Characterization and Antioxidant Activity of Mahogany Bark Extract-loaded Chitosan Nanoparticles

Syamsul Falah, Sulistiyani, and Dimas Andrianto

Abstract

Nanoparticles-based drug delivery has been recognized to improve the solubility of poorly water-soluble drugs, prolong the half-life of drug systematic circulation by reducing immunogenicity, and releases drugs at a sustain rate. The present study reports on the characterization of mahogany bark extract-loaded chitosan nanoparticles and their antioxidant activity. Mahogany bark meal was extracted in boiled water for four hours. Chitosan-sodium tripolyphosphate (STPP) nanospheres were sonicated with ultrasonicator to obtain chitosan-STTP nanocapsules for 30 and 60 min and then were dried with spray dryer. The chitosan-STPP nanocapsules loaded by mahogany extract were then analysed for surface morphology and physical state by scanning electron microscope (SEM) and X ray diffraction (XRD), respectively. Antioxidant activity of the nanoparticles was evaluated by scavenging the 1,1-diphenyl-2-picrylhydrazyl (DPPH) using free radical method. Based on SEM data, the nanoparticle shapes were viewed to adhere to spherical shape. Spherical chitosan-STTP nanoparticles loaded with mahogany bark extract were obtained in the size range of 480 ~ 2000 nm and 240 ~ 1000 nm for 30 and 60 min of ultrasonication time, respectively. The antioxidant activity of the nanoparticles was lower than that of the native mahogany bark extract.

Keywords: Mahogany, chitosan, nanoparticles, spray drying, ultrasonication.

Introduction

Nanotechnology is an important and interesting study in physics, chemistry, biology, and engineering sciences. Japan and the United States are the leading countries on the nanotechnology research. Nanobiosystem and biomedical studies have been the priority research project in some countries like USA, England, Japan, Australia, and China (Malsch 2005). Nanoparticles-based drug delivery has been recognized to improve the solubility of poorly water-soluble drugs, prolong the half-life of drug systematic circulation by reducing immunogenicity, and release drugs at a sustain rate.

Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. Nanoparticles are defined as particulate dispersion or solid particles with a size in range of 10 ~ 100 nm (Mohanraj and Chen 2006). Nanoparticles can be prepared from natural product polymers as well as synthetic polymers. In this study, chitosan was used to perform the chitosan nanoparticles. Chitosan is a natural polymer derived from chitin by deacetylation. Chitosan is regarded as biodegradable, biocompatible, non-immunogenic, and noncarsinogenic, making it suitable for pharmaceutical application (Hejazi and Amiji 2003).

The hot water extract of mahogany bark was used to load the chitosan nanoparticles. Previous study of mahogany showed that the three high antioxidant compounds were isolated and identified from the acetone extracts, i.e. catechin, epicatechin, and swietemacrophyllanin (Falah *et al.* 2008). In addition, mahogany bark extracts have antidiabetic activity on the diabetic rats (Falah *et al.* 2010). The objectives of the present study were to prepare the mahogany bark extract-loaded chitosan nanoparticles and characterize thus prepare chitosan nanoparticles for their surface morphology, physical state, and to assess the antioxidant activity of the mahogany bark extract-loaded chitosan nanoparticles.

Materials and Methods

Preparation of Mahogany Bark Extract

Bark samples were obtained from Sumedang District area. The bark was chopped from the mahogany logs to yield bark chips. The bark chips were milled with Willey mill to get the 40 ~ 60 mesh bark meal in size. An amount of 2 kg of the bark meal was extracted by boiled water at 100ºC for 4 h, and then evaporated with rotary vacuum evaporator to afford the mahogany bark hot water extract (MBWE).

Preparation of MBWE-loaded Chitosan Nanoparticles

A 4 g of chitosan was dissolved in 200 mL of 2% acetic acid to afford chitosan solution (2%, v/v). The solution was divided into two Erlenmeyer flasks. A 50 mL of 0.5% wt/vol aqueous solution of sodium tripolyphosphate (STPP) was added dropwise to the chitosan solution in each flask. A 1 mL mahogany extract was added to one of those two flasks. Both flasks were sonicated for 30 and 60 min with ultrasonicator at an output power of 150 W, 20 KHz. The chitosan-STPP-MBWE suspension was then spray dried to obtain the STPP cross-linked chitosan nanosphere loaded with MBWE.

Surface Morphology of the Chitosan-STPP Nanoparticles

The surface morphology of the spray dried chitosan-STPP nanoparticles loaded with a MBWE was examined by means of a scanning electron microscope (SEM). The powders were previously fixed on a brass stub (Ø 1 cm) using double-sided adhesive tape and then were made electrically conductive by coating with a thin layer of platinum for 30 sec under the pressure of 2 Pa and at 30 mA. Photographs were taken at an excitation of voltage of 10 kV and at 10.000X magnification.

Detection of Nanoparticles Functional Groups by Fourier Transform Infrared (FTIR)

A 2 mg of the nanoparticles was mixed with 100 mg of KBr and then was pressed to make a pellet form. The formed pellets were assessed with infra red light at 400 ~ 400 cm^{-1} of wavelength range to detect the functional groups of samples.

X-Ray Diffraction (XRD) Study

The physical state of MBWE in the chitosan-STPP matrix was assessed by XRD study. A 200 mg of the samples was pressed in a 2 x 2.5 cm of alumunium plate and then was analyzed by XRD instrument at 1.5406 Å of wavelength.

DPPH Scavenging Activity Assay

The samples were re-dissolved in methanol and concentrations of 25 μgml^{-1} of each sample were used. In a total volume of 2 mL, the assay mixture contained 1000 μl of the samples and 1000 μl of DPPH (125 μM in methanol). The assay mixture was shaken and allowed to stand at room temperature in darkness for 30 min. The absorbance was then measured at 517 nm in a spectrophotometer. Rutin was used as a positive control. The capacity to scavenge the DPPH radical was calculated as follows:

$$\text{DPPH radical scavenging (\%)} = (1 - A/Ao) \times 100$$

where Ao is the absorbance of the mixture without a sample and A is the absorbance of the mixture with a sample after 30 min.

Results and Discussion

MBWE-loaded Chitosan Nanoparticles

Preparation of the mahogany bark extract-loaded chitosan nanoparticles was done by comparing the time of ultrasonication. Ultrasonication time of 30 and 60 min was performed to determine the size of the nanoparticles and the homogenity of the solution. The chitosan dissolved in 2% acetic acid was then mixed with 0.5% STPP. The addition of STPP was to form an ionic crosslinking between chitosan molecules, which can be used as an absorbent material (Mi *et al.* 1999). Phosphate has a function, among others, improve emulsification and reduce the use of surfactants.

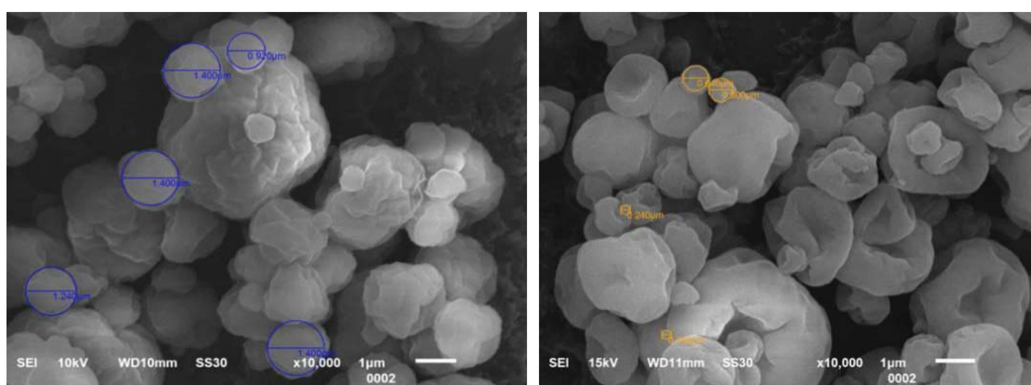
Sonicated chitosan nanoparticles were then dried using a spray dryer. Spray drying was undertaken to transform the liquid into the powder samples due to thermal effects (Figure 1).

Surface Morphology and Particle Size

SEM photographs of the mahogany bark extract-loaded chitosan nanoparticles with 30 min of ultrasonication time showed a variety of size in the range of 480 ~ 2000 nm while the nanoparticles with 60 min of ultrasonication time showed the size in the range of 240 ~ 1000 nm (Figure 2). The diversity of MBWE-loaded chitosan nanoparticle size in this study is quite large. The size difference is due to the effect of the ultrasonication time. Surface morphology of the mahogany bark extract-loaded chitosan nanoparticles is spherical. The result of the 30 min ultrasonication time have a smooth surface and a convex while the morphology of nanoparticles with a time of 60 min ultrasonication have irregular shapes with rough and wrinkled surface. Only a few are spherical with a smooth surface and convex. The materials that have been coated with chitosan on the surface of the ball are smooth and convex, while the chitosan without stuffing has a rough surface and concave. The chitosan nanoparticles not loaded by mahogany bark extract were caused by the length of ultrasonication time (60 min) which led to rupture and then chitosan cannot encapsulate the extract.

A B C

Figure 1. Spray dryed-chitosan nanoparticles. Chitosan nanoparticles (A), MBWE-loaded chitosan nanoparticles with 30 min (B) and 60 min (C) of ultrasonication time.



A B

Figure 2. Scanning electron microscope images of mahogany bark extract-loaded chitosan–STPP nanosphere with 30 min (A) and 60 min of ultrasonication times (B) (Magnification: 10,000X).

Functional Groups of the MBWE-loaded Chitosan Nanoparticles

The transmittance graph of FTIR resulted in the chemical profiles on different spectral pattern and a distinctive feature. Pure chitosan has a typical functional groups of amide group (-NH_2) and hydroxyl (-OH) while the mahogany bark extract has a particular groups of -OH, C=O, and C=C. In this study, the extract from bark of the mahogany was successfully coated by chitosan. This is evidenced from the graphs of FTIR that mahogany bark extract-loaded chitosan nanoparticles with ultrasonication time of 30 and 60 min have a graphical form similar to nanochitosan (Figure 3).

The Mahogany bark extract having functional group of C=O appeared at wavenumber of 1734 cm^{-1}, while the mahogany bark extract nanoparticles with a 30 min of ultrasonication time appeared at wavenumber of 1736 cm^{-1}. In addition, the functional group C=C of the mahogany bark extract emerging at wave number of 1615 cm^{-1} compared to that of the nanoparticles with a 30 min of ultrasonication time which at the wavenumber of 1650 cm^{-1}. Functional groups of C=O emerged at the wavenumber of 1282 cm^{-1} and 1251 cm^{-1} for the mahogany bark extract and chitosan nanoparticles with a 30 min of ultrasonication time, respectively. However, the C ≡ C functional groups in the chitosan nanoparticles have shifted compared to the standard nanochitosan. Wavelength shifting is due to the interaction among functional groups other than the functional groups of chitosan by the addition of STPP and ultrasonication time (Table 1).

These results may indicate that the chitosan nanoparticles with 30 min of ultrasonication time were successfully loaded by the mahogany bark extract. It is based on a typical function groups which are owned by the bark extract and mahogany bark extract nanoparticles with a 30 min of ultrasonication time. This data is also supported by the results of SEM images for mahogany bark extract-loaded chitosan nanoparticles with 30 min of ultrasonication time.

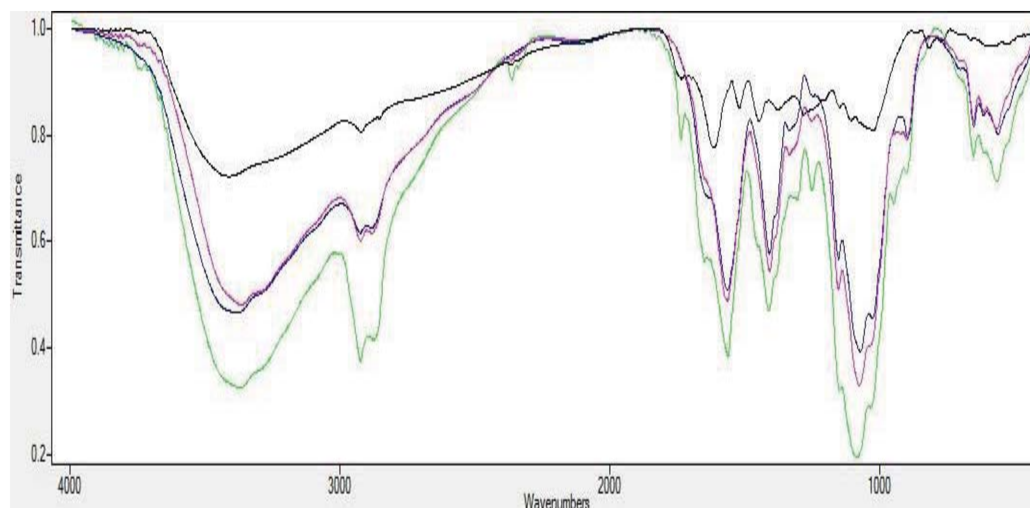


Figure 3. FTIR transmittance of mahogany bark extract (black), chitosan standard (blue), mahogany bark extract-loaded chitosan nanoparticles with 30 min (green) and with 60 min (pink) of ultrasonication time.

Table 1. Wavelength number of functional groups presenced at the mahogany extract, chitosan, and the mahogany bark extract-loaded chitosan nanoparticles with 30 and 60 min of ultrasonication time.

Functional Groups	Wavelength Number (cm^{-1})				
	Mahogany	Chitosan	Ultrasonication time 30 min	Ultrasonication time 60 min	Reference (Creswell *et al.* 1991)
Stretch –OH	3411	3374	3364	3366	3750~3000
Stretch C-H	2920	2923	2923	2922	3300~2900
Stretch C≡C	-	2123	2362	-	2400~2100
Stretch C=O	1734	1763	1736	-	1900~1650
Stretch N-H	-	1564	1562	1564	1660~1500
Stretch C=C	1615	-	1650	-	1675~1500
Bending C-H	1448	1409	1411	1408	1475~1300
Bending C-O	1282	-	1251	-	1300~1000
Bending C=C	817	896~653	949-653	899-653	1000~650

Physical State of the Chitosan Nanoparticles

XRD analysis of the chitosan–STPP nanospheres was performed in order to characterize the physical state of the loaded extract in the chitosan–STPP matrix. The characteristic of XRD spectra of the mahogany bark-loaded chitosan-STPP nanospheres, with 30 and 60 min of ultrasonication time are presented in Figure 4.

The XRD analysis of the MBWE-loaded chitosan nanoparticles with both 30 and 60 min of ultrasonication time showed an amorphous nature. The property was characterized by the diffraction peak at around 20º. Diffraction peaks of the nanoparticles with the 30 and 60 min of ultrasonication time were at 21.06º and 21.80º, respectively. The degree of crystallinity values obtained from the nanoparticles with the 30 and 60 min of ultrasonication time were 46.59 and 58.68%, respectively. Those data showed that the crystallinity is high enough. It can be ascertained that the mahogany bark extract had loaded the chitosan nanoparticles.

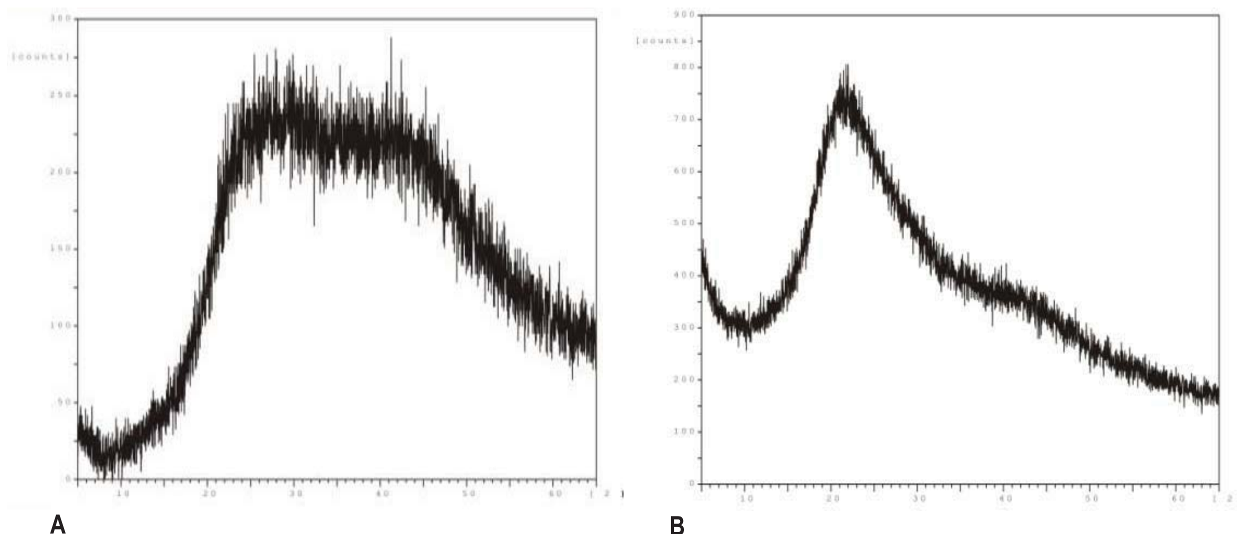


Figure 4. Patterns of x-ray diffraction of the MBWE-loaded chitosan nanoparticles with 30 min (A), and 60 min of ultrasonication time.

Antioxidant Activity of the Mahogany bark extract-loaded Chitosan Nanoparticles

Mahogany bark extract nanoparticles with 30 and 60 min of ultrasonication time had IC_{50} values of more than 100 ppm. IC_{50} values for nanochitosan and chitosan were at concentrations above 1000 ppm. Hanani *et al.* (2005) stated that a substance has a strong antioxidant activity if it has an IC_{50} value of less than 200 ppm. IC_{50} value of the average of all samples is shown in Table 2.

Table 2. IC_{50} value of the samples.

Extracts	IC_{50} (ppm)
Rutin	9.55
Hot Water Extract	18.15
Nanoparticles (30 minute)	>100
Nanoparticles (60 minute)	>100
Nanochitosan	>100
Chitosan	>1000

DPPH scavenging activity assay showed that mahogany bark extract had high activity compared to that of rutin as reference. In addition, the native mahogany bark extract had higher activity than that of the mahogany bark extract-loaded chitosan nanoparticles. However, the extract encapsulated by chitosan nanosphere showed a low activity even with the samples was sonicated at 30 and 60 min of ultrasonication time. The small IC_{50} values of chitosan, nanochitosan, mahogany bark extract-loaded chitosan nanoparticles with 30 and 60 min of ultrasonication time can be caused by the degree of deacetylation of chitosan samples, the molecular weight of chitosan, and the incubation time after addition of DPPH (Yen *et al.* 2008).

Chitosan had very low DPPH scavenging activity at 25 ppm of concentration and less soluble in methanol. The activity increased slighty after performing in nanoparticles form than bulk material of chitosan (Figure 5). Therefore, it needs time to release the extract for scavenging the free radical DPPH. The antioxidant activity of chitosan samples with an incubation time of 120 min is more effective than chitosan samples with an incubation time of 60 and 90 min (Yen *et al*. 2008).

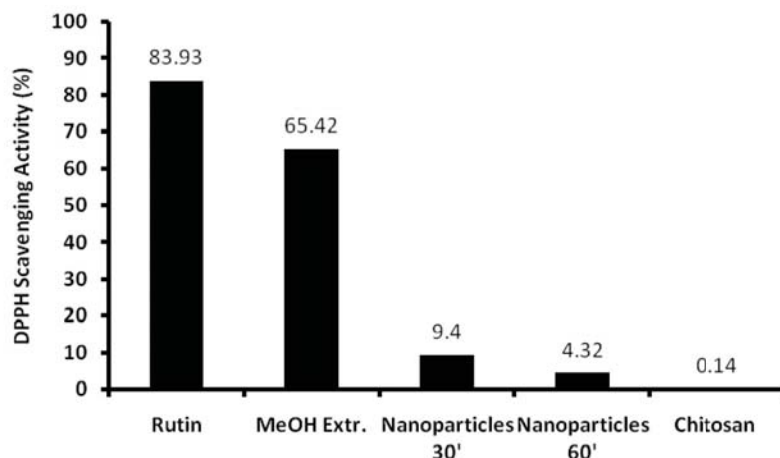


Figure 5. DPPH scavenging activity of the MBWE-loaded chitosan nanoparticles, mahogany bark extract, and rutin as a standard (at concentration 25 ppm).

Conclusions

Chitosan nanoparticles were successfully loaded with mahogany bark extract in the range of 480 ~ 2000 nm and 240~1000 nm in particle size at 30 and 60 min of ultrasonication time, respectively. The DPPH scavenging activity of the mahogany bark extract-loaded chitosan nanoparticles was lower than that of the native mahogany bark extract.

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Syamsul Falah, Sulistiyani, and Dimas Andrianto
Department of Biochemistry, Faculty of Mathematics and Natural Sciences
Bogor Agricultural University
Jalan Agathis Gd. Fakultas Peternakan
Lt. 5 Wing 5 Kampus IPB Darmaga, Bogor Jawa Barat
Tel : +62-251-8423267
Fax : +62-251-8423267
E-mail : syamsulfalah@yahoo.com