic aroma. It has rejuvenating, boosting and energizing properties, which makes it a natural mind-blowing fragrance. Jasmine is primarily used in the perfume industry and has a very high commercial value due to its therapeutic
properties. The production of perfume from Jasmine fragrance has even started in 1860 [3] and continually expands over the years. The pleasant smell from this flower is coming from the bioactive compounds such as linalool, franesene, and benzyl acetate. However, the extracted Jasmine flower is easy to degrade due to the adversely environmental effect such as undesirable effect of light, heat, pressure, and oxygen [4] that will lead to the decreasing of shelf life.

In order to minimize the compound lost, a proper control after the extraction process is necessary. In this work, the extracted bioactive compound is subjected to the encapsulation process due to the ability of the encapsulated matrix to minimize the permeability of oxygen, water, bacteria and other factor that might change or degrade the compound structure. Encapsulation process has been applied to many areas especially in pharmaceuticals and food industries due to the ability of encapsulation to avoid the adverse factor or condition, increase the stability of bioactive compound [5], enhance bioavailability, and improve the control release of bioactive compounds [6].

Various kind of encapsulation methods have been explored and applied for commercial purpose i.e spray drying and freeze drying. However, due to the heat sensitive issue applies to the extracted bioactive compound, the application of spray drying that involves heat is not preferable. Meanwhile, freeze drying is a simple technique for encapsulating water-soluble essences and natural aromas, as well as drugs, but the major disadvantages of freeze drying are it consumes high energy and take a long processing time [7].

Therefore, an alternative encapsulation method is required to ensure the practicality and the survival of the bioactive compound during and after the encapsulation process. To date, due to the emerging of technology, electrospray has been found to be an alternative method for encapsulation process without the thermal application. The process also provides many advantages such as producing fine monodispersed droplet within nanometer scale, minimum droplet aggregation, controlled charged droplets, and high deposition efficiency due to the charged carries by the produced droplets [8].

Since no thermal application is needed, the drying process that occurs during the electrospray process is due to the evaporation and droplet fission that continuously take place along the process. The evaporation of water from the droplet increases the charge concentration on the droplet surface and lead to the droplet fission. These two processes occurred repeatedly until a solid body is obtained and deposited onto the aluminium collector or substrate.

The application of electrospray is quite well known in the pharmaceutical area to increase the bioavailability of the drug. However, the application of electrospray for the encapsulation of bioactive compound is limited and lack of literature review. Thus, this study is focusing on the encapsulation of bioactive compounds extracted from Jasmine flower using β-CD via the electrospray technology. In general, cyclodextrin is considered as a good encapsulating agent because of its advantages such as extremely low toxicity, excellent biocompatibility and non immunogenicity [9]. In addition, β-CD was specifically chosen as encapsulating agent in this study compared to others is due to its’ ability to form inclusion complexes with many organic and inorganic compounds within the cavity of their ring structure that composed of D-glucose units and has been demonstrated from our previous work [10].

The drying process that occurs during the electrospray process is due to the high electrostatic charged droplet fusion that produces progeny droplet when exceeding the Rayleigh limit condition. As a result, the repeatable droplet fission has caused the water evaporation until a solid body is obtained as the deposit.

2. Methodology

2.1 Preparation of Jasmine extract

Raw material in this study which is Jasmine flowers were obtained from Universiti Putra Malaysia (UPM) garden, and the β-CD that was used as encapsulating agent was purchased from Sigma Aldrich (M) Sdn. Bhd.
The extracted Jasmine compound was obtained by sonicating 3 g of Jasmine flower in 20 ml of deionized (DI) water. Total sonication process was conducted for 10 minutes with 15 seconds pause-mode after each 1 minute to prevent the heat to develop during the process. The ultrasonic process increases the solution’s temperature due to the vibration of ultrasonicator’s probe. Therefore, it is important to ensure the temperature of solution is always at the average of 20°C. In order to achieve this target, the solution was put in a 50 ml beaker, and this beaker was put in a bigger beaker that can fit the 50 ml beaker. Wet tissue that was submerged in iced water was placed between these two beakers to control the solution temperature from increasing and it needed to be changed for every 1 minute.

The extracted Jasmine compound consists of inconsistent particle size distribution after the ultrasonic process. To separate the large and fine particles, the extracted Jasmine flower was centrifuged at 10000 rpm, 20°C, for 30 minutes. Figure 1 shows all the steps to obtain the extracted Jasmine compound.

![Figure 1](image)

**Figure 1.** Experimental setup for extraction of bioactive compound from Jasmine flower

### 2.2 Preparation of Jasmine’s extracts with β-CD.

In order to prepare the mixture, 1 g of β-CD was mixed with 10 ml of extracted Jasmine compound, and was stirred for 1 day. To ensure the well mixture of β-CD and Jasmine solution, the β-CD was added bit by bit into the solution. Ultimately, two samples were prepared for electrospray, which are pure extracted Jasmine compound (non-encapsulated), and the mixture of extracted Jasmine with β-CD (encapsulated).
2.3 Electrospray

As shown in Figure 2 above, electrospray setup consist of electrostatic generator that supply positive charge to the needle, syringe that loaded with jasmine’s extract solution, needle, syringe pump, retort stand, substrate, ring electrode, and the negative power supply to the aluminium substrate. The electrostatic generator supplied high voltage positive charge to the needle and directly to the solution. An intense electrostatic field is formed at the tip of the needle by applying a few kilovolts potential difference between the needle and the surrounding environment. At the tip of the needle, the strong electric field pulls liquid-phase ions toward the liquid-gas interface. The liquid surface at the tip of the needle forms a cusp, called a Taylor cone. The Taylor cone is developed from the combination of hydrodynamic and electrostatic forces, which resulting of fine spray jet and able to produce charged droplets. Evaporating charged droplets quickly reach a point at which they are no longer stable. This condition is known as “Rayleigh instability”. At this stage, evaporation process is continuously occurs along the deposition process and reduces the size of droplet. The reduction size of droplet creates the repulsion effect, where the positive charge tends to repel with each other and produce more droplet fission. The evaporation and droplet fission will continuously occur until the droplets are completely dry and transformed into very fine solidified particles before deposited onto the aluminum substrate that was supplied with the contradict charge (negative). The illustration of the mechanism is shown is Figure 3. The conditions of electrospray in this work are tabulated in Table 1.

![Figure 2. The electrospray experimental setup for producing solidified particles](image)

![Figure 3. The mechanism of droplet evaporation and fission along the deposition process](image)
Table 1. Condition of Electrospray

| Parameter                                      | Value          |
|------------------------------------------------|----------------|
| Voltage (kV)                                   | 4 – 5          |
| Flow rate (ml/hr)                              | 0.2            |
| Distance from needle tip to aluminium collector (cm) | 30             |
| Electrospray duration (hr)                     | 1              |

The non-encapsulated and encapsulated particle from electrospray were analysed by Gas Chromatography Mass Spectrometry (GCMS) – Head space, Scanning Electron Microscopy (SEM), Field Emission Scanning Electron Microscopy (FESEM), Image J for particle size distribution, Fourier Transform Infra-Red (FTIR), and Transmission Electron Microscopy.

3. Result and Discussion

3.1. GCMS-Headspace

The purpose of conducting GCMS – Head space was to determine the component of bioactive compounds existed in the Jasmine flower. Figure 4 and Table 2 below show the components of bioactive compound existed in the Jasmine flower.

![Figure 4](image)

Figure 4. Peaks of bioactive compound from Jasmine flower by GCMS-Headspace

Table 2. Components existed from the extraction of Jasmine flower

| Time (min) | Compound         |
|------------|------------------|
| 1.01       | Ethanolamine     |
| 1.38       | Alanylglycine    |
| 7.53       | 3-Hexenyl acetate|
| 10.57      | Ocimene          |
| 12.73      | Linalool         |
| 22.60      | Acetic acid      |
| 23.52      | Hexanoic acid    |
| 25.41      | β-Cubebene       |
| 45.49      | α-Fransesene     |

As observed above, there are many compounds existed in the Jasmine flower. However, based on the research done by [11], the constituent that give essence and has the heat sensitive characteristic (easy to volatile) are lie to the linalool and α -Frnesene.
3.2. SEM and FE-SEM

SEM and FE-SEM were used to observe the morphology of the encapsulated and non-encapsulated particle after the electrospray. Figure 5 shows the morphology of particle for non-encapsulated and encapsulated compound.

| SEM | FE-SEM |
|-----|--------|
| Non-encapsulated particle | ![Image](image1.png) |
| Encapsulated particle | ![Image](image2.png) |

**Figure 5.** The morphology of solidified particles through SEM and FE-SEM

From SEM, it can be observed that the sphere particle shapes were produced after electrospray process. However, further observation by FE-SEM indicates that the non-encapsulated figure were not in sphere from, more porous, and aggregated compared to the encapsulated ones. In contrast, the particle distributions of encapsulated compounds were observed to be finer, solid-looks and less aggregation between the deposits.

In order to justify the morphology obtained by SEM, high magnification (50,000x) observation was performed using FE-SEM. Based on the FE-SEM images, it can be clearly seen that the non-encapsulated particles indicate a porous-look, poor sphericity, and seems not fully dry when reached the substrate surface. However, the encapsulated particles were deposited in solid form and expected to have the high crystallinity index compared to the non-encapsulated ones. From the FE-SEM images, it can be hypothesized that the non-encapsulated particles were unable to fully dry when undergoes the deposition process even the distance to collect both samples are exactly the same. The encapsulated compound is expected to be fully encapsulated since only crystal look is obtained and no porous form deposits were noticed on the substrate surface. It can be concluded that the Jasmine flower extracts were fully surrounded by β-CD.
3.3. *Particles size distribution* 

The particle size distribution was analysed by using the ImageJ software. By using the software, the distribution of non-encapsulated and encapsulated compound were compared and analyzed.

![Graph 1](image1.png)  
**Figure 6.** Particle size distribution for non-encapsulated and encapsulated compound collected at 30 cm distance from the needles tip.

Based on the graph above, the particle size distribution of encapsulated Jasmine extracts is finer and it has sharp distribution compared to the non-encapsulated compound. This information can be proved through the data of average particle size ($d_{mean}$). Due to the wider distribution of non-encapsulated compound, the average particle size is bigger than the encapsulated compounds which are 55.12 nm and 36.81 nm respectively.

Since the evaporation is taken place along the electrospray process until the droplets become smaller droplets [12], the different in the obtained results were justified through evaporation rate.
The proposed equation rate to be used is:

\[ E_R = \frac{\Delta V}{t} \]  

Where,

- \( \Delta V \): Volume difference
- \( t \): Time taken
- \( E_R \): Evaporation rate

Therefore;

| Type of compound   | Evaporation rate (nm³/s) |
|--------------------|-------------------------|
| Non encapsulated   | 1.11x10¹¹               |
| Encapsulated       | 1.05x10¹¹               |

The evaporation rate of encapsulated compound is slower than non-encapsulated compound is due to the water percentage presence in the initial droplet. Initially, encapsulated compound has 75 wt % of water, while non-encapsulated compound was having 85 wt % of water. Earle (2013) reported that when the water at the surface is gradually removed, the moisture content inside the material is decreased and cause the remaining water inside the droplet to be strongly bounded to its solid compound [13]. Thus, the encapsulated particle took low evaporation rate compared to the non-encapsulated ones. In addition, even though the non-encapsulated compound has higher evaporation rate, but that rates is not high enough to allow the droplet to fully dry before reaching the collector. The distance need to be increased in order to allow more water to be evaporated and transform the evaporating droplet into a solid form.

3.4. FTIR analysis

The FTIR identify the presence of functional group in the compound’s structure.

![FTIR spectra for Extracted Jasmine, β-CD, and Encapsulated Compound](image)

As shown in Figure 7, the encapsulated compound consists of identical β-CD between 1100 and 1700 cm⁻¹. The encapsulated compounds also consist of bioactive compound extracts which indicates the broad peaks between 2800 and 3500 cm⁻¹. The β-CD peak that appeared similarly at the
encapsulated compound were represented by the wave number of ~1100 cm$^{-1}$ and ~1000 cm$^{-1}$ that corresponding to the C-C and bending vibration of O-H respectively [11]. Meanwhile, the similarities peak between extracted Jasmine and encapsulated compound were represented by the wave number of ~3500 cm$^{-1}$ and ~2800 cm$^{-1}$ that indicates the appearance of OH stretching and C-H stretching. Those functional groups are believed to be signified to the linalool and α-franesene. The similar peak from β-CD and extracted bioactive compound that appear at the encapsulated compound was evidence to the encapsulation of bioactive compound with β-CD.

4. Conclusion
The encapsulation of bioactive compound with β-CD without any thermal-assisted was demonstrated in this research work. The evidences can be seen through SEM, FE-SEM, and FTIR. From SEM result, the morphology of encapsulated compound is found to be finer, solid looks, and less aggregation compared to the non-encapsulated compound. Additionally, the crystal-look is obtained around the spherical shape of encapsulated compound through FESEM that confirms the presence of β-CD. Besides that, the encapsulation of Jasmine extract with β-CD can also be proved through the similarity peak from Jasmine extract and β-CD in the encapsulated peak. The inclusion of β-CD in every single droplet has improves the solidification process compared to the solidification of Jasmine extracts only.

5. References
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