

Research Article

Encapsulation of Willow Bark Extract with Potato Starch by Spray Drying

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Abstract

Willow bark extract has been known to have anti-inflammatory and anti-nociceptive functions for ancient times and used for traditional medicine. However, it has some drawbacks to apply in clinic application. Encapsulation is an excellent way to solve these problems. In this study, willow bark extract was encapsulated by starch as a wall material in order to improve bioavailability and stability of bioactive materials. Spray drying was applied to obtain powder composed of willow bark extract and starch. Particle size and morphological characteristics were investigated by scanning electron microscopy (SEM). The encapsulation was confirmed by Fourier transform infrared (FTIR) spectroscopic analysis and X-ray diffraction (XRD). Thermal properties, hygroscopicity and storage stability were also studied. The result revealed that it was possible to encapsulate bioactive materials in willow bark extract with starch. Encapsulated powder showed small size at micro level. The particles were spherical and amorphous shape with wrinkled surface. In addition, encapsulated powder exhibited better thermal and storage stability than free willow bark extract powder. The results of this study indicate that encapsulation with starch could improve bioavailability and stability of willow bark extract. This result will provide preliminary information for encapsulating bioactive materials in herb extract by using starch through spray drying.

Keywords

Willow Bark Extract, Starch, Encapsulation, Spray Drying, Bioavailability

1. Introduction

Willow, belonging to Salicaceae family contains notable amount of bioactive compounds [18]. Especially, willow bark, known to have anti-inflammatory and anti-nociceptive functions has been used as traditional medicine since ancient times [15, 20]. It was regarded that pharmaceutical activity

of willow bark was attributed to salicylate compounds after the discovery of aspirin. However, pharmacological studies have indicated that not only salicylate derivatives but also other active components of willow bark (e.g. polyphenols, flavonoids) play an important role in the analgesic and an-

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ti-inflammatory activity [14, 22]. Willow bark extracts contain different kinds of phenolic compounds such as gallic acid, caffeic acid, canillin, catechin, epigallocatechin gallate, rutin, quercetin and so on [5]. Therefore, willow bark extract may have different mechanism from aspirin [22].

Although bioactive compounds in willow bark extract exhibit various physiological activities, they have some drawbacks in clinic application. For example, saligenin, hydrolysis form of salicin in the stomach, is known to damage the gastrointestinal mucosa [3]. In addition, phenolic compounds in extract generally have low bioavailability and stability, which hinders their clinic application [16]. Willow bark extract is also difficult to handle because it is in liquid form.

The encapsulation approach could provide a perfect solution to these problems. Encapsulation has been studied for many years in food and medicine industry. It enables to improve bioavailability and stability of bioactive materials, to modify sensory properties of stimulant ingredients and to control release of drug [9]. In encapsulation process, selection of wall material is important. Wall materials in pharmaceutical and food industry should be GRAS (Generally Regarded As Safe), therefore natural polymers such as starch, Arabic gum, maltodextrin, pectin, cellulose and protein are currently available due to their non-toxicity, bioavailability and low cost. Among them, starch has gained a growing interest of scientists due to its advantages such as biocompatibility, low cost and hypoallergenic character [9, 10]. Previous studies on starch as a coating material have proved that starch has good characteristics such as high encapsulation efficiency, oxidation stability, releasing parameters and cellular absorption. Over the last decade, the research of using starch as a wall material has focused on multiple starch source and its modified forms to encapsulate active materials at micro- and nanoscale with and without other polymers [7-11].

Starches with type B crystallinity, such as potato starch could trap and protect bioactive molecules [9]. Researches proved that potato starch could produce more homogenous particles for encapsulating of phenolic compounds compared to maltodextrins [1].

Spray drying is the most used encapsulation technique due to its efficiency, continuous production and ease of industrialization. It is a technological process in which liquid product is changed into a powder in a single processing step [9]. Spray dryers utilize atomizer or spray nozzle to disperse the liquid. Water evaporation occurs when spray and drying medium contact. This technology is well established and straightforward.

In our preliminary experiment, we obtained yellow powder from willow bark extract by spray drying. However, this powder has some intrinsic drawbacks such as stickiness, hygroscopicity and astringency. Therefore, high molecular weight encapsulating agents such as starch should be used together to solve such problems.

There have been attempts to encapsulate bioactive mole-

cules using starch as a wall material. For example, yerba mate extracts, gulupa seed extracts, polyphenols from olive pomace, black berry extract, green tea bioactive compounds were successfully encapsulated by starch [8, 10, 11, 17, 21, 23]. However, to our knowledge, this is the first report to encapsulate willow bark extract by spray drying. The present study aimed to obtain powder from willow bark extract with starch by spray drying and characterize encapsulated material.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

Folin-Ciocalteu phenol reagent, salicin and gallic acid were purchased from Sigma Chemical Co. (USA). Native potato starch (amylose content 20-25%) was purchased from Samjiyon Potato Flour Production Factory (DPRK). All other reagents were of analytical grade.

2.1.2. Plant Material and Preparation of Willow Bark Extract

For the willow bark extraction, 2-year-old shoots of the willow trees (*S. Koraiensis* Anderson) growing around the riverside of Taedong in Pyongyang were harvested in April 2023. The shoots were cut to 30-cm-long pieces and immediately debarked. The obtained willow bark was dried in an air circulation oven (101-1AE, China) at 40 °C until constant weight and ground in a mill. The samples were packed in plastic bags and stored at 4 °C until preparation of willow bark extract.

Willow bark extract was prepared according to the methodology with some modification [20]. Briefly, ten kilogram of dry willow bark was added into a 100 L extraction system and filled with deionized water. The extraction temperature was 90 °C and the extraction time was 60 min. Finally, the extract was filtered and stored at 4 °C before experiment. Dry matter content of the prepared willow bark extract amounted to 3.5 wt%.

2.2. Methods

2.2.1. Encapsulation of Willow Bark Extract by Spray Drying

Potato starch (20 g) was suspended in 300 mL of distilled water. The mixture was gelatinized at 80 °C for 20 min with continuous stirring. Then, 600 mL of willow bark extract was dropped to the mixture under the constant stirring. The solution obtained was sprayed by a spray dryer (LPG-5, China). The inlet and outlet temperatures were 150 °C and 80 °C, respectively. Compression air pressure and feed velocity were 0.4 MPa and 2 L/h, respectively.

On the other hand, the same amount of willow bark extract

used for encapsulation was sprayed without starch addition to obtain willow bark extract powder. This free extract powder was used for control.

2.2.2. Characterization of the Encapsulated Powder

- 1) Salicin & total polyphenol content and encapsulation efficiency.

Encapsulation Efficiency (EE%) was measured according to the method [7]. Briefly, a certain amount of encapsulates was dissolved in water. This water suspension was centrifuged at 12 000 rpm for 10 min. The supernatant solution was collected and filtered with a 0.45 μ m FH membrane (Millipore, USA). Salicin content in supernatant (salicin residual) was quantified by HPLC (Shimadzu, Japan) according to the previous report [5]. Total polyphenol content in supernatant (polyphenol residual) was evaluated by Folin-Ciocalteu [8]. Total polyphenol was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried powder.

The Encapsulation Efficiency was calculated by using the following equation:

$$\text{EE (\%)} \text{ of salicin} = (\text{salicin content initial} - \text{salicin residual}) / (\text{salicin initial}) \times 100$$

$$\text{EE (\%)} \text{ of total polyphenols} = (\text{Phenolic compounds initial} - \text{Phenolic compounds residual}) / (\text{Phenolic compounds initial}) \times 100$$

- 2) Moisture content. The moisture content of the encapsulated powder was determined according to the method described by [11]. Samples were placed in an oven at 105 $^{\circ}$ C until reaching constant weight.
- 3) Morphology and particle size. Morphological feature and particle size of the spray dried powder were characterized by a Jeol scanning electron microscopy model JSM-6610A (SEM, JEOL, USA) at an accelerating voltage of 10 kV. The samples were coated with gold and examined at 200 \times , 500 \times , 1600 \times magnifications. Approximately 100 particles from different powders were measured to calculate their average diameter.
- 4) Zeta-potential. Zeta potential of encapsulated particles was determined to characterize particle suspension by zeta potential analyzer (Malvern, UK).
- 5) Fourier transform infrared (FTIR) spectroscopic analysis. FTIR experiments were performed to identify the functional groups present in native starch, willow bark extract and encapsulated particles. This experiment was carried out using Fourier Transform Infrared Spectrometer (Nicolet, USA) in the range of 400-4000 cm^{-1} .
- 6) X-ray diffraction (XRD). Willow bark extract, native starch and encapsulated powder were analyzed by an X-ray diffractometer (Siemens D5000, Germany) using a $\text{CuK}\alpha$ radiation source (40 KV, 40 mA). The scan range was 5 $^{\circ}$ to 70 $^{\circ}$ and the speed was 0.5 degrees with 2 θ /min.
- 7) Thermal stability and degradation behaviors. The

thermal stability and degradation behaviors were investigated by thermogravimetry/derivative thermogravimetry analysis. Each graph was obtained from a TGA 50-H (Shimadzu TGA 50-H, Japan). Approximately 7 mg of encapsulated powder, starch and willow bark extract were placed in aluminum pans and heated from 20 $^{\circ}$ C to 900 $^{\circ}$ C at a rate of 20 $^{\circ}$ C/min.

- 8) Hygroscopicity. Hygroscopicity was measured according to previous methodology with some modification [10]. Ten gram of encapsulated and free extract powder was placed in a container at 25 $^{\circ}$ C with a saturated NaCl solution, corresponding to a 75.3% relative humidity environment. The samples were weighed after 3 days. The hygroscopicity was measured in gram of adsorbed water per 100 g of dry solid.
- 9) Storage stability. Storage stability of the encapsulated powder and the control (free willow bark extract) were tested according to previous method [11]. Briefly, an amount of 10 g of encapsulated powder and willow bark extract powder were placed in closed petri dishes and stored in the dark at 4 $^{\circ}$ C for 50 days. Every 10 days, samples were taken and measured the total polyphenol content by Folin-Ciocalteu method.
- 10) Statistical analysis. Each assay was performed in triplicate (n=3). Results were expressed as the mean \pm standard deviation (SD). Analysis of variance (ANOVA) was used to evaluate the comparison of formulations.

3. Result and Discussion

3.1. Salicin, Total Polyphenol Contents and Encapsulated Efficiency

Spray drying is a powerful technology to encapsulate bioactive molecules. However, the main disadvantage of spray drying is the use of high temperature, which is not suitable for heat sensitive ingredients. On the other hand, if the temperature is too low, the produced powder may exhibit high moisture content. In previous literature, the author used a low temperature of 130 $^{\circ}$ C for preserving nutraceutical features of green tea extract [4]. Therefore, taking into account the heat stability of salicin (around 190 $^{\circ}$ C) and polyphenols in willow bark extract, the inlet temperature was set to 150 $^{\circ}$ C. After spray drying, the moisture content of the powder obtained was below 5% (3.7 \pm 0.3%), which implies good stability. The encapsulation efficiency was 75.71 \pm 0.02% (polyphenols) and 57.8 \pm 0.25% (salicin), respectively.

3.2. Particle Size and Morphology of Encapsulated Powder

Particle size plays an important role in the stability of the systems. The particle size and distribution are closely related

to the releasing behavior. The SEM result indicated that spray dried powder had micro level size with the average diameter of 17.9 μm , ranging from 8.2 to 27 μm .

The shape and morphology of the encapsulated particles were shown in Figure 1. As can be seen in Figure 1, the encapsulated particles had a regular, spherical shape with extensive dented surface. Formation of dented surfaces was presumed due to the shrinkage of the particles during the drying process [13].

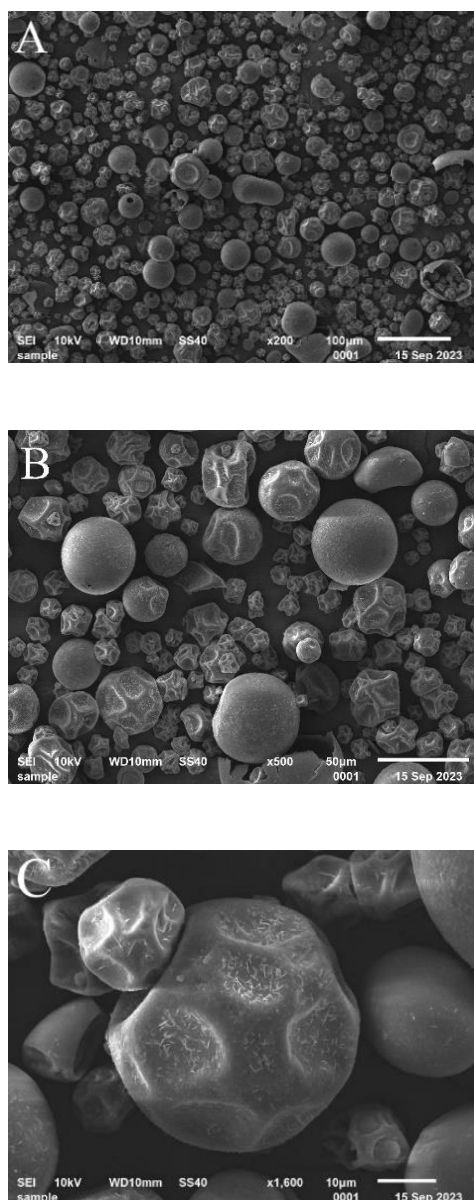


Figure 1. Scanning Electronic Microscopy of encapsulated powder. (A) 200 \times (B) 500 \times (C) 1600 \times Magnification.

3.3. Zeta-Potential

Zeta-potential is an important parameter to evaluate suspension of particles. High zeta potential values mean electrostatic repulsion, which is ideal for the stability. Zeta-potential

of encapsulated particle was -18.5 mV (Figure 2), similar to -20.1 mV published in previous result where catechin was encapsulated by using starch [2].

If the zeta potential of a solution is between -18 mV and +18 mV, this solution may be instable and inappropriate for intravenous administration. However, because our goal was to develop willow bark extract carrier for oral administration, we assumed that it does not cause any serious problem. Negative charge of the particle may be attributed to the particles with loading negative charged polyphenols.

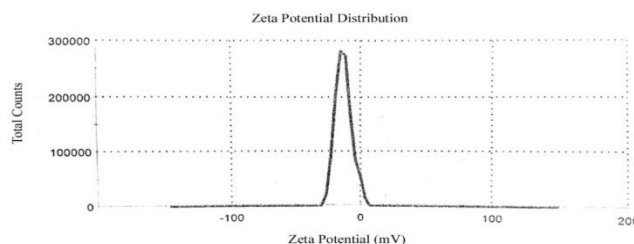


Figure 2. Zeta-potential of encapsulated particles.

3.4. FTIR

Figure 3 depicts the FTIR spectra of native starch, willow bark extract and encapsulated particles. As shown in Figure 3, the FTIR spectra of native starch and encapsulated particles showed similar intensity bands with slight differences in some peaks. The broad absorption peak around 3400 cm^{-1} was attributed to hydroxyl group and its inter and intramolecular interaction. Compared to starch and willow bark extract, in encapsulated particle, a shift towards lower frequency (3403 cm^{-1}) was observed, suggesting the formation of intermolecular hydrogen bonds.

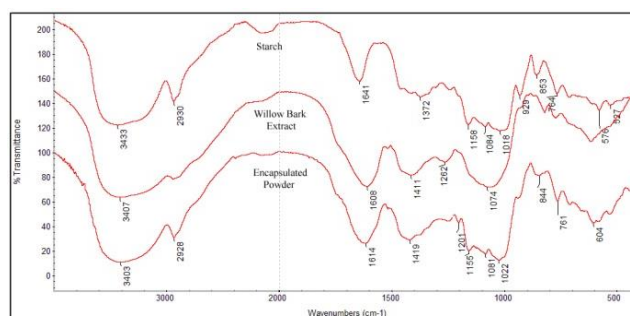


Figure 3. FTIR spectra of starch, willow bark extract and encapsulated powder.

The other characteristic bands at around 2900 cm^{-1} were assigned to $-\text{CH}_2$ stretching vibrational modes and that at around 1640 cm^{-1} was attributed to bound water. Willow bark extract showed several characteristic bands: the broad band around 3400 due to O-H stretching of hydroxyl groups; a band around

1620, 1450, 1270 and 1070 due to the stretching of aromatic rings. Notably, willow bark extract and encapsulated extract showed almost identical characteristic spectrum, especially from 800-1500 cm^{-1} (fingerprint region), indicating that integrity of willow bark extract was preserved after spray drying and bioactive molecules were incorporated in the starch [4].

3.5. XRD

Generally, solubility of substances in amorphous state is higher than the one in crystalline state. Since solids in amorphous state have randomly arrangement of molecules and atoms compared to ordered arrangement in the case of a crystal, lower energy is required to dissociate them [12].

Figure 4 depicts the X-ray diffraction pattern of native starch, willow bark extract and encapsulated powder. As shown in Figure 4, the native starch exhibited a strong reflection peak at around 16.65° and several weak peaks at around 14° , 23° and 26° , indicating the existence of crystal structure. However, these peaks disappeared after encapsulation and diffused, fluctuated halo shape was observed. The XRD patterns demonstrated that encapsulation by spray drying could decrease crystallinity of starch. Similar results were found in previous report [19].

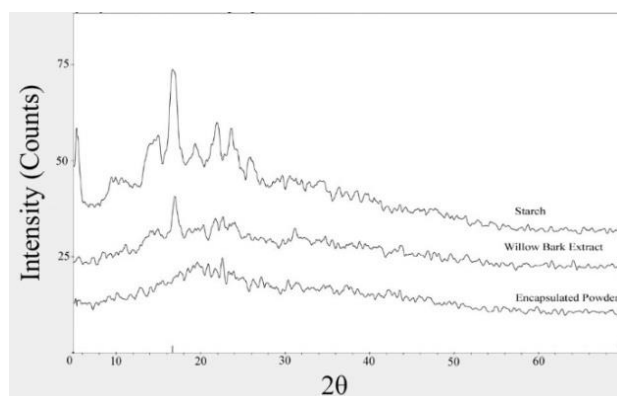


Figure 4. XRD of native starch, willow bark extract and encapsulated powder.

3.6. Thermogravimetric Analysis/Derivative Thermogravimetry (TGA/DrTGA)

The thermogravimetric properties of three samples-willow bark extract, starch and encapsulated powder were described in Figure 5. The thermal degradation of starch and encapsulated powder showed three stages while free willow bark extract showed constant decrease profile with multiple steps. In TGA figure of starch and encapsulated powder, the first mass loss around 140-150 $^\circ\text{C}$ could be attributed to the loss of volatile compounds, especially dehydration of samples. The second stage may correspond to degradation of the glycoside bonds of starch. The final stage may be due to thermal degradation of glucose ring of starch and the decomposition of residual carbon

[6]. Notably, thermal degradation of encapsulated powder occurred earlier than that of starch, which implies particles loaded willow bark extract were more thermally instable than native starch. However, in comparison of free willow bark extract, degradation of encapsulated powder was initiated at a higher temperature, indicating that a thermal protection of willow bark extract could be achieved by encapsulation process under around 200 $^\circ\text{C}$. This result was similar to previous report [11].

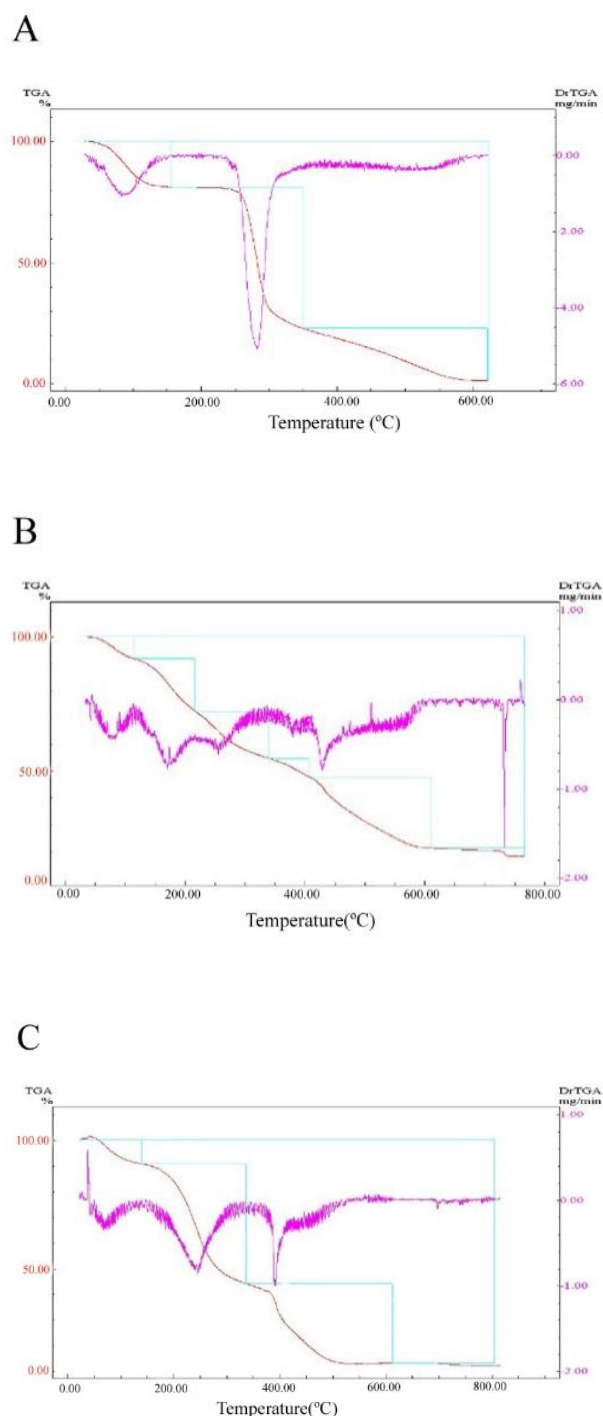


Figure 5. Thermogravimetric properties of starch (A), willow bark extract (B) and encapsulated powder (C).

3.7. Hygroscopicity

The willow bark extract powders and encapsulated powders were stored in 75.7% relative humidity at 25 °C. After 3 days in this condition, the willow bark extract powders absorbed water, causing hardening and darkening of the powders. It could be due to carbohydrates in willow bark extract. In contrast, encapsulated powder stayed low moisture content and original color. At this point, measured hygroscopicity of willow bark extract powder and encapsulated powder was 26.7 ± 0.5 and 14.9 ± 0.4 g/100 g, respectively. The lower hygroscopicity of encapsulated powders showed its good storage stability.

3.8. Storage Stability

The changes of total polyphenols in willow bark extract and encapsulated powder for the period of 50 days were shown in Figure 6. Total amount of polyphenols in willow bark extract decreased as time went by whereas it was relatively stable in the case of encapsulated powder. From this result, it is possible to induce that starch could provide the protective effect on the phenolic compounds during the storage. These results were consistent with previous reports [11].

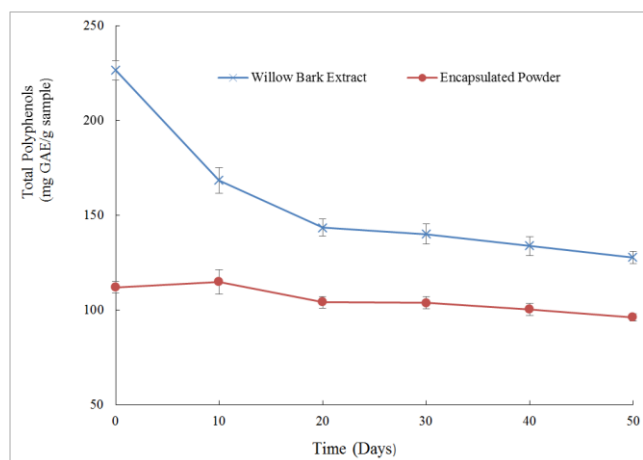


Figure 6. Storage stability of willow bark extract and encapsulated powder at 4 °C for 50 days. Values represent the mean \pm standard deviation.

4. Conclusion

The result of present study proved that starch can trap bioactive compounds like salicin and polyphenols in willow bark extract. Spray-drying of willow bark extract with potato starch produced small, homogeneous and stable powder suitable for oral administration. In addition, FTIR showed that the intrinsic feature of willow bark extract was preserved after spray drying. Crystallinity decreased after encapsulation. The obtained powder exhibited low moisture and hygroscopicity and showed good stability during a long storage

time. In the future, several parameters relating to spray drying should be optimized to maximize pharmaceutical activity of willow bark extract.

Abbreviations

FTIR	Fourier Transform Infrared
SEM	Scanning Electron Microscopy
XRD	X-ray Diffraction

Author Contributions

Su-Chol Rim designed manuscript and performed experiment. Hang-Ryol Mang corrected and finalized manuscript. Il-Gon Kim conceptualized and performed experiment. Yong-Il Hwang, Il-Jin Un and Ryong-Hyon Gong analyzed data, revised draft and supervised.

Conflicts of Interest

The authors declare no conflicts of interests.

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