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VOL 13, NO 01 (2019)

ASIAN JOURNAL OF PHARMACEUTICS

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Development of Andrographolide-chitosan Solid Dispersion System: Physical Characterization, Solubility, and Dissolution Testing

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Abstract

Objective: The purpose of this study was to determine the effect of various chitosan concentration on the psychochemical characteristic, the solubility, and dissolution of andrographolide-chitosan solid dispersion system. **Materials and Methods:** Andrographolide-chitosan solid dispersion system was prepared by solvent wetting-spray drying method in various drug to polymer ratio of 1:1, 1:2, 1:3, 1:4, and 1:5. The results were evaluated for morphology, physical state, drug content, solubility, and dissolution rate. **Results and Discussion:** The andrographolide-chitosan solid dispersion system particles showed spherical shapes analyzed by scanning electron microscopy. Infrared spectra of solid dispersion systems showed a similar absorption pattern as the drug compound. Differential thermal analysis and X-ray diffraction analysis showed a reduction in melting point and crystal intensity. The study revealed a 1.75 times increase in andrographolide-chitosan solid dispersion system solubility and 3.1 times for dissolution rate compared to andrographolide compound, respectively. **Conclusion:** An increase in chitosan amount in andrographolide-chitosan solid dispersion system resulted in decreasing crystallinity and a melting point of andrographolide, which had a positive effect on increasing solubility and dissolution rate.

Key words: Andrographolide, chitosan, solid dispersion, solvent wetting method, spray-drying

INTRODUCTION

Andrographolide possesses several important biological activities, including hepatoprotective, immune system enhancer, anti-inflammatory, antioxidant, antidiarrheal, and antimalarial agent. Low andrographolide solubility ($3.29 \pm 0.79 \mu\text{g/mL}$) in water and very lipophile character ($\log P = 2.632 \pm 0.135$) result in a very low bioavailability (2.67%).^[1-3] One method that can be used to improve solubility is the preparation of nanoparticles, whereas the reduction of particle size can elevate surface area and increase solubility. However, the lack of this system is at very small sizes can cause agglomeration and aggregation, to prevent this problem a solid dispersion system is chosen.^[4] Solid dispersion is the distribution of one or more active ingredients in the carrier in the form of an inert matrix prepared by a melting method, solvent wetting method, or combination.^[5,6] Solid dispersion systems have advantages such as particle size reduction, improving the

wetting process, physical change, and molecular dispersed that change the solubility of the drug in water and easily applied and produced in comparison to prodrug.^[6,7] The use of polymers as carriers of solid dispersion systems can reduce the crystallinity of the drug ingredients become more amorphous. The amorphous formation can reduce the melting point of medicinal ingredients, so the energy needed to dissolve the drug ingredients becomes lower than the crystal shape that has a regular lattice.^[8]

Chitosan is a natural polymer derived from chitin derivatives which have N-deacetylation. This polymer is widely used due to its biocompatible, biodegradable, and non-toxic

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Received: 14-11-2018

Revised: 02-01-2019

Accepted: 27-01-2019

effect.^[9] Chitosan is hydrophilic because it has an amine group that is easily protonated.^[10] The formation of a solid dispersion system from chitosan polymer extend the surface area, inhibit recrystallization, wetting effect, solubilization, and dissolution rate so that the drug bioavailability was increased.^[7,11]

Solid dispersion system was prepared by the spray drying method, which the drug and polymers dissolved in each solvent and dried by spray drying. This process was able to reduce high humidity and prevents chemical damage to the drug.^[7,8] The aim of this study is to determine the effect of increasing chitosan amount on physical characteristics, solubility, and dissolution of andrographolide-chitosan solid dispersion system prepared by the spray-drying method. The evaluation includes morphology, X-ray diffraction (XRD), melting point, solubility test, and dissolution rate were conducted.

MATERIALS AND METHODS

Andrographolide (RD Health Ingredients Co., Ltd.); Chitosan (87.5% degree of deacetylation, viscosity 1% 18.16 mPa.s, China Eastar Group Co., Ltd.); methanol pro analysis, NaOH, KH_2PO_4 , and aquadest were used.

Preparation of andrographolide-chitosan solid dispersion system

Andrographolide-chitosan solid dispersion system was formed by the solvent wetting method and spray dried. The solid dispersion were prepared with andrographolide-chitosan ratio of 1:1, 1:2, 1:3, 1:4 and 1:5. Chitosan was dissolved in 50 mL of 0.3% acetic acid solution. Andrographolide was dissolved in 10 mL of methanol. The solution of chitosan was added into andrographolide solution and stirred at a speed of 500 rpm for 1 h then spray dried. The process parameters were as followed: nozzle diameter 1.0 mm, inlet temperature 100°C, flow rate 3 mL/min, and 2 bar pressure.

Morphology evaluation

The shape and surface morphology of the particles was observed using scanning electron microscopy. Particles were embedded in a holder made of aluminum and coated with gold-palladium before analysis. Pictures were taken at various magnifications at 20.00 kV.

Fourier transform-infrared (FT-IR) analysis

Particles were made as a pellet by mixing with KBr powder then pressed with a hydraulic pump to form a transparent pellet. Sample observation was conducted at wavelength 4000–450 cm^{-1} (Jasco FT-IR 5300, Easton MD, USA).

Differential thermal analysis (DTA)

Thermal analysis of the sample was conducted with DTA (DTA FP-65 P-900 Thermal, Mettler Toledo, USA). About 5 mg of particles were placed in a crucible pan, sealed and observed for its thermogram. The thermogram was recorded at temperature 5–250°C with a heating rate 10°C/min.

XRD analysis

XRD analysis was conducted by Philips X'Pert X-ray diffractometer to evaluate sample's crystallinity. The light source employed was $\text{K}\alpha$ Cu Ni. The voltage and the current were set at 40 kV and 40 mA. Samples were analyzed at 2θ and angle between 5 and 40°. The diffractogram of the andrographolide-carboxymethyl chitosan particles was compared with diffractogram of andrographolide substance.

Drug content

The andrographolide content in solid dispersion system was determined by ultraviolet (UV)-Visible double-beam spectrophotometer (Hitachi UH5300, Japan) at a wavelength of 225 nm. An amount of 10.0 mg of sample was carefully weighed and dissolved in 5 ml of methanol, filtered with a 0.45 μm filter membrane and then analyzed at wavelength 225 nm. The analysis was replicated three times.

The drug content was calculated using the equations below:

$$\% \text{ Drug content} = \frac{\text{drug amount}}{\text{particle weight}} \times 100\%$$

Solubility test

Solubility test was carried out in 15 ml phosphate buffer, pH 7.0 \pm 0.05. The sample put into a shaker water bath with 120 rpm speed, at a temperature of 37.0 \pm 0.5°C. Samples were taken 1 mL and filtered with 0.45 μm Millipore filter paper. The absorbance was observed using UV-Visible double-beam spectrophotometer (Hitachi UH5300, Japan) with a wavelength of 225 nm. Solubility test was replicated 3 times.

Dissolution test

The dissolution test was carried out in 50 ml of phosphate buffer pH 7.0 \pm 0.05 at 37.0 \pm 0.5°C, and 120 rpm at water bath shaker. The andrographolide-chitosan, solid dispersion system, was weighed equivalent to 5.0 mg andrographolide and put into the media. 2.0 ml sample was taken intervals of 5, 15, 30, 60, 120, 180, 240, and 300 minutes. After sampling, 2.0 ml media were added. Samples were analyzed by UV-Visible double-beam spectrophotometer (Hitachi UH5300, Japan) at a wavelength of 225 nm. The amount of andrographolide dissolved from

the andrographolide substance and solid dispersion system of andrographolide-chitosan were calculated. The dissolution test was carried out 3 times of replication.

RESULTS AND DISCUSSION

Morphology evaluation

The results of the morphological evaluation of andrographolide-chitosan solid dispersion system with the ratio of 1:1, 1:2, 1:3, 1:4, and 1:5 can be seen in Figure 1. It was concluded that the higher the ratio of chitosan used in the formula, the more spherical shapes were formed and less andrographolide crystals were found.

FT-IR analysis

IR spectrum of andrographolide, chitosan, and andrographolide-chitosan solid dispersion system was presented in Figure 2. The spectrum of chitosan (b) showed

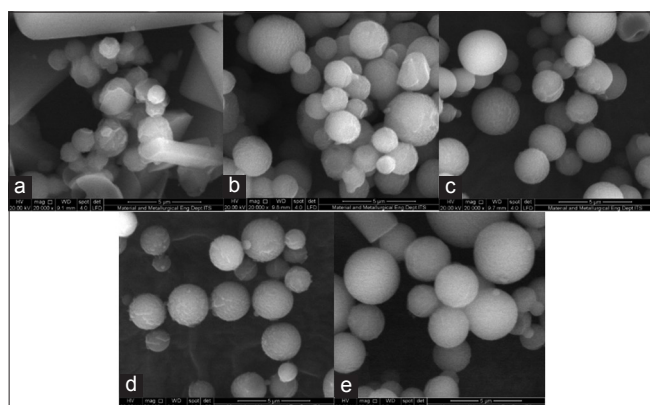


Figure 1: Scanning electron microscopy micrograph of andrographolide-chitosan solid dispersion system with various drug-polymer ratio (a) 1:1, (b) 1:2, (c) 1:3, (d) 1:4, and (e) 1:5 with $\times 20000$ magnification

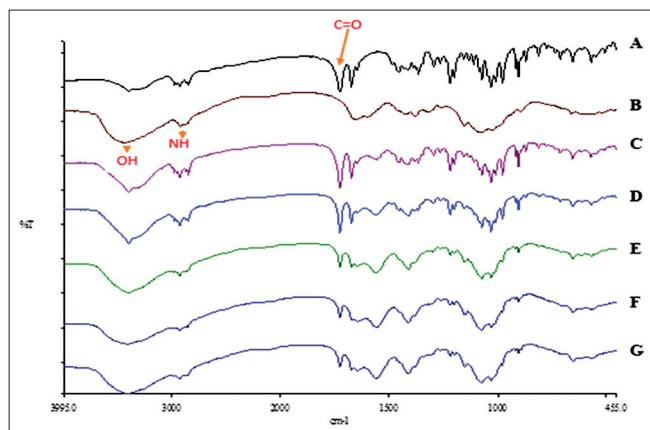


Figure 2: Infrared spectrum of (a) andrographolide, (b) chitosan, and andrographolide-chitosan solid dispersion system with various drug-polymer ratio: (c) 1:1, (d) 1:2, (e), 1:3 (f) 1:4, and (g) 1:5

a broadband at $3353\text{--}3284\text{ cm}^{-1}$ attributed to O-H and N-H stretching vibrations of the functional group engaged in intramolecular hydrogen bonding between chitosan molecules.^[12] There was a sharp peak found at wave numbers 1727.3 cm^{-1} which is the specific C=O spectrum from andrographolide (a), and also at $1725, 1680\text{ cm}^{-1}; 1640, 1480\text{ cm}^{-1}; 1220, 1240\text{ cm}^{-1};$ and $980, 1040, 1090\text{ cm}^{-1}$, may be due to the presence of C=O, C=C, C-O-C of lactone ring, and O-H group of alcohol, respectively, which also present in the molecular structure of andrographolide.^[13,14] An absorption of amide and amine groups was occurred in solid dispersion system (C to G spectra). The widening and shifting of the peak indicate that the intermolecular hydrogen bonds were formed between andrographolide and chitosan.^[15]

Thermal analysis using DTA

The results of the thermal analysis in Figure 3 showed the high and sharp melting peak of andrographolide occurred at 231.6°C . Andrographolide has an endothermic peak at 230°C .^[16] In the DTA thermogram of chitosan, there was a widened endothermic peak at 175°C . Endothermic peak

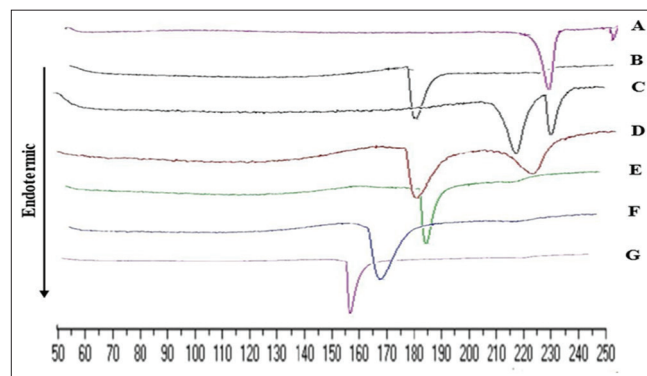


Figure 3: The differential thermal analysis thermogram of (a) andrographolide, (b) chitosan, and andrographolide-chitosan solid dispersion system with various drug-polymer ratio: (c) 1:1, (d) 1:2, (e) 1:3, (f) 1:4, and (g) 1:5

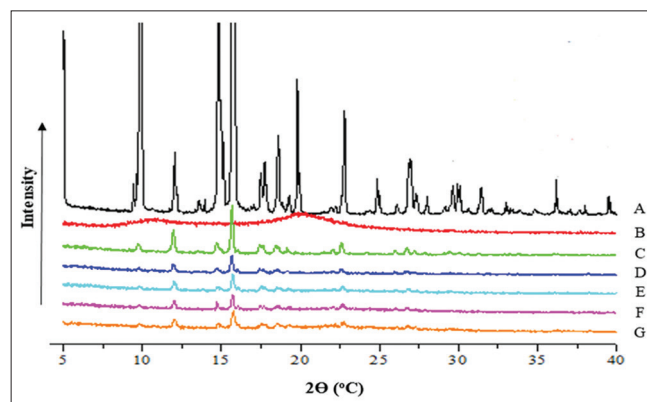


Figure 4: X-ray diffractograms of (a) andrographolide, (b) chitosan, and andrographolide-chitosan solid dispersion system with various drug-polymer ratio: (c) 1:1, (d) 1:2, (e) 1:3 (f) 1:4, and (g) 1:5

shifting was found in thermogram C to G, with lower melting point shown at 214.9°C and 227.4°C, 179.5°C and 218.8°C, 184.0°C, 166.4°C, and 155.1°C in andrographolide-chitosan solid dispersion system 1:1, 1:2, 1:3, 1:4, and 1:5, respectively. These result demonstrated the changes of crystalline form of andrographolide to amorphous form.

XRD analysis

Figure 4 showed the andrographolide diffractogram which has high intensity at $2\theta = 9.83^\circ$, 14.81° , 15.69° , and 15.85° . Andrographolide has a specific peak at $2\theta = 12.2$; 13.3 ; 15.4 ; and 25.9° .^[16] Chitosan diffraction was plateau-shaped with no apparent diffraction peaks; this indicated an arrangement of molecules that tend to be amorphous. In the solid dispersion system, there was a decrease in diffraction intensity which showed the reduction in particle size and change in the physical state of the crystal to an amorphous state.^[15,17]

Drug content

Drug content evaluation was carried out with spectrophotometry UV-Visible method. Determination of the content was carried out on an andrographolide-chitosan solid dispersion system 1:3 (F3), 1:4 (F4), and 1:5 (F5) because it only showed a single endothermic peak, low XRD, and spherical photomicrographs. The result of andrographolide content from the andrographolide-chitosan solid dispersion system for F3, F4, and F5 was $31.19 \pm 0.19\%$, $26.02 \pm 1.05\%$, and $28.47 \pm 0.36\%$, respectively.

Solubility test

The results of the solubility test in Table 1 showed that the solubility of F3 formula increased by 1.32 times, where F4 and F5 formula multiplied by 1.75 times than the andrographolide substances. The increase in andrographolide solubility was caused by the dispersion of andrographolide between chitosan matrixes so that it showed amorphization, increasing surface area, and andrographolide wetting ability.^[18]

Dissolution test

The dissolution test was carried out on a phosphate buffer pH 7.0 ± 0.05 . Figure 5 showed that the andrographolide dissolved from the solid dispersion increased compared to andrographolide substances.

The andrographolide dissolution rate was $3.79 \pm 0.64\% \text{ mg/min}^{-1}$, whereas the F3 solid dispersion system was $10.02 \pm 0.60 \text{ mg/min}^{-1}$, F4 $10.17 \pm 0.48 \text{ mg/min}^{-1}$, and F5 increased up to $12.08 \pm 0.74 \text{ mg/min}^{-1}$. Statistical analysis indicated that the release rate of andrographolide and solid dispersion systems in all formula was significantly different, whereas the solid dispersion systems of F3 and F4 were significantly different with F5 [Table 2].

These results indicated that the formation of a solid dispersion system improved the dissolution rate of andrographolide because an increase in surface area which raises the dissolution rates, inhibit recrystallization, wetting, and solubilization effects.^[18] In accordance with DTA thermogram and XRD diffractogram in Figure 3 and 4, the solid dispersion system showed a transformation of crystallinity to an amorphous state. The amorphous formation was able to reduce the melting point of the drug, so lessen energy was needed to dissolve than the crystal shape which has a regular lattice.^[8]

CONCLUSION

The formation of solid andrographolide-chitosan dispersion system affected drug characteristics, such as decreasing of drug crystallinity and lower melting point, caused a good impact to andrographolide solubility and dissolution rate. The solubility and dissolution rate of andrographolide was enhanced up to 1.75 times and 3.1 times compared to andrographolide substances, respectively.

ACKNOWLEDGMENT

This research was financially supported by The Directorate General of Strengthening for Research and Development,

Table 1: Solubility of andrographolide and solid dispersion system of andrographolide-chitosan with various drug-polymer ratio: (F3) 1:3, (F4) 1:4, and (F5) 1:5. in phosphate buffer media pH 7.0 ± 0.05

Sample	Drug solubility (ppm) \pm SD
Andrographolide	277.21 \pm 5.14
F3	374.31 \pm 3.32
F4	481.56 \pm 5.58
F5	484.82 \pm 0.63

SD: Standard deviation

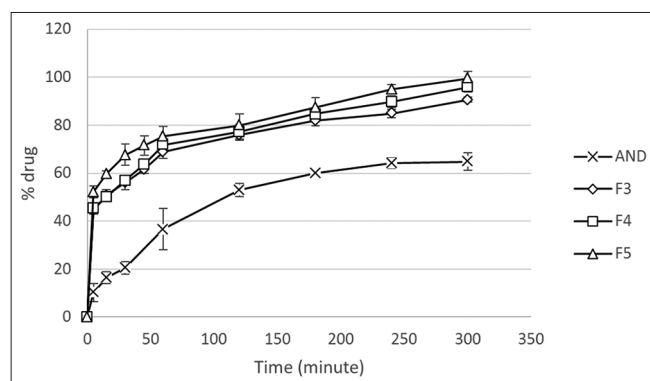


Figure 5: Dissolution profile of andrographolide (AND) and solid dispersion system of andrographolide-chitosan with various drug-polymer ratio: (F3) 1:3, (F4) 1:4, and (F5) 1:5 in phosphate buffer media pH 7.0 ± 0.05 at $37.0 \pm 0.5^\circ\text{C}$ ($n = 3$)

Table 2: The result of one-way ANOVA ($P=0.05$) statistical analysis of andrographolide and andrographolide-chitosan solid dispersion system release rate

Sample	n	Release rate (mg/mL min ^{1/2})	ANOVA	
			Result	Conclusion
Andrographolide	3	3.79±0.64**	F=107.399 P=0.000	Significantly different
F3	3	10.02±0.60**		
F4	3	10.17±0.48		
F5	3	12.08±0.74**		

**Indicates significant difference between sample groups. N=sample group

Ministry of Research, Technology, and Higher Education through the research grant of PDUPT 2018.

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Source of Support: Nil. **Conflict of Interest:** None declared.