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Contributed Paper

Solid-state Characterizations of the Inclusion Complexes between Warfarin Sodium and β-Cyclodextrin

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ABSTRACT

The sodium salt of warfarin has become the most widely used in order to improve the physicochemical properties of warfarin such as solubility, dissolution rate and mechanical properties. Nevertheless, warfarin sodium may be precipitated in the gastrointestinal tract leading to erratic in drug absorption. The purpose of this study is to investigate the inclusion complexes between β-CD and warfarin sodium by spray drying technique. The inclusion complexes were prepared at various β-CD and warfarin sodium mole ratio of 1:0.5, 1:1 and 1:1.5. The inclusion complexes were characterized by Infrared Spectroscopy (FT-IR), Nuclear Magnetic Resonance (NMR), and X-ray Diffractrometer (XRD). The presence of warfarin sodium in the inclusion complexes was confirmed when the bands of C=O vibration of warfarin sodium (1670 cm^{-1}, 1610cm^{-1}) were shifted upon inclusion complex compared to the bands in the free warfarin sodium (1720cm^{-1}, 1660cm^{-1}). In the 1H NMR spectrum of the β-CD-warfarin sodium (β-CD-WNa) inclusion complexes, the upfield shift of the protons lying on the inner surface of β-CD (H-3, H-5 and H-6) results from shielding effects, suggesting that warfarin sodium was included into the cavity of β-CD. These results confirmed the presence of the inclusion of warfarin sodium into the β-CD cavity. Furthermore, the morphology of spray dried powders was investigated by using SEM. The spray dried powder appeared in spherical shape with roughness.

Keywords: warfarin sodium, β-cyclodextrin, spray-drying, inclusion complexes

1. INTRODUCTION

CDs are cyclic oligosaccharides that produced by enzymatically catalyzed degradation of starch forming like a doughnut-shaped ring structure [1-3]. The most remarkable of CDs in industry are α-CD, β-CD and γ-CD, which consist of glucopyranose units 6, 7 and 8, respectively. The CDs have a hydrophilic exterior and a hydrophobic cavity that can accommodate a variety of suitable guest molecules. The

inclusion complex phenomena results in significant changes of solubility, dissolution rate, bioavailability, stability and reactivity of the guest molecules without any chemical modification [4-6]. b-CD and γ-CD are also listed in the generally regarded as safe (GRAS) list of the FDA for use as a food additive [7]. However, β-CD is the most widely used due to its availability, reasonable price and comparable cavity size for a variety of guest.

Warfarin (pK_a = 4.79) is an anticoagulant drug, which competitively depresses the synthesis of vitamin K-dependent coagulation factors [8]. Warfarin is almost insoluble in water and acidic media which are significantly affected on its bioavailability. The sodium salt of warfarin has become the most widely used in order to improve the physicochemical properties of warfarin such as solubility, dissolution rate, hygroscopicity, chemical stability, crystal form and mechanical properties [9]. However, warfarin sodium may be precipitated in the gastrointestinal tract leading to erratic in absorption. To overcome this limitation, the warfarin sodium complexation with β-CD has been employed in this study to enhance its physicochemical properties of the drug at low pH value.

In this study we aimed to investigate the inclusion complexes between warfarin sodium with β-CD by spray-drying technique. The inclusion complexes were prepared at various mole ratios of β-CD and warfarin sodium (1:0.5, 1:1 and 1:1.5). The inclusion complexes were characterized by Nuclear Magnetic Resonance (NMR), Infrared Spectroscopy (FT-IR) and X-ray Diffractrometer (XRD). Furthermore, the morphology of spray dried powders was investigated by using scanning electron microscope (SEM).

2. MATERIALS AND METHOD

2.1 Materials

β-CD was purchased from WACKER (The Sun Chemical Co.,LTD., Bankok, Thailand). Warfarin sodium was obtained from the Government Pharmaceutical Organization (GPO) (Bangkok, Thailand). Deuterium oxide 99.99 atom % D (D_2O) was purchased from Sigma Aldrich.

2.2 Method

2.2.1 Preparation of solid binary system

The preparation of solid binary system between β-CD and warfarin sodium was performed by spray drying technique. The β-CD were dissolved in water at 20 wt%. Warfarin sodium was added into β-CD slurry at the warfarin sodium: β-CD mole ratio of 0.5, 1, 1.5 and 2 (pH=8.3). The inclusion complexes were prepared by vigorously stirred at 25°C. After 18 h of agitation, the resulting solutions were drying by using a Büchi mini spray dryer model B-290 (Büchi Lab, Switzerland) with a standard two-fluid nozzle (0.7 mm diameter). Spray drying process conditions were as follows: atomizing air volumetric flow rate 10 m^3/hr, feed rate 10 mL/min, aspirator rate 100%, and inlet temperature was 160°C.

2.2.2 Nuclear Magnetic Resonance (NMR)

The ^{1}H-NMR spectra were recorded on AVANCE AV 500MHz spectrometer (Bruker, Switzerland) in D_2O. All measurements were performed at 25°C, using the pulse accumulation of 64 scans and LB parameter of 0.30Hz.

2.2.3 Fourier transformation-infrared (FTIR) spectroscopy

Infrared spectra were obtained by using Nicolet 6700 spectrometer (Thermo Company, USA). The samples were previously ground and mixed thoroughly with potassium bromide. The KBr disks were prepared by compressing the powder under mechanical force of 15t for 30 sec in a hydraulic press. The spectra were recorded by using standard spectral collection techniques and the rapid scan software in OMNIC7.0. Thirty two scans were obtained with a resolution of 4 cm^{-1}, from 4000 to 400 cm^{-1}.

2.2.4 X-ray diffractometry (XRD)

The powder X-ray diffraction patterns were recorded by using a Rigaku X-ray diffractometer, TTRAX III Multipurpose System model. The X-ray diffractometer was operated with an anode current of 10 mA and accelerating voltage of 20 kV. The data collection was performed using CuKα radiation scanning from 2 to 42° 2θ range by 0.02° at a scan speed of 2 sec/step.

2.2.5 Scanning electron microscopy (SEM) morphology

Surface morphology of spray dried powder was visualized by SEM (HITACHI S-3400N, Japan). A small amount of powder was mounted on a brass stub using a double-sided adhesive tape and vacuum-coated with a thin layer of gold by sputtering (HITACHI S-3400N, Japan). The samples were analyzed by operating at an accelerating voltage of 20 kV.

3. RESULTS AND DISCUSSION

Molecular encapsulation between β-CD and warfarin sodium can occur both in solution state and in solid state. The equilibrium between complexes and non-complex of warfarin sodium appeared in the solution state whereas free warfarin sodium was aggregated outside of the β-CD cavity during spray drying process. There are many techniques providing to investigate the inclusion complexes between β-CD and warfarin sodium. In this work, we employed Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier transform infrared spectroscopy (FTIR) and X-ray diffractiometry (XRD) as major techniques to observe the presence of the inclusion complexes. 1H-NMR can be directly used to observe the inclusion of warfarin sodium into β-CD cavity [10,11]. Figure 1 shows 1H-NMR spectrum of an inclusion complex between warfarin sodium and β-CD (mole ratio 1:1) in D_2O. This spectrum was compared to the spectrum of a β-CD solution in order to identify line shifts that could provide evidence for complexation phenomena. The change in chemical shift of all β-CD protons and the presence of inclusion complexes were presented in Table 1. 1H-NMR spectra of the inclusion complexes between β-CD and warfarin sodium was significantly up-field shifted in comparison with those of β-CD. In addition, the H-3 and H-5 atoms of β-CD which are located on the interior of the cavity showed more up-field shifts from 3.843 to 3.734 ppm and 3.734 to 3.582 ppm, respectively. The high-field shift changes in the β-CD cavity protons can only be attributed to the penetration of the warfarin sodium into the β-CD cavity, resulting in the formation of β-CD-WNa inclusion complexes. On the other hand, the H-1, H-2 and H-4 atoms which are located on the exterior of the cavity showed only minimal up-field shifts.

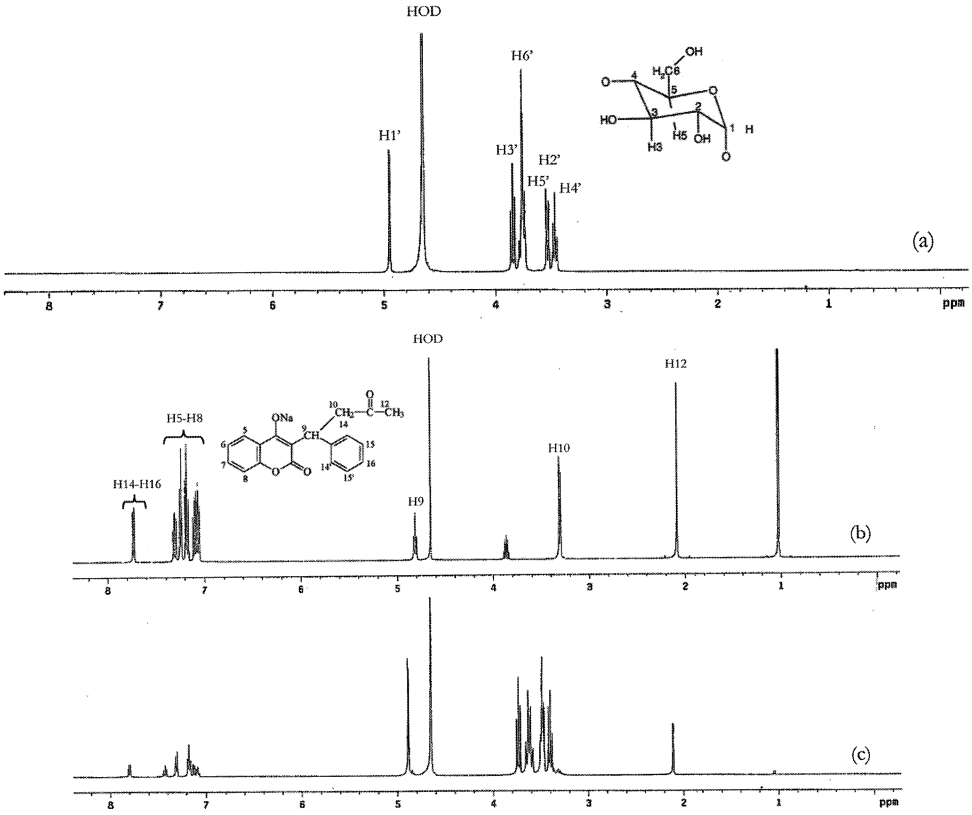

Figure 1. ^{1}H-NMR spectra of β-CD (a), warfarin sodium (b) and inclusion complexes between warfarin sodium and β-CD at mole ratio of 1:1 (c).

Table 1. The ^{1}H-NMR spectroscopy chemical shifts (ppm) of β-CD and the inclusion complexes between warfarin sodium and β-CD (at mole ratio of 1:1).

	β-CD (δ_0)	β-CD-WNa	Dδ (δ-δ_0)
H-1'	4.946	4.891	-0.055
H-2'	3.516	3.463	-0.053
H-3'	3.843	3.734	-0.109
H-4'	3.463	3.401	-0.062
H-5'	3.734	3.582	-0.152
H-6'	3.758	3.633	-0.125

Fourier transform infrared spectroscopy (FT-IR) has been used to characterize the interaction between β-CD and warfarin sodium in the solid state [10]. The chemical interaction between the warfarin sodium and b-CD often leads to identifiable changes in the infrared (IR) profile of complexes. The FT-IR spectra of β-CD (a) and warfarin sodium (b) were compared with the inclusion complexes (c-e) between β-CD and warfarin sodium (Figure 2). The result showed that the FT-IR of β-CD bands at 3340 cm^{-1} due to O-H stretching vibrations and the vibration of the -CH and CH2 group appears at the 2910 cm^{-1} region. The symmetrical bending band of water in β-CD hydrate appears at 1630 cm^{-1}. For the FT-IR spectrums of warfarin sodium, the

carbonyl vibration bands were observed as a series bands at 1720, 1660 cm^{-1} due to the C=O stretching. The analysis of the spectra from all inclusion complexes from spray dried powder shows the carbonyl stretching band shifted to a lower frequency (1670 and 1610 cm^{-1}). These results proved the presence of the solid complexes formation and suggested the carbonyl group may be not fully included within the β-CD cavity [12].

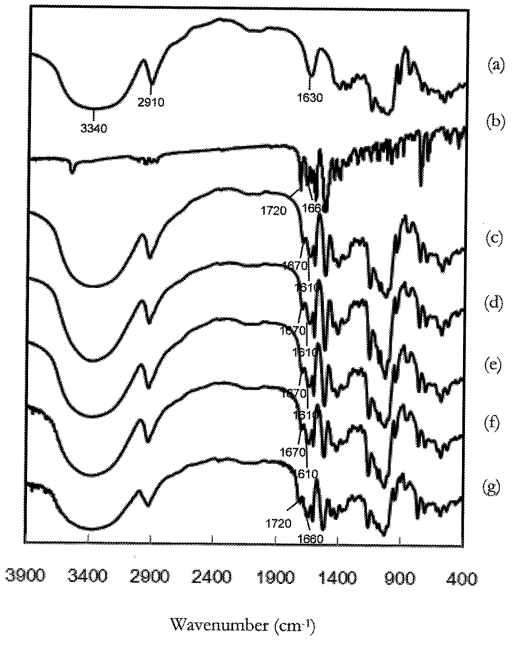

Figure 2. FT-IR spectra of β-CD (a), warfarin sodium (b), inclusion complexes between β-CD and warfarin sodium at mole ratio of 0.5:1 (c), 1:1 (d), 1.5:1 (e), 2:1 (f) and and physical mixture between warfarin sodium and β-CD at mole ratio of 1:1 (g).

Moreover, X-ray powder diffraction pattern was used to confirm the results of ^{1}H-NMR and FT-IR analysis. Figure 3 shows X-ray diffraction profiles of β-CD (a), Warfarin sodium (b), inclusion complex between -CD and warfarin sodium at mole ratio of 0.5:1 (c), 1:1 (d), 1.5:1 (e) and physical mixture between warfarin sodium and β-CD (f). The reduction of the degree of crystallinity of the warfarin sodium was found. A reduced number of signals were noticeable in the inclusion complexes, thus indicating a greater amount of amorphous form of the inclusion compounds, compared to the free warfarin sodium. However, the physical mixture between β-CD and warfarin sodium show the

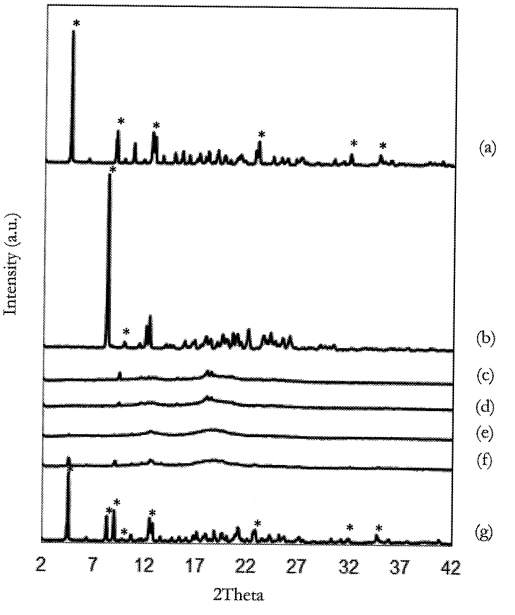

Figure 3. X-ray diffraction profiles of β-CD (a), warfarin sodium (b), inclusion complexes between β-CD and warfarin sodium at mole ratio of 0.5:1 (c), 1:1 (d), 1.5:1 (e), 2:1 (f) and and physical mixture between warfarin sodium and β-CD at mole ratio of 1:1 (g).

diffraction profiles same as β-CD and warfarin sodium.

The external structure of the spray dried particles was investigated by using scanning electron microphotographs. Figure 4 showed the SEM photograph of β-CD, warfarin sodium and spray dried powder of inclusion complexes between β-CD and warfarin sodium at mole ratio of 0.5:1, 1:1, 1.5:1. The SEM photographs of

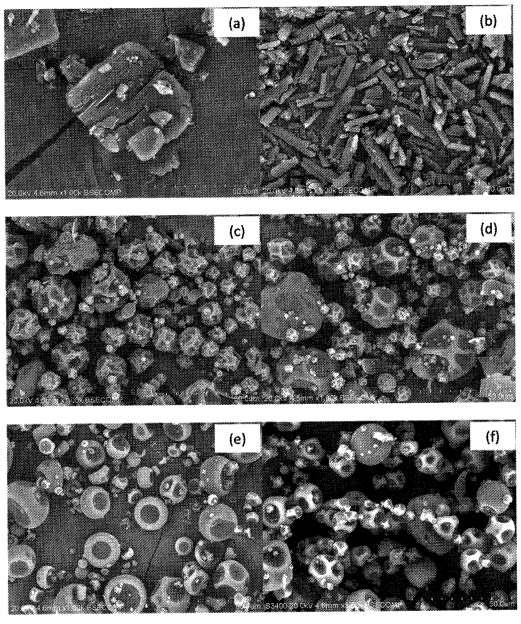

Figure 4. SEM photographs of β-CD (a), warfarin sodium (b) and spray dried powder of inclusion complexes between β-CD and warfarin sodium at mole ratio of 0.5:1 (c), 1:1 (d), 1.5:1 (e) and 2:1 (f).

native βCD appeared as in crystalline form as well as warfarin sodium. In contrast, spray dried powder of inclusion complexes between β-CD and warfarin sodium were found in spherical shape.

4. CONCLUSIONS

The inclusion complexes between warfarin sodium with β-CD were prepared by spray-drying technique. The characterization of inclusion complexes were successfully carried out by using Nuclear Magnetic Resonance (NMR), Infrared Spectroscopy (FT-IR) and X-ray Diffractrometer (XRD). These three techniques confirmed the formation of inclusion complexes in the solution state and in the solid state where a presence of carbonyl group of warfarin sodium penetrated into the β-CD cavity.

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REFERENCES

[1] Szejtli J., Past, present, and future of cyclodextrin research, *Pure Appl. Chem*, 2004; **76**: 1825-1845.

[2] Li Z., Wang M., Wang F., Gu Z., Du G., Wu J., Chen J., γ- Cyclodextrin: A review on enzymatic production and applications, *Appl Microbiol Biotechnol*, 2007; **77**: 245-255.

[3] Astray G., Gonzalez-Barreiro C., Mejuto J.C., Rial-Otero R. and Simal-Ga'ndara J., A review on the use of cyclodextrins in foods, *Food Hydrocolloids*, 2009; **23**: 1631-1640.

[4] Karathanos V.T., Mourtzinos I., Yannakopoulou K., Andrikopoulos N.K., Study of the solubility, antioxidant activity and structure of inclusion complex of vanillin with β-cyclodextrin, *Food Chem.*, 2007; **101**: 652-658.

[5] Sajeesh S. and Sharma C.P., Cyclodextrin-insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery, *Int. J. Pharma.*, 2006; **325**: 147-154.

[6] Redenti E., Szente L, and Szejtli J., Cyclodextrin Complexes of Salts of Acidic Drugs, Thermodynamic Properties, Structural Features, and Pharmaceutical Applications., *Journal of Pharmaceutical Sciences*, 2001; **90**: 979-986.

[7] Loftsson T., Jarho P., Másson M., Järvinen T., Cyclodextrins in drug delivery., *Expert Opin. Drug Deliv*, 2005; **2**: 335-351.

[8] Gao D., Physical Chemical Stability of Warfarin Sodium, *AAPS Pharmsci*, 2001; 3(1).

[9] Serajuddin A.T.M., Salt formation to improve drug solubility, *Advanced Drug Delivery Review*, 2007; **59**: 603-616.

[10] Cruz J.R., Becker B.A., Morris K.F. and Larivea C.K., NMR characterization of the host-guest inclusion complex between β-cyclodextrin and doxepin, *Magnetic Resonance in Chemistry*, 2008; **46**: 838-845.

[11] Ali S.M., Maheshwari A., Fozdar B.I., ^{1}H NMR spectroscopic study of complexation of citalopram with β-cyclodextrin in aqueous solution, *Magnetic Resonance in Chemistry*, 2007; **45**: 253-256.

[12] Sal□stio S.P., Feio G., Figueirinhas J.L., Pinto J.F. and Marques H.M.C., The influence of the preparation methods on the inclusion of model drugs in a β-cyclodextrin cavity, *Eur J Pharm Biopharm*, 2009; **7(2)**: 377-386.

[13] Chen M., Diao G. and Zhang E., Study of inclusion complex of β-CD and nitrobenzene, *Chemosphere*, 2006; 63: 522–529.