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Supaporn Ketpitthava

Suchada Sukrong

Walaisiri Muangsiri

Nontima Vardhanabhuti1

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See next page for additional authors

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PRELIMINARY STUDY OF SPRAY DRYING AND FREEZE DRYING PROCESSES ON PHYSICOCHEMICAL PROPERTIES OF ENCAPSULATED ROSMARINIC ACID

Supaporn Ketpitthaya[1], Suchada Sukrong[2], Walaisiri Muangsiri[1], Nontima Vardhanabhuti[1], W. John Kao[3], Narueporn Sutanthavibul[1] and Phanphen Wattanaarsakit[1]

[1]*Department of Pharmaceutics and Industrial Pharmacy,* [2]*Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.*
[3]*Department of Biomedical Engineering, School of Pharmacy, Department of Surgery, University of Wisconsin-Madison, USA.*

KEYWORDS: rosmarinic acid, encapsulation, spray drying, freeze drying, sodium alginate

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INTRODUCTION

Rosmarinic acid (RA) is a polyphenol ester found in a variety of herbal plants especially in the Lamiaceae group such as rosemary, sage, spanish sage, and oregano [1]. RA has a variety of bioactivities, especially antioxidant and anti-inflammatory properties [1,2]. It also can break up amyloid-beta conglomerates of Alzheimer's Disease in laboratory studies, and it has anti-viral properties with effects against Herpes Simplex [3,4]. RA is soluble in organic solvent such as ethanol, but slightly soluble in water. Since RA is unstable to heat and oxidation [5], it is suitable to use as the model substance for herb extract to study of the formulations and encapsulation process. Encapsulation is a process by which active substances are incorporated inside particles in order to protect them from external environment or to control the release of active substances from the microparticles [6]. Among various encapsulation techniques; coacervation, ionic gelation, emulsion etc., spray drying (SD) and freeze drying (FD) are the two processes notably used to support an industrial production and also to increase the stability of active substances [9-11,13]. In this study, sodium alginate (AG) was used as a biocompatible polymer to encapsulate rosmarinic acid and to protect it from external environment [7,8,12]. Spray drying and freeze drying processes were used and compared as encapsulation techniques. The factors of encapsulation processes and polymer concentrations on physicochemical properties of the encapsulated RA were investigated.

MATERIALS AND METHODS

Materials Rosmarinic acid (purity: 96%) and sodium alginate were purchased from Sigma-Aldrich. Mannitol, calcium chloride dihydrate and trisodium citrate were purchased from Ajax Finechem.

Methods

Preparation of encapsulated RA microparticles The formulations of encapsulated RA prepared by spray drying and freeze drying processes were shown in Table 1.

Table 1 Formulations and encapsulation processes of rosmarinic acid.

Spray drying			Freeze drying	Polymer concentration
Blank (without RA)	Non-crosslinked	Crosslinked	Crosslinked	
SD1-NB	SD1-NC	SD1-C	FD1-C	AG 1%
SD2-NB	SD2-NC	SD2-C	FD2-C	AG 2%

Spray drying process Non-crosslinked spray dried RA microparticles (SD-NC) were prepared by adding 75 mg of rosmarinic acid into 30 ml of polymer solution at concentrations and ratios as shown in Table 1 to make the ratio of RA polymer by weight equal to 1:4 in all formulations. The obtained solution was then vigorously stirred by magnetic stirrer and sprayed into the chamber at the temperature of 130°C with the spray rate of 2 ml/min. The outlet temperature was kept at 70-75 °C. Non-crosslinked spray dried microparticles without RA (SD-NB) were also prepared as control. Crosslinked spray dried RA microparticles (SD-C) was obtained by dispersing the non-crosslinked microparticles into 30 ml of 5% w/v calcium chloride solution for 5 min then washed 3 times with methanol and 2 times with purified water and was dried by freeze drying.

Freeze drying process Crosslinked freeze dried RA powders (FD-C) were prepared at the same concentration and ratio of polymer to rosmarinic acid as in the spray drying process but with the addition of mannitol at the concentration of 3% w/v into the solution followed by 500 µl of 5% w/v calcium

chloride as crosslinking agent. After crosslinking, freeze drying process was performed to make a matrix of encapsulated RA and the obtained cake matrix was sieved by using mesh no. 30.

Characterization of encapsulated RA

Particle size and morphology Particle size was measured by light scattering using Malvern® Mastersizer2000. Ethanol was used as solvent to avoid polymer swelling. Particle morphology was observed by Scanning electron microscope (SEM).

Differential scanning calorimetry analysis (DSC) Thermal analysis by DSC was carried out to determine the transition energy of the encapsulated samples. The samples were run at a scanning rate of 10◦ C per min and within a temperature range of 25-250 ◦ C.

Entrapment efficiency High Pressure Liquid Chromatography (HPLC) was used to analyze the entrapment efficiency (% EE) of the encapsulated RA, using Cosmosil®C18 column. The mobile phase solution was prepared by using methanol and water at the ratio 35:65 with 0.1% v/v of acetic acid. The flow rate of mobile phase was controlled to be at 1 ml/min and at 35◦C. 10 µl of the filtered solution was injected into HPLC and equipped with UV detector at the wavelength of 320 nm. Analytical method validation was carried out for accuracy, precision, specificity and linearity. The sample for analyzing was prepared by dispersing 30 mg of the encapsulated RA in 5 ml of 3% w/v sodium citrate solution for 12 hours. The solution was then diluted with methanol to 50 ml and centrifuged at 3,200 rpm for 15 minutes. The obtained solution was filtered through 0.22 nylon syringe filter and analyzed by HPLC.

In vitro release study *In vitro* release study of the encapsulated RA was performed using modified Franz diffusion cell with cellulose acetate membrane. Phosphate buffer pH 5.5 and pH 7.4 were used as donor and receptor medium, respectively. The temperature of receiver medium was maintained constant at 32±0.5◦C. The 10 mg of encapsulated RA particles was dispersed onto the donor compartment and 2 ml of dissolution fluid was periodically withdrawn from the receptor compartment which replaced with the same amount of fresh medium and assayed by HPLC.

RESULTS

The particle size results from SD and FD processes were shown in Figure 1. From SD process, the sizes of microparticles were ranged within 15 to 52 µm and were found to be decreased with the increasing of polymer concentrations as can be seen in all formulations of SD-NC, SD-C and the control (SD-NB). The sizes of crosslinked spray dried microparticles were found to be larger than those of non-crosslinked spray dried microparticles. The particle sizes of FD powders which were produced by sieving the cake matrix were found to be larger than SD particles.

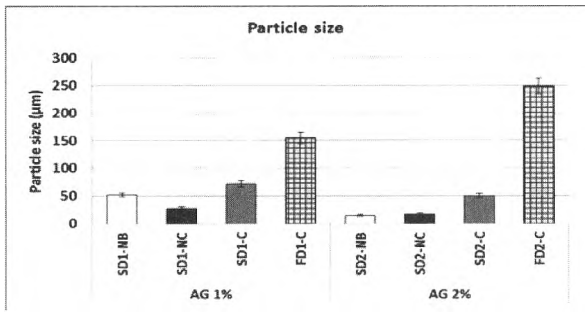

Figure 1 Particle size of non-crosslinked and crosslinked spray dried microparticles and freeze dried powders.

The particle morphology from SEM was shown in Figure 2. It was found that spray drying process created spherical shape microparticles where as freeze drying process, after cake matrix sieving, created flatted shape powders. Figure 3 showed the surface appearance of FD powders. The surface of FD2-C powder was found to be smoother than FD1-C.

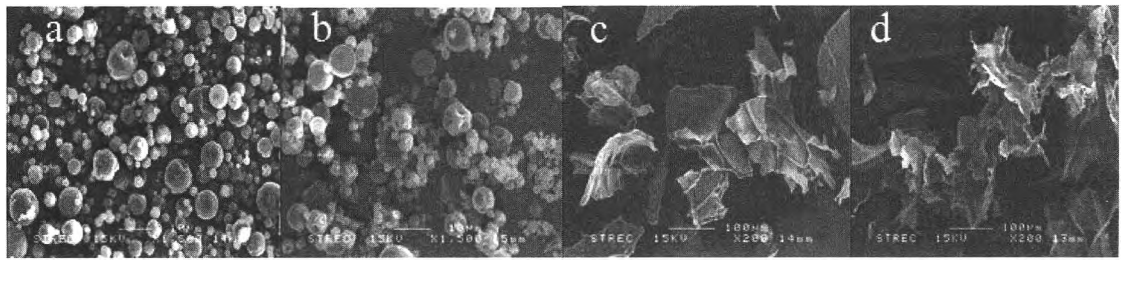

Figure 2 SEM images of spray dried microparticles and freeze dried powders. (a; SD1-NC, b; SD2-NC, c; FD1-C, d; FD2-C)

The results from Figure 4 showed that the melting endothermic peak of rosmarinic acid at 170°C was seen in physical mixture with polymer while the peak was disappeared in all formulations through spray drying and freeze drying process.

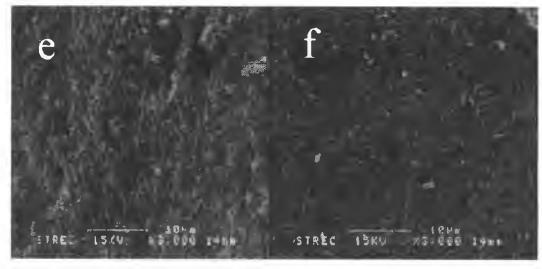

Figure 3 SEM images of surface of freeze dried powders (e; FD1-C, f; FD2-C).

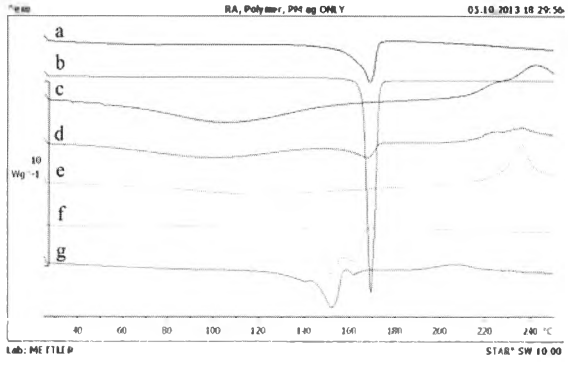

Figure 4 DSC thermogram of physical mixture of rosmarinic acid with polymer (PM), encapsulated spray dried microparticles and freeze dried powders (a; rosmarinic acid, b; mannitol, c; polymer AG, d; PM of RA+AG 1:1, e; SD1-NC, f; PM of RA+mannitol 1:1, g; FD1-C).

Table 2 Entrapment efficiency of the spray dried microparticles and freeze dried powders.

Process	Spray drying				Freeze drying	
Formulation	SD1-NC	SD2-NC	SD1-C	SD2-C	FD1-C	FD2-C
% EE (mean±SD)	71.97±3.04	75.59±5.49	1.71±0.08	2.13±1.01	103.9±3.76	97.71±5.85

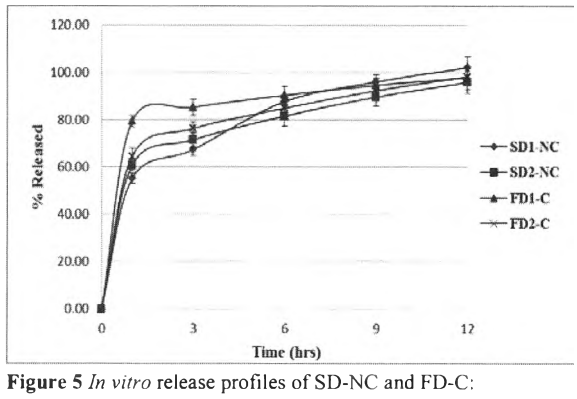

Figure 5 *In vitro* release profiles of SD-NC and FD-C: (♦) SD1-NC, (■) SD2-NC, (▲) FD1-C, (x) FD2-C.

Entrapment efficiency (% EE) results were shown in Table 2. It was found that the crosslinking process reduced the % EE of SD-C significantly to lower than 5%. As well, the % EE of the freeze drying process (FD-C) was found to be higher than those of spray drying process (SD-NC). The release profiles of rosmarinic acid from the encapsulated particles were shown in Figure 5. FD1-C showed the fastest release profile. The profiles of both SD1-NC and SD2-NC have similar result but SD2-NC seem to have slightly faster release than SD1-NC at the initial phase.

DISCUSSION

From spray drying process, using a hot gas for rapid drying, the obtained microparticles are spherical shape and free-flowing. In order to make a stronger shell matrix, a crosslinking of microparticles was performed by adding calcium into an alginate structure to form a stable network interaction [6,7]. However, there is also an interaction between the particles during a crosslinking process which resulted in an agglomeration of those particles. It was also found that the crosslinking process for spray dried microparticle affected the entrapment efficiency of rosmarinic acid. This was caused by the rosmarinic acid loss in the process of crosslinking [8]. As for the freeze drying process, which is a drying technique worked by freezing the samples and then reducing the pressure for water removing by sublimation into gas, the encapsulated cake matrix was obtained. The powders obtaining from sieving step has a flatted shape and less in free-flowing property than the particles from spray drying process. The spray dried microparticles obtained tend to be smaller with increasing in polymer concentrations. Also the surface

roughness of particle from freeze drying was reduced. This might be caused by higher polymer concentrations which affected the viscosity and strong structure in the solution and resulted in lesser change in the structure of polymer surface during freeze drying process. Rosmarinic acid was encapsulated and homogeneously dispersed in the polymer matrix as solid dispersion in amorphous state through spray drying and freeze drying processes. The amorphous state of RA in polymer matrix might cause this effect from the large amount of RA dissolved and lost during dispersion in crosslinking solution. Entrapment efficiency of encapsulated RA freeze dried powders was higher than spray dried microparticles since there was no rosmarinic acid loss in the process. From the release studies, RA was remarkably increased at the initial phase and then sustained release from the encapsulated particles of both processes. The result showed that higher polymer concentrations provided more sustained release profiles. However, the smaller particle size with the higher surface area might have a possibilty to enhance the RA dissolve rate as observed from the result of SD2-NC at initial phase. Apart from the surface area, the more surface roughness and porosity from freeze drying process give a better performance of release profile compared with those from spray drying process.

CONCLUSION

Rosmarinic acid, which was used as a model of natural bioactive substances in this study, can be encapsulated in the particles made of sodium alginate. Sodium alginate, which is an anionic polysaccharide with linear chain copolymer and the property of biocompatibilty, biodegradability and nontoxicity, can be performed as a polymer for encapsulation by using spray drying and freeze drying process. The non-crosslinked SD and crosslinked FD are suitable processes to make the encapsulated RA particles and have a potential to be further developed for pharmaceutical applications or drug delivery systems especially in the area of encapsulation of active substances.

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