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Synthesis and characterization of curcumin-phosphatidylcholine complex via solution enhanced dispersion by supercritical CO₂

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Abstract

In order to enhance the bioavailability of poorly water-soluble curcumin, solution enhanced dispersion by supercritical carbon dioxide (CO₂) (SEDS) was employed to prepare curcumin-phosphatidyl choline complex. The reaction parameters were varied and investigated. The typical parameters were determined as follow: P = 200 bar, T = 60 °C, gas flow rate 10 mL.min⁻¹, molar ratio of phosphatidylcholine:curcumin 2:1, ratio of dichloromethane/scCO₂ 15 % (w/w). The characteristics of complexation product were measured such as the water solubility, natural stability, UV-Vis, FTIR and DSC. The FE-SEM image shows that curcumin-phosphatidylcholine complex appeared in two types of spherical shape with the big particle size of complex approximately 0.9 μm and nano curcumin with particles size of approximately 100 nm. This study revealed that supercritical CO₂ technologies had a great potential in fabricating complex and improving the bioavailability of poorly water-soluble drugs.

Keywords. Curcumin, water soluble curcumin, phosphatidylcholine, CO₂ supercritical fluids.

1. INTRODUCTION

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl) -1-6-heptadiene-3,5-dione), derived from the rhizome of the plant *Curcuma longa*, is a yellow-orange polyphenol compound [1, 2]. It is widely used as a spice, food preservative, flavoring and coloring agent. Evidence suggests curcumin has the potential to prevent or treat various pathophysiological processes, including cardiovascular disease, carcinogenesis, wound healing and inflammation [1]. However, the low water solubility and low stability are two obstacles that inhibit the high activated property of curcumin [2, 5]. Seeking to address these problems, recent studies have explored a number of delivery systems such as micelles, plasma proteins, cyclodextrins, and polymer nanoparticles [3].

Some typical techniques have been used for the preparation of curcumin encapsulated in Phosphatidylcholine (PC), the part of soy lecithin. The PC, an essential part of the cell membrane used in phytosomes technology, acts as a carrier and also nourishes the skin [9]. The curcumin, just like another dietary phenolic, shows polar groups (two phenolic hydroxyls and one enolic hydroxyl) that can interact via hydrogen bonding and polar interactions with complementary groups, like the polar head of PC. The soy lecithin has a highly

polarized head, with the negative charge of a phosphate group and the positive charge of the choline ammonium group, and can complex a variety of poorly soluble phenolics, including curcumin [12]. The details of some complexes in the daily dose and specific indications showed higher aqueous and n-octanol solubility and higher antioxidant activity than that of pure curcumin at all dose levels tested [10].

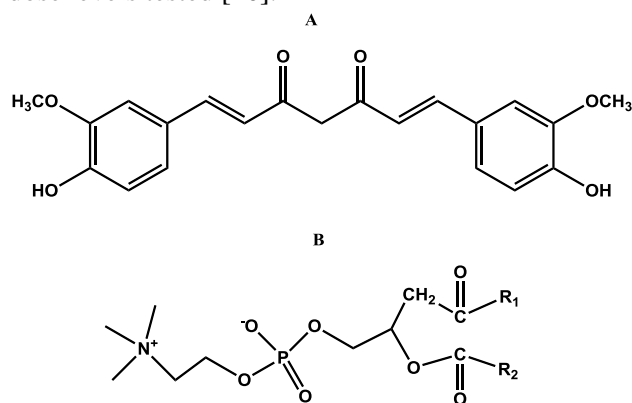


Figure 1: Structure of (A) curcumin and (B) phosphatidylcholine compounds

However, there are some drawbacks in the complex preparation such as the energy drying and the toxic solvent residues of VOC solvents [6, 9]. In

this point of view, supercritical CO₂ (SCCO₂) is ideal. Recently, supercritical carbon dioxide (scCO₂) technologies have shown great potential in the field of particle formation, especially drug micronization. The scCO₂ technologies present many advantages, including low critical conditions ($T_c = 304.1\text{ K}$, $P_c = 7.38\text{ MPa}$), no solvent residue, non-flammability, low cost, and being environmentally benign [4, 7, 8].

In this work, we used SCCO₂ as solvent and solution enhanced dispersion method for the preparation of curcumin-phosphatidylcholine complex. The reaction was conducted in batch condition using high pressure induced autoclave. The reaction parameters such as reactant ratio, reaction temperature, pressure, and reaction time were investigated. The addition of dichloromethane showed significant influence to the reaction yield. UV-Vis spectrometer, HPLC, FTIR, DSC, FE-SEM instruments were used to measure the reaction yield, product solubility, and other properties.

2. MATERIALS AND METHOD

2.1. Materials

Curcumin (Cur, 96 %) from Wacker (Germany), phosphatidylcholine (99 %) from Indian, dichloromethane (HPLC grade, Merck), acetonitrile (HPLC grade, Merck), double distilled de-ion water (DI), CO₂ (99.99 %, Air Liquid) were purchased and used as received.

2.2. Synthesis curcumin-phosphatidylcholine complex in CO₂ media

The equipment includes a volumetric pump, a 100 mL stainless steel autoclave laid on a magnetic stirrer, a depressurization system with a two-way coaxial nozzle and a temperature heating. The schematic diagram of the SEDS process is shown in figure 2. In a typical experiment, 0.48 g curcumin was dissolved in 50 mL absolute dichloromethane. 10 mL of the 20 % phosphatidylcholine dichloromethane solution was dropwise added. The resulting mixture was sonicated at 55W for 3 min to generate the final emulsion then added to the autoclave. The autoclave was closed carefully and connected to the experiment system. The reactor was filled up with SCCO₂. The heat was slowly increased, the pressure was fixed and the reaction mixture was kept with continuous stirring by the magnetic stirrer. When the desired experimental conditions were reached, curcumin complex in the autoclave was injected into the high-pressure vessel via a stainless steel coaxial nozzle simultaneously with other supercritical CO₂ flow, using a high-pressure liquid pump. During the dispersion process, the precipitation temperature and pressure were maintained as described above. After all the solution was delivered into the high-pressure vessel, the CO₂ flow was stopped and the CO₂ in the high-pressure vessel was slowly depressurized to atmospheric pressure. The curcumin-phosphatidylcholine complex was then collected on the filter at the bottom of the high-pressure vessel for further characterization.

- (1) CO₂ liquid vessel
- (2) Cooler
- (3) High pressure pump
- (4) Heater exchanger
- (5) Magnetic stirrer
- (6) Nozzle
- (7) High pressure vessel
- (8) Valve on/off
- (9) Pressure gauges
- (10) Backflow valve
- (12) Temperature control
- (13) Gas flowmeter

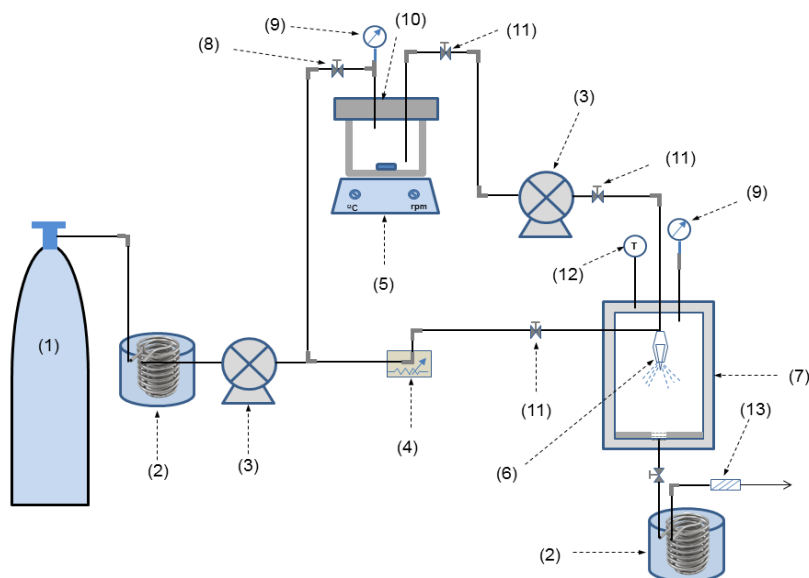


Figure 2: Schematic diagram of the SEDS process

2.3. Determination of the reaction yields, solubility, and natural light stability

The reaction yield was calculated based on the relative amount of insoluble curcumin in product and that in the solid material mixture. For that, 15 mg of red powder product was dissolved in 10 mL of DDW with sonication for 30 sec. The solution was then centrifuged at 9,000 rpm for 5 min, the supernatant was decanted. This work was repeated 2 more times until no more product be dissolved. The remaining solid in the centrifuge tube is dissolved by acetonitrile/DDW solvent (1:1 volume) mixture. The solution is transferred to the volumetric flask and added solvent to 25 mL. This solution is measured the UV absorption at 425 nm and calculated the amount based on premade calibration curve.

The reaction yield is calculated based on the equation:

$$H \% = \frac{Cur_{tot} - Cur_{fr}}{Cur_{tot}} \times 100$$

with Cur_{tot} and Cur_{fr} are the amount of curcumin in solid material before reaction and amount of curcumin insoluble in the product, respectively.

The water solubility of curcumin in the complex product was measured by dissolving 25 mg of red powder product in 10 mL of DDW with sonication for 30 sec. The solution was then centrifuged at 9,000 rpm for 5 min, the saturation solution was decanted. All clear supernatant solution was collected and added DDW to 25 mL in a volumetric flask. The measurement was conducted on HPLC equipped with a Zorbax Eclipse Plus C18 (4.6 x 250, 5 μ m) column using DAD detector and the amount of curcumin is calculated based on the premade calibration curve. The measurements concentration of curcumin in saturation solution under sun light at room temperature in one week was conducted by UV absorption at 425 nm based on premade calibration curve.

2.4. Characterisation of curcumin-phosphatidylcholine complex

2.4.1. Thermal analysis

Thermal analysis was carried out on about 3 mg sample by using a Differential Scanning Calorimetry (DSC, Model 204 F1 Phoenix, NETZSCH, Germany) from 40 to 220 °C at a heating rate of 5 °C/min under an N₂ gas stream.

2.4.2. FTIR spectroscopy

Study of the complex product was conducted with FTIR Tensor 27, Bruker, Germany spectrophotometer. The samples were combined with potassium bromide, and the mixtures of the samples and potassium bromide were pressed into a transparent tablet. The FTIR spectra of the samples were recorded with the wave number ranging from 4,000 to 400 cm⁻¹.

2.4.3. FE-SEM

The surface morphology of curcumin nanoparticles was visualized by scanning electron microscopy (FE-SEM) (JSM-7401F, JEOL, Tokyo, Japan). These samples were then gold sputter-coated under argon prior to imaging. Complex sample was made conductive by sputtering a thin layer of gold onto their surface. The particle size and particle size distribution of the samples were analyzed by Analysis Station JED-2300 software from the SEM photographs.

3. RESULTS AND DISCUSSION

3.1. Influence of reaction parameters on yield of product

The influence of temperature on the reaction was investigated and shown in Fig. 4A. The reaction yield increased with increasing the temperature. However, when further increased the reaction temperature more than 60 °C, reaction yield decreased. For the other later investigations, we choose the reaction temperature at 60 °C. Pressure built up inside the reactor is due to the occupancy of SCCO₂ in all the reactor volume. The pressure rapidly increased when increasing the temperature. Influence of pressure to reaction yield was shown in Fig. 4B. When carried out the reaction without using SCCO₂ and the reaction temperature at 60 °C, we did not observed the indication of pressure on pressure gauge. At the same time, the yield of reaction was rather low. In the presence of SCCO₂, the reaction yield dramatically increased. However, when the pressure was controlled in the range of 30 to 300 bar the reaction yield slightly increased. We used the pressure of 200 bar for all other later studies. The reaction time effect is shown in Fig. 4F. Interestingly the complexation occurred and got the yield up to 82 % within the first hour of reaction time. With keeping the reaction time longer, reaction yield increased slowly. The highest yield was obtained at 99.8 % after 3 hours. The relationship of curcumin and phosphatidylcholine molar ratio in the starting materials with the reaction yield is shown in

Fig. 4D. The highest yield was obtained at the ratio of 1:2. When we kept increasing the amount of phospholipid in the ratio from 2.0 to 3.0, the yield almost did not increase further. In this work, the use of supercritical CO₂ as a solution enhancing dispersion showed strong positive effect. In this

case, the reaction yield dramatically increased to 84.5 % compared to 65.6 % in case of without using scCO₂. The investigation results are shown in Fig. 4C. The highest effect is shown at the gas flow rate 10 L/min. The yield even decrease if increase gas flow rate.

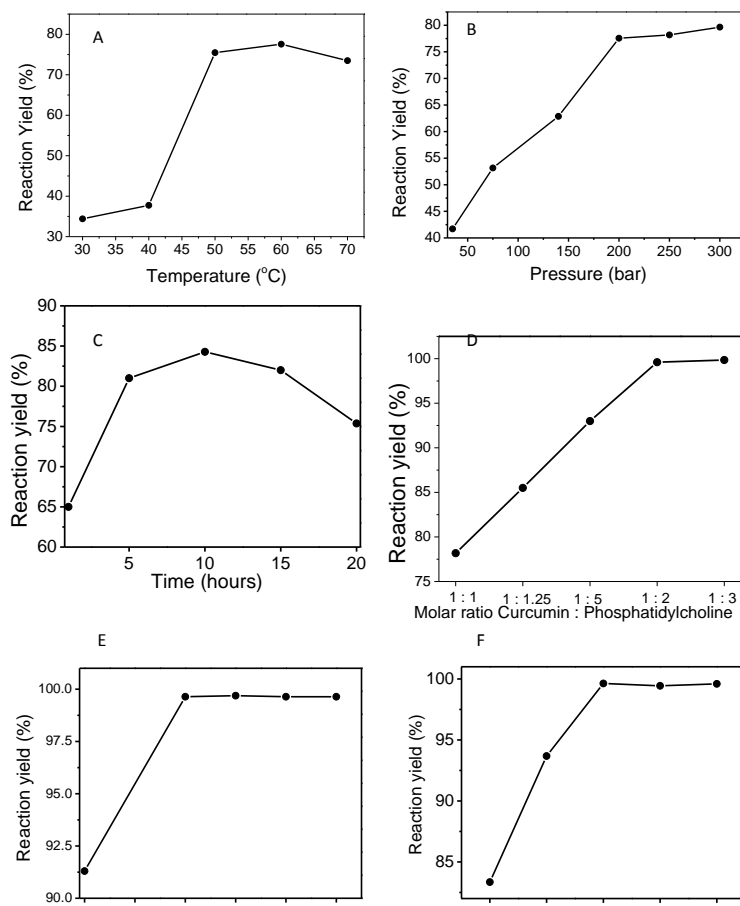


Figure 4: The influences of reaction yield by the reaction condition such as temperature (A), pressure (B), gas flow rate (C), the molar ratio of phosphatidylcholine with respect to curcumin (D), the ethanol amount (E) and reaction time (F)

The influence of dichloromethane in the reaction is shown in Fig. 4E. By physical mixing and the dichloromethane excited as absorbed dichloromethane in phosphatidylcholine, the encapsulate almost did not occur. When dichloromethane added, the reaction yield dramatically increased and got the highest of 99.4 % with ratio ^mdichloromethane/^mCO₂ (15 %) was added into the reactant mixture. With the addition of dichloromethane, it probably partially dissolved the surface of reactants and increased the reaction on the fresh surface. Moreover, the dichloromethane soluble product of this surface reaction easily diffused out of surface and enhanced the surface dissolving. As the result of this interaction, the

equilibrium of this reaction shifted toward the complexation formation direction.

3.2. Solubility and natural light stability

The improvement of solubility of curcumin complexation product in water is the main goal to be achieved. As the result of the investigation, the solubility of curcumin is obtained 198 µg/mL in DI water corresponding to 2.5×10^{-4} mol/L while we observed only the trace amount of free curcumin in DI water. Based on the calculation, the water solubility of complexed curcumin is approximately 4000 times enhanced compared to that of intrinsic curcumin. High stability of the curcumin as shown

in the sample of the complex indicating a very good, after 3 days, the concentration of curcumin retention was 192 $\mu\text{g}/\text{mL}$, corresponds 96.9 %.

Continued survey to after one week, curcumin retention was 93.5 %. By on the facts about reaction yield and stability of the complex, it shown the role of phosphatidylcholine with complex system. Almost all of phospholipid and phosphatidylcholine can form micelles with curcumin or another drug, and enhance solubility and stability of them [9, 10].

3.3. UV-Vis absorption spectrum

The complexation obviously changed the solubility as well as the spectrum of product with respect to that of free curcumin. Fig. 6A-B show the photograph pictures of phosphatidylcholine and complexation product in DDW. Free curcumin is clearly insoluble. In contrast, complexation product in DDW is quickly dissolved and formed a clear yellow color solution as shown in Fig. 6B. Consistent with these images, the UV-Vis absorption spectrum of product in Fig. 6C clearly showed the characteristic absorption peak of curcumin at 425 nm.

3.4. DSC

DSC thermograms of each starting material, physical mixing sample, and complexation product are shown and indicated in Fig.5. The thermogram curve of phosphatidylcholine showed the endothermic peak starting from 150 $^{\circ}\text{C}$ to 170 $^{\circ}\text{C}$. While the endothermic peak of free curcumin appeared a sharp peak at 175-178 $^{\circ}\text{C}$ as the melting point and high crystallinity. We did not observe the peak as the absorbed water loss in both cases. In the case of physical mixing sample, two peaks in the region 150-164 $^{\circ}\text{C}$ and 174-178 $^{\circ}\text{C}$ corresponding to phosphatidylcholine and curcumin respectively were absorbed. With the complexation product, this thermogram is almost flattened, and the endothermic peak due to the molten curcumin disappeared. This result reveals that the initial crystalline structure of curcumin was collapsed. In the molecular level, curcumin molecules in the product are disorder arrangement. Based on DSC characterizations, it is high possibility that curcumin was encapsulated in the phospholipid micelles.

3.5. FTIR spectroscopy

Figure 7 represents a graph of the FTIR analysis of the curcumin, phosphatidylcholine, physical mixing, and complex. In the spectra of free curcumin and

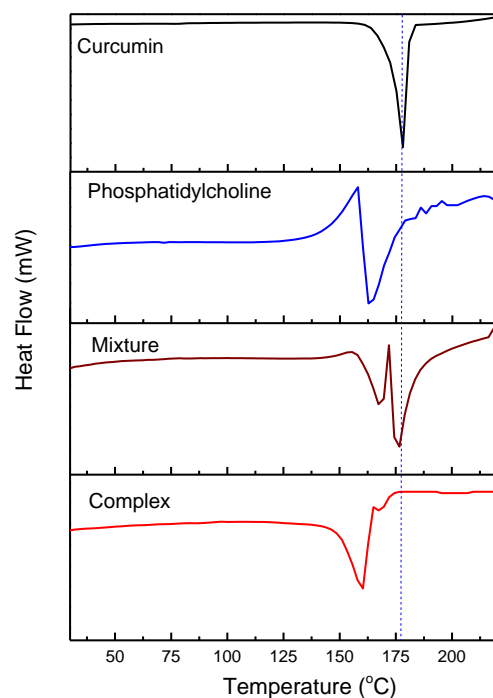


Figure 5: The DSC curve of curcumin, phosphatidylcholine, phosphatidylcholine physical mixing and complex as indicated

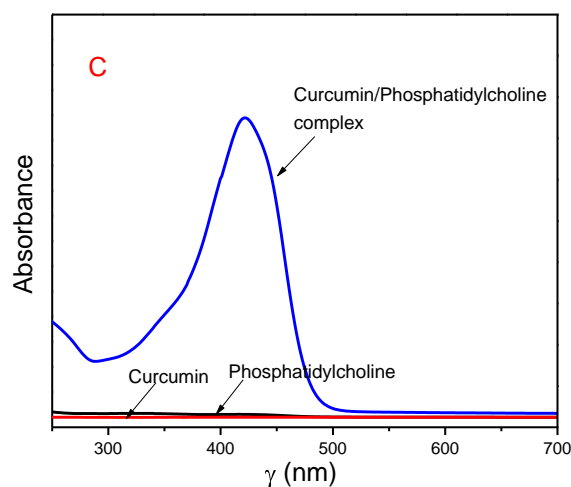
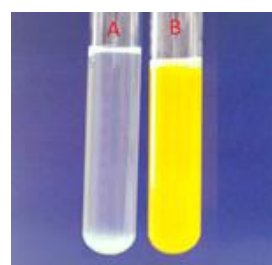


Figure 6: The photograph pictures of phosphatidylcholine in water (A), complex in water (B), the UV-Vis absorption spectra of complex, curcumin free and phosphatidylcholine in H_2O as indicated (C)

physical mixing sample, the vibration peaks at 1628 and 1601 cm^{-1} corresponding to the C=O, and C=C vibration mode of aromatic ring respectively are quite sharp. However, those of peaks are broader and lower intensity in both the case of Cur/PC complex. The vibration peak of C=C of the aromatic ring in curcumin and physical mixing at 1628 cm^{-1} shifted to 1631 cm^{-1} , peak 1601 cm^{-1} shifted to 1588 cm^{-1} in case of complex [13, 14]. The vibration peak of C=O, and C-C=O in physical mixing at 1510 cm^{-1} shifted to 1515 cm^{-1} , the intensity of this peak decreased obviously. The peak at 1280 cm^{-1} due to

vibration mode of C=C-H and aromatic C-O stretching are clearly seen in the spectra of curcumin and physical mixing shifted to 1264 cm^{-1} , suggesting that the interaction to one or both phenol group with phosphatidylcholine occurred in complex formation. The peak at 1228 cm^{-1} corresponding to antisymmetric PO_2^- stretching provides information about the interfacial region of the phospholipid splitting into two separate bands at 1242 and 1203 cm^{-1} and lower intensity in spectrum of complex [9]. This results indicate when curcumin molecule was encapsulated in phosphatidylcholine there is

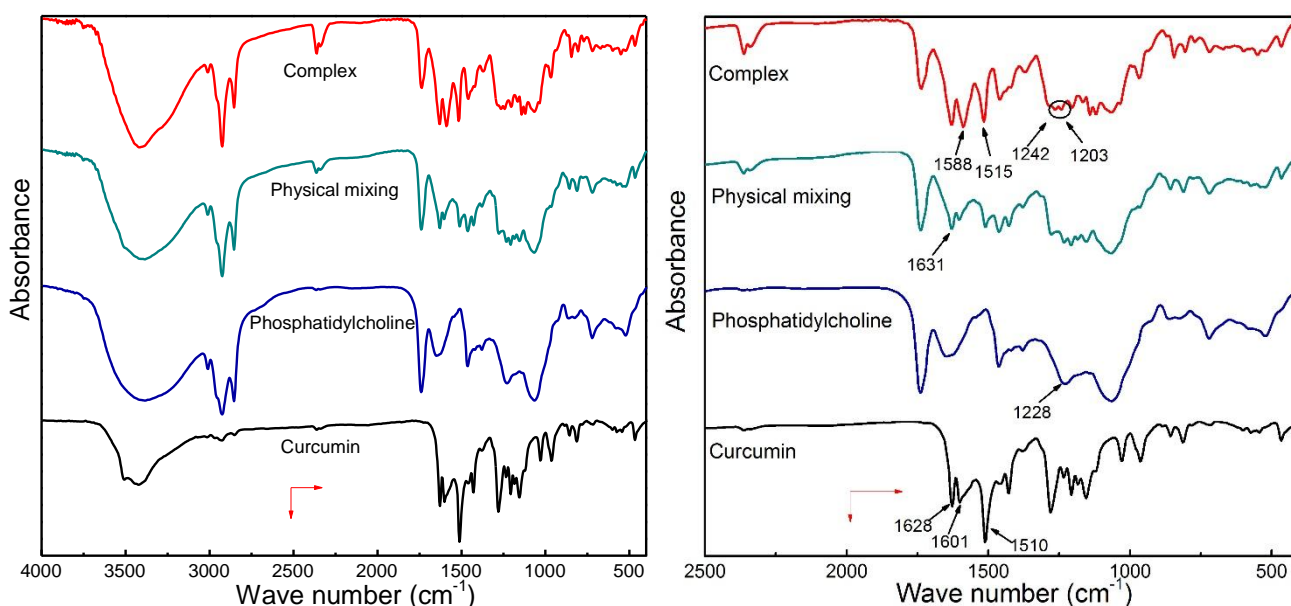


Figure 7: The FTIR absorption spectra of curcumin-phosphatidylcholine complex, physical mixing, curcumin free and phosphatidylcholine as indicated

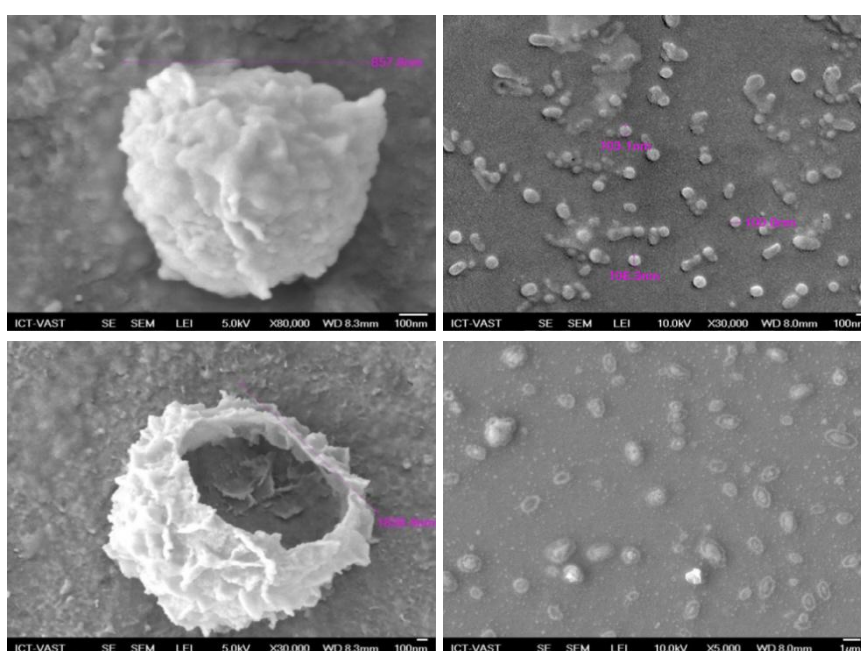


Figure 8: FE-SEM image of curcumin-phosphatidylcholine complex

interaction of curcumin with PO_2^- group of phosphatidylcholine membrane for formation phytosome structures [11].

3.6. FE-SEM

Figure 8 shown the SEM micrographs of the curcumin-phosphatidylcholine complex fabricated by the SEDS process. It can be concluded that phytosomes possessed an irregular spherical morphology with a mean particle size from 857 to 1,824 nm.

Moreover, in the complex there is the presence of curcumin nanoparticles with particles size approximately 100 nm. FE-SEM image indicates, that curcumin was encapsulated on phosphatidylcholine membrane and phytosome structure was formed. In liposome structure the active principle is dissolved in an internal pocket or floats in the layer membrane, while in phytosomes the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane [9]. Evidently, the SEDS process is an effective way to prepare curcumin nanoparticles. Moreover, by adjusting the different process parameters of the SEDS process, curcumin nanoparticles with controllable particle size can be prepared successfully [7, 8].

4. CONCLUSIONS

In this work, we have reported the research result in the preparation of complex curcumin-phosphatidylcholine. The reaction is conducted in the SCCO_2 condition at $P = 200$ bar, $T = 60$ °C, gas flow rate $10 \text{ mL}\cdot\text{min}^{-1}$, molar ratio of phosphatidylcholine:curcumin 2:1, ratio of dichloromethane/ scCO_2 10 % (w/w) temperature 60 °C, to reach high inclusion yield. The complexation product is highly soluble in water and high potential to bring to practical application.

The morphology of complex was determined by FE-SEM. According the FE-SEM image, almost of phosphatidylcholine molecules were in form of complex with curcumin. FTIR results showed there was interaction of curcumin molecule to PO_2^- group of phosphatidylcholine membrane for phytosomes formation.

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