Formation of β-Cyclodextrin Inclusion Enhances the Stability and Aqueous Solubility of Natural Borneol

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Abstract: The aims of this study were to optimize the preparation conditions of natural borneol/β-cyclodextrin (NB/β-CD) inclusion complex by ultrasound method, and to investigate its improvement of stability and solubility. The complex was characterized by different various spectroscopic techniques including Fourier transform infrared spectroscopy, X-ray diffractometry, and differential scanning calorimetry. The results demonstrate that NB could be efficiently loaded into β-CD to form an inclusion complex by ultrasound method at a molar ratio of 1 : 1 and mass ratio of 1 : 6. The complex exhibited different physicochemical characteristics from that of free NB. Typically, formation of β-CD inclusion significantly enhanced the stability and aqueous solubility of NB.

Keywords: β-cyclodextrin; inclusion complex; Natural borneol; ultrasound method

Practical Application: Natural borneol (NB) has the potential to be widely used in the fields of medical and functional food, due to its specificity. However, the disadvantages of unstability in the preparation and storage process due to its easy sublimation and the low water solubility limit its application. This research provides an effective way to improve the solubility and stability of NB by preparing NB/β-CD inclusion complex. Furthermore, theoretical basis is also provided for the application development of NB.

Introduction

Borneol ($C_{10}H_{18}O$), a bicyclic monoterpenoid alcohol and one of the valuable medical materials, senior aromatic spices, and chemical materials, has been widely used in food and drug industries, typically in folk medicine in China and India. Its applications include treatment of abdominal pain, particularly stomachache (Wang and others 2006), injuries, burns, rheumatic pains, and ulcerations of the mouth, ear, eye, or nose. It has also been used in aromatherapy (Svoboda and Hampson 1999), and as an additive for foods and cosmetic products, such as candy, beverage, toothpaste, perfume, and skin care products (Kotan and others 2007).

There are two different types of borneol: synthetic borneol (SB) and natural borneol (NB). SB is a mixture of *d,l*-borneol and isoborneol. NB is mostly composed of *d*-borneol with >95.0% in weight content. Previous studies showed that SB was slowly degraded for storage and noxious camphor content might be as high as 45% to 97% (Zeng and He 2004), whereas NB was steady and nontoxic. Therefore, an effective strategy to avoid the toxic effects of camphor is to use NB instead of SB in medicine and food additive.

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Recently, many researchers have shown that NB is an excellent penetration enhancer. Wu and others (2001) have reported that NB can enhance the trans-oral mucosal absorption of insulin. Wu and others (2006) have also reported that NB can accelerate the absorption of puerarin and timolol maleate through the cornea *in vitro*. With anti-inflammatory, antibacterial, and permeation enhancing effects, NB has the potential to be widely used in the medical and functional food fields. However, the disadvantages of unstability in the preparation and storage process due to its easy sublimation with low water solubility have limited its application. To improve the solubility of bioproducts, various techniques such as solid dispersion and inclusion complexations, have been developed. Among these techniques, inclusion complexation with cyclodextrins (CDs) and their derivatives are the most useful method to enhance the aqueous solubility of poorly water-soluble bioproducts (Lee and others 2008).

CDs are a family of macrocyclic oligosaccharides known as α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), and γ-cyclodextrin (γ-CD), which are composed of six, seven, or eight α -(1,4) linked glycosyl units, respectively (Liu and Zhu 2007). β-CD is shaped like a truncated cone with a relatively hydrophobic cavity surrounded by the secondary -OH in the wider rim and the primary -OH in the smaller rim (Kadri and others 2005). The most notable feature of β-CD is its ability to form solid inclusion compounds (host-guest complexes) with a very wide range of solid, liquid, and gaseous compounds by molecular complexation, while the solubility, chemical reactivity, and thermal property have been shown to change dramatically (Li and Xu 2010). Thus, in the pharmaceutical and food processing industry, β-CD has been used to enhance the solubility, stability, and bioavailability of

doi: 10.1111/j.1750-3841.2012.02713.x

Journal of Food Science A Publication of the Institute of Food Technologists

bioproducts (Li and others 2005). Many techniques such as coprecipitation, grinding, and ultrasound have been developed to prepare inclusion complex. However, coprecipitation and grinding have some disadvantages such as low inclusion ratio and total recovery and high time and power consumption. In contrast, preparation of the inclusion complex using ultrasound method showed high inclusion ratio and total recovery, and the operation is very simple and easy to control (Liu and Zhu 2007).

In the present study, β-CD was used as a host molecule to prepare NB/β-CD inclusion complex to improve its stability and aqueous solubility by ultrasound method. The complexes were confirmed by different analytical techniques including Fourier transform infrared spectroscopy (FT-IR), X-ray diffractometry (XRD), and differential scanning calorimetry (DSC). Furthermore, the solubility and release behavior of the inclusion complexes of NB/β-CD were investigated. Our results demonstrate that formation of β-CD inclusion enhances the stability and aqueous solubility of NB.

Material and Methods

Materials

NB (chemical purity, with *d*-borneol >99.90%, molecular weight 154.24) was obtained from the Natural Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China. β-CD was purchased from Aldrich Chemicals Co. (Milwaukee, WI, USA). All other chemicals and solvents were of analytical grade and purchased from China National Medicine Co., Ltd. (Chongwen district, Beijing, China).

Preparation of NB/β-CD inclusion complex

The complex of NB and β-CD was prepared by using an ultrasound method. Briefly, β-CD was dissolved in 100 mL of distilled water. NB dissolved in 20 mL ethanol was slowly added into the β-CD solution with continuous agitation. The resultant mixture was treated by SB-3200 ultrasonic (Xinzhi Biotechnology, Ningbo, China). The final solution was maintained overnight at 4 °C. The cold precipitated NB/β-CD complex was recovered by filtration. The precipitate was washed with distilled water once and with 30% ethyl acetate twice to clear NB which was absorbed by the surface of β-CD and dried in a vacuum freeze dryer at −30 °C for 24 h. The final dry complex powders were stored in an airtight glass desiccator at room temperature. NB content among the inclusion complex was measured by gas chromatography (GC). The inclusion ratio of NB was calculated as follows:

$$\text{Inclusion ratio}(\%) = [\textit{NB content of inclusion complex (mg)}/\textit{NB content(mg)}] \times 100 \quad (1)$$

The total recovery was calculated according to the following equation:

$$\text{Total recovery}(\%) = [\textit{recovered power (mg)}/\textit{initial } (NB + \beta - CD)\ (mg)] \times 100 \quad (2)$$

Preparation of NB and β-CD physical mixture

β-CD was pulverized in ceramic mortars. The calculated amounts of NB and β-CD with a mass ratio of 1 : 6 were mixed together by a spatula until a homogeneous mixture was obtained.

Optimization of the preparation of inclusion complex

An orthogonal $L_9(3)^4$ test design was used for optimizing the preparation conditions. In this study, the ultrasonic method was used to prepare the inclusion complex. Nine extractions were carried out with mass ratio of NB and β-CD as 1 : 4, 1 : 6, and 1 : 8, with ultrasonic time 60, 80, and 100 min, and with ultrasonic temperature at 40, 60, and 70 °C, respectively, on the basis of the single-factor test. All the factors and levels were listed in Table 1.

Determination of NB in the inclusion complex

NB (250 mg) was dissolved in anhydrous ethanol (50 mL). Then 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mL solution were taken out and diluted to 10 mL with anhydrous ethanol, respectively. These samples were analyzed by GC (Agilent N9890, Palo Alto, Calif., USA). The concentration (X) and the peak area (Y) of NB had a good relationship as follows:

$$y = 1307x + 10.14, \quad R = 0.9994 \quad (3)$$

A 100 mg of the inclusion complex sample and 20 mL of ethanol were added into a 50 mL stoppered centrifuge tube bathed into an ultrasonic wave cleaner. NB was extracted by ethanol from the inclusion complex for 30 min under the ultrasonic condition. The supernatant containing NB was obtained by centrifugation at 2500 rpm for 10 min. The content of NB in ethanol was determined by GC with the calibration curve of NB.

Physicochemical characterization

GC. Quantitative analysis of NB was performed using a GC (Agilent N9890) with a split/splitless injection port and a flame ionization detection system (FID), under the following operational conditions: column HP-5MS fused silica capillary column (30 m×0.25 mm i.d.; 0.25 μm). Pure nitrogen (99.999%) was used as the carrier gas at a constant flow of 7.8 mL/min, and an injection volume of 1 μL was employed with an injector temperature of 250 °C. The oven temperature was initiated at 100 °C (held for 1 min), then raised at the rate of 10 °C/min to 170 °C, and held for 5 min at this temperature. Detector and column temperatures were 270 and 100 °C, respectively.

DSC. DSC analysis was carried out for NB, β-CD, their physical mixture, and the inclusion complex with a TA DSCQ100 differential calorimeter calibrated with indium (TA, New Castle, PA, USA). Each sample (2–5 mg) was heated in a crimped aluminum pan at a scanning rate of 10 °C /min from 0 to 250 °C under a nitrogen flow of 40 mL/min. An empty pan sealed in the same way was used as reference.

XRD. The powder X-ray diffraction was recorded using D8 ADVANCE diffractometer (Bruker, Karlsruhe, Germany), operated at a voltage of 40 KV and a current of 40 mA. NB, β-CD, their physical mixture, and the inclusion complex were analyzed in the 2 h angle range of (5–55) °C and the process parameters were set as scan step size of 0.02 °C and scan step time of 17.7 s.

Table 1–Factors and levels for orthogonal test.

	Level		
Variable	**1**	**2**	**3**
Mass ratio of NB and β-CD	1 : 4	1 : 6	1 : 8
Ultrasonic time (min)	60	80	100
Ultrasonic temperature (°C)	40	60	70

FT-IR. The FT-IR spectra of NB, β-CD, their physical mixture, and the inclusion complex were collected between 4000 and 800 cm^{-1} (mid infrared region) on a Tensor37 FT-IR spectrophotometer (Bruker) with 256 scans at a resolution of 4 cm^{-1}. NB was recorded on KBr plates. The physical mixture of β-CD and NB and their inclusion complex were ground with spectroscopic grade potassium bromide (KBr) powder and then pressed into 1 mm pellets (2 mg of sample per 200 mg dry KBr).

Phase solubility of NB/β-CD complex

Phase solubility studies were carried out according to the method described by Higuchi and Connors (1965). Excess amounts of NB (5 mg) were added to 5 mL of water solutions of β-CD at different concentrations ranging from 0 to 10 mM. Then the suspensions were sealed and shaken at (25 ± 2) °C for 72 h. After equilibrium was reached, the samples were filtered through 0.45 μm membrane filter and properly diluted. A small volume of the filtrates was withdrawn and determined for NB by GC (Agilent N9890). All samples were prepared in triplicate. The phase solubility profile was obtained by plotting the solubility of NB compared to the concentration of β-CDs. The apparent stability constant (K_c) of NB and β-CD complex can be calculated from the slope and the intercept of the linear segment of the phase solubility line, according to the following equation:

$$K_c = k/S_0(1-k) \quad (4)$$

where S_0 is the intrinsic solubility of NB in deionized water in the absence of β-CD and k is the slope of the straight line.

Release of NB from NB/β-CD complex

Release of NB from the inclusion complex at different conditions. Comparative tests involving the stability of physical mixture and inclusion complex were tested at 25, 60, and 80 and in the 3000LX light incubator, respectively. After a fixed period of time (days 0, 1, 2, 3, 4, and 5), the retention amount of physical mixture and inclusion complex were measured, respectively, to evaluate the embedding effect of the inclusion complex.

***In vitro* release study.** The *in vitro* release of NB from the inclusion complex and physical mixture were determined according to the method described by Haroun and El-Halawany (2010). Briefly, 50 mg NB/β-CD inclusion complex and physical mixture were placed in 300 mL of an acidic dissolution medium containing 0.05M sodium chloride that was adjusted to pH 1.5 by HCl at (37 ± 0.5) °C. The study was carried out by using RC-3-type Intelligent Dissolution Rate Test Apparatus (Xing Tianguang, Tianjin, China) with a speed of 100 rpm. At different time intervals (0, 10, 30, 60, 90, 120, and 150 min), 1 mL samples of the release medium were taken out and analyzed by GC (Agilent N9890), and the volume was replaced with 1 mL of fresh acidic dissolution medium (pH 1.5) after each estimation. The dissolution experiment was carried out in triplicate. The data represent the average from three independent experiments.

Statistical analysis

The obtained data were expressed as the mean ± standard deviation of triplicate determinations. Statistical analysis was performed using the software SPSS 13.0

Results and Discussion

Preparation of inclusion complex

In recent years, the inclusion complexes of β-CD have been successfully used to improve solubility, chemical stability, and bioavailability of a number of poorly soluble compounds. Various known methods used for the formation of the inclusion complexes such as coprecipitation, neutralization, kneading, spray drying, freeze-drying, solvent evaporation, and ball-milling and sealed-heating in the laboratory have extensively been studied (Yamada and others 2000). The preparation of inclusion complex was widely performed using the coprecipitation method in laboratory, due to its advantages of easy observation of the complex forming and the guest disappearing during the inclusion (Hedges 1998). In this study, the ultrasound method was selected to prepare the NB/β-CD inclusion complex. Through experiments designed based on orthogonal test, the optimum condition of the preparation of inclusion complex was 1 : 6 mass ratio of NB and β-CD, with ultrasonic treatment for 60 min at 40 °C. Under the optimum conditions, the total yield of NB/β-CD complex was 89.22%, and the inclusion ratio of NB was 96.53%. (Hadaruga and others 2007) reported that the nanoencapsulation yields of *A. sativum* L. bioactive compounds/α- and β-CD were >60%, the higher ones obtained for the case of β-CD. The driving forces between CDs and bioproducts that have been proposed to justify the complex formation are hydrogen bonds, van der Waals forces, hydrophobic interactions, and the release of "high-energy water" molecules from the cavity (Salústio and others 2009).

Physicochemical characterization of NB/β-CD complex

DSC analysis. DSC can be used for the recognition of inclusion complexes. When guest molecules were embedded into β-CD cavities, their melting, boiling, or sublimating points generally shifted to different temperature or disappeared (Marques and others 1990). The thermal curves of NB, β-CD, the physical mixture of NB and β-CD, and NB/β-CD were shown in Figure 1. The DSC results presented in Figure 1D showed an endothermic peak for NB at 216 °C, corresponding to the melting point. The thermogram of β-CD showed a wide endothermic peak at approximately 152 °C (Figure 1B). The broad endothermic peak was related to dehydration of water molecules that bind to cyclodextrin molecules (Kohata and others 1993; Marini and others 1996). The peaks at approximately 110 °C were observed for the case of the physical mixture of NB and β-CD (Figure 1A). This might be due to the elimination of included water molecules with different strengths of interaction with the CD ring (Kohata and others 1993). DSC curve of the physical mixture of NB and β-CD was a superimposition of individual components of NB and β-CD. A different pattern was observed in the thermogram of the NB/β-CD inclusion complex (Figure 1C). The endothermic peak at approximately 152 °C originally in the β-CD is slightly shifted to a higher temperature at 163 °C for the inclusion complex system, which can be explained by a major interaction between NB and β-CD. The exothermic peak associated with melting point of NB was not present in the DSC spectrum of the NB/β-CD complex, indicating that NB was protected from melting, as it was located inside the β-CD cavity, which offers an indirect proof of the formation of the NB/β-CD inclusion complex.

XRD analysis. XRD is a useful method for the detection of CD complexation in powder or microcrystalline states. The diffraction pattern of the complex was supposed to be clearly distinct from that of the superimposition of each of the components

if a real inclusion complex was formed (Veiga and others 1996). The peaks of the sample were intense and sharp, indicating its crystalline natural. As shown in Figure 2A, some sharp peaks at the diffraction angle of 2θ, 15.28 and 17.66, were present in the X-ray diffractogram of NB powder, suggesting that the powder was present as a crystalline material. The X-ray diffractogram of the physical mixture (Figure 2B) showed approximate superimposition of the individual patterns of both β-CD and NB. The β-CD crystallinity peaks remained detectable in the physical mixture with NB. The X-ray diffractogram of the inclusion complex (Figure 2D) showed a new diffraction peak. Some sharp peaks originally found at the diffraction angles of 2θ, 15.28 and 17.66, in the NB samples disappeared or weakened, and a few new sharp peaks at diffraction angles of 2θ, 5.37, 13.09, 14.24, and 16.12, appeared in the X-ray diffractogram of the complex sample, suggesting the formation of the NB/β-CD inclusion complex.

FT-IR analysis. As a confirmation technique for the formation of an inclusion complex, FT-IR had been shown to support the evidence for a complex formation between bropirimine and β-CD in solution and in the solid state (Ahmed and others 1991). The FT-IR spectra of NB, β-CD, the physical mixture of NB and β-CD, and NB/β-CD inclusion complex were present in Figure 3. The FT-IR spectrum of β-CD (Figure 3A) showed prominent absorption bands at 3381 cm^{-1} (for O-H stretching vibrations), 2922 cm^{-1} (for C-H stretching vibrations), 1153 cm^{-1} (for C-O stretching vibrations), and 1024 cm^{-1} (C-O-C stretching vibrations). The FT-IR spectrum of NB (Figure 3D) showed prominent absorption bands at 3350 cm^{-1} (for O-H stretching vibrations) and 2945 cm^{-1} (for C-H stretching vibrations).

The FT-IR spectrum of the physical mixture (Figure 3B) showed approximate superimposition of the individual patterns of both β-CD and NB. However, the FT-IR spectrum of the

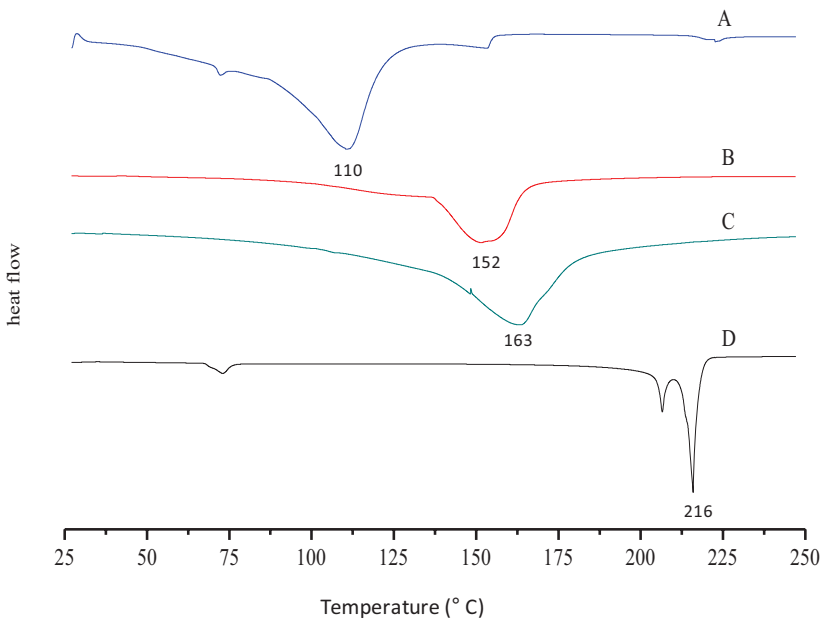

Figure 1–DSC thermograms of (A) NB and β-CD physical mixture, (B) β-CD, (C) NB/β-CD inclusion complex, and (D) NB.

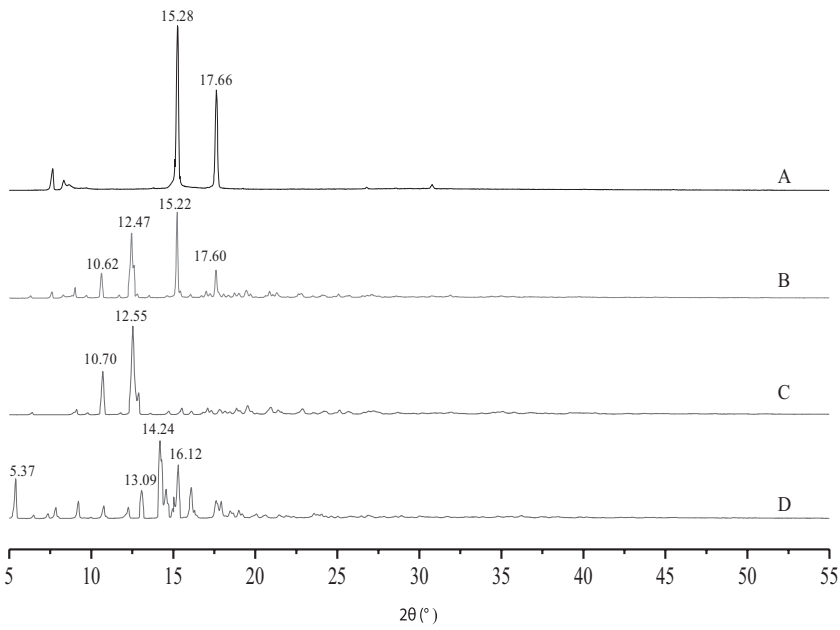

Figure 2–Powder X-ray diffraction patterns of (A) NB, (B) NB and β-CD physical mixture, (C) β-CD, and (D) NB/β-CD inclusion complex.

NB/β-CD inclusion complex showed no features similar to pure NB (Figure 3C). The bands located at 2945, 1463, 1381, and 1062 cm^{-1} of NB disappeared, and the NB bands were obscured by very intense and broad β-CD bands. However, the absorption bands at 3381 and 2922 cm^{-1} of β-CD were shifted toward these lower frequencies at 3354 and 2885 cm^{-1} of NB/β-CD, respectively. These changes may be related to the formation of intramolecular hydrogen bonds between NB and β-CD.

Phase solubility of NB/β-CD complex

The stoichiometry of NB/β-CD complex was determined by the solubility technique. The phase solubility profile of NB/β-CD complex was presented in Figure 4, which could be classified as B_s-type according to Higuchi and Connors (1965). The solubility of NB increased with the increment of the β-CD concentration in the range of 0–5 mM. When the concentration of β-CD was higher than 5 mM, the solubility of NB began to decrease. It might lead to the formation of a water-insoluble substance (Manolikar and Sawant 2003). According to Higuchi and Connors's theory (1965), the 1 : 1 molar ratio of the inclusion complex was achieved from the initial ascending part of the curve, a nearly straight line with the slope of 0.9703. The regression equation was as follows:

$$Y = 0.9703X + 0.8432, \quad R = 0.9993 \tag{5}$$

where Y is the concentration (in mM) of NB and X is the concentration (in mM) of β-CD. The apparent stability constant $K_{1:1}$ was obtained to be 38.812 M^{-1} according to Eq.(4), which indicated that the interactions between NB and β-CD are very strong. Such result was in agreement with those obtained from other inclusion complexes where the β-CD was bound to the small hydrophobic molecules (Fernandes and others 2002). He and Li (2009) reported that the apparent aqueous solubility of borneol could be enhanced about 70 times by complexation with methyl-β-CD by supercritical carbon dioxide processing.

Release of NB from the inclusion complex

Figure 5A illustrated the effect of temperature at 25 °C on the volatility rate of physical mixture and physical complex. The physical mixture had a degradation of 24.89% within 5 days, whereas there was no change of the inclusion complex. The results showed that at room temperature, the NB/β-CD complex was very stable, and no NB release from the complex was observed within the tested time.

Figure 5B illustrated the effect of temperature at 60 °C on the volatility rate of physical mixture and physical complex. The physical mixture had a severe degradation of 100% within 5 d, whereas there was no obvious change of the inclusion complex with only a decrease of 24.90%. The results showed that the effect of heat on the inclusion complex was insignificant, but the effect of heat on free physical mixture was apparent. The reason may be that the inclusion complex offered a powerful protection for NB. Hence, the stability of the inclusion complex against heat was significantly enhanced after the NB and β-CD formed inclusion complex.

Figure 5C illustrated the effect of temperature at 80°C on the volatility rate of physical mixture or physical complex. After 5 days, the volatility rate of the inclusion complex increased slowly at this temperature, while the volatility rate of physical mixture reached 100%. It was noteworthy that when the temperature was at 60 °C, the physical mixture had a 100% loss, but the complex with β-CD reduced this figure to only 45.10%. Probably, β-CD inhibited the sublimation of NB after the NB was included in the

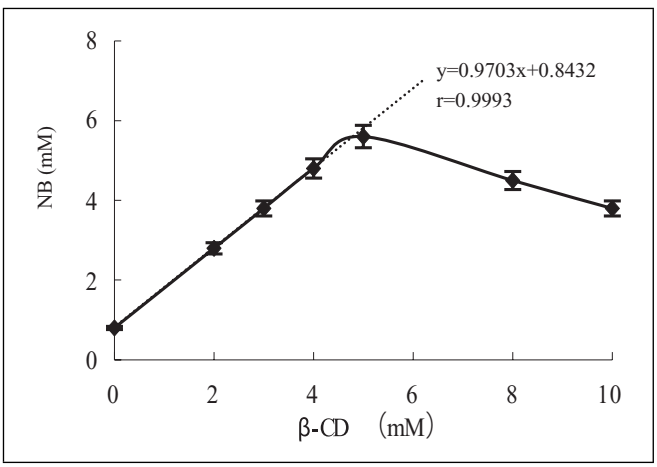

Figure 4–Phase solubility profile of NB/β-CD complex.

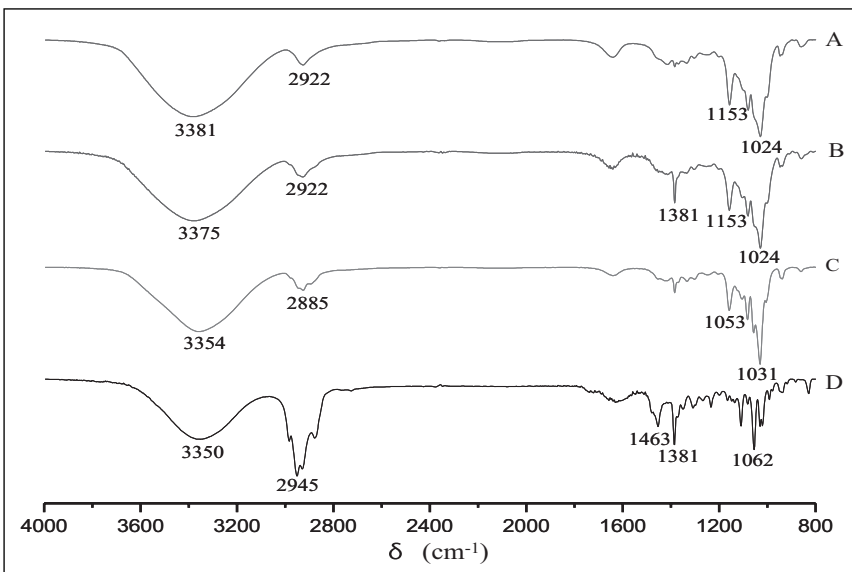

Figure 3–FT-IR spectra of (A) β-CD, (B) NB and β-CD physical mixture, (C) NB/β-CD inclusion complex, and (D) NB.

cavity of β-CD. It was indicated that the physical stability of NB was also greatly improved after NB and β-CD formed inclusion complex.

Figure 5D illustrated the effect of light at 3000LX on the volatility rate of physical mixture or physical complex. The results indicated that the volatility rate of physical mixture increased more rapidly than the inclusion complex with the increasing of days. The effect of light on the inclusion complex was less substantial than that on physical mixture. The volatility rate of physical mixture reached 67.29% within 5 d, while that of inclusion complex was only 18.60%. It was likely that β-CD inclusion provided some protection from light that was harmful to the stability of the physical mixture. Therefore, the stability of NB against light was also greatly enhanced as a result of the formation of NB/β-CD inclusion complex.

The *in vitro* release profile of NB from the NB/β-CD complex and the physical mixture in an acidic medium containing 0.05 M sodium chloride which was adjusted to pH 1.5 by HCl at (37 ± 0.5) °C was shown in Figure 6. The profile was characterized by an initial fast release phase followed with a delayed release which reached the plateau level of 86.21% and 9.53% of cumulative release rate. The releasing contents of NB from the complex and mixture were 30.02% and 6.03% in the first 10 min, 63.21% and 8.01% after 30 min, and 86.21% and 9.53% after 150 min, respectively. The accelerating release of NB in an acidic dissolution medium might be mainly attributed to the diffusion and inclusion of NB from the surface and cavities of β-CD.

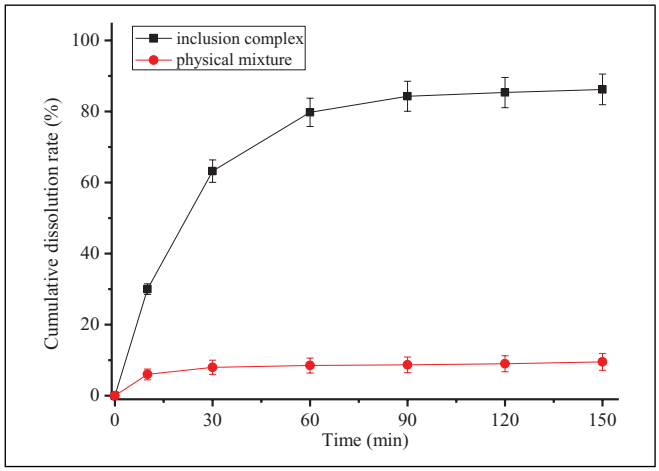

Figure 6–Dissolution profiles of NB of inclusion complex and physical mixture.

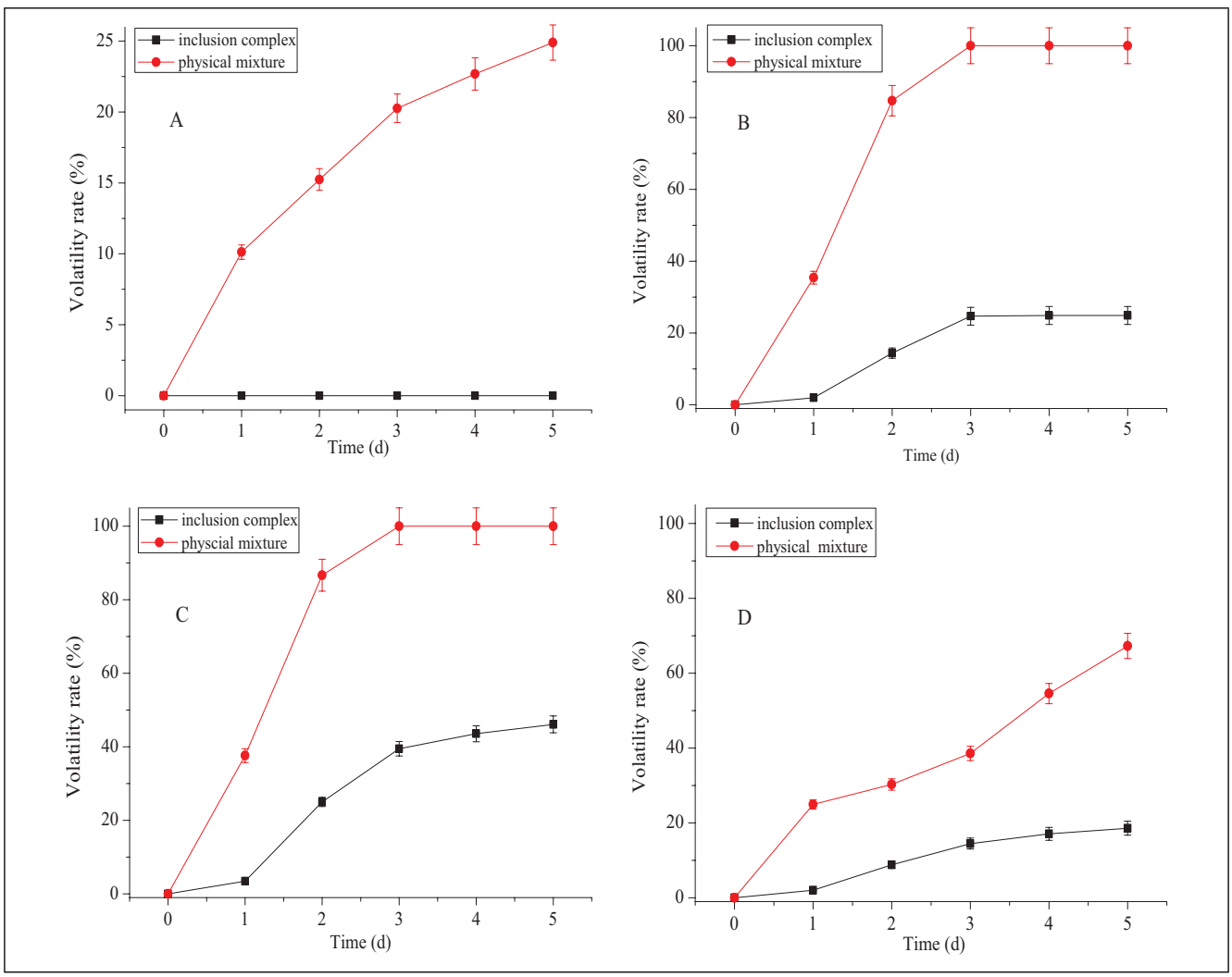

Figure 5–Stability of NB and β-CD physical mixture and NB/β-CD inclusion complex at a temperature of (A) 25 °C, (B) 60 °C, (C) 80 °C; and (D) in the 3000LX light incubator.

The untreated NB was difficult to be dissolved in water, and the aqueous solubility of pure NB is about $(4.0\pm0.4)\times10^{-3}$ mol/L (He and Li 2009). For the physical mixture of NB and β-CD, 6.03% and 9.53% dissolution were observed at 10 and 150 min, respectively. The increase in dissolution rate observed for physical mixture may be explained with the enhancing solubilization of the drug in aqueous β-CD solutions (Corrigan and Stanley 1982; Naidu and others 2004; Lu and others 2009) and the hydrophilic effect of the carrier, which can reduce the interfacial tension between the poorly soluble NB and the dissolution medium, thus leading to a dissolution (Mura and others 2002; Li and others 2005). However, after forming inclusion complex, the dissolution rate of borneol was significantly increased with over 79.78% dissolution within 60 min. Since NB formed an inclusion complex with β-CD at a mass ratio of 1/6, the enhancement of NB dissolution was more significant than the physical mixture.

Conclusion

In this study, NB was efficiently complexed with β-CD to form an inclusion complex by ultrasound method with a molar ratio of 1 : 1. The optimum method was 1 : 6 (g/g) mass ratio of NB/β-CD with ultrasonicated for 60 min at 40 °C. Under this optimum condition, the total yield of NB/β-CD complex was 89.22%, and the inclusion ratio of NB was 96.53%. The results of DSC, XRD, and FT-IR showed that NB/β-CD complex had different physicochemical characteristics from free NB. The stability and aqueous solubility of NB were significantly increased by inclusion with β-CD. The NB release rate from the NB/β-CD complex can be well controlled.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (nr 31101278 and 31130042), the Fundamental Research Funds for the Central Universities (2011ZM0099), and the National Science and Technology Support program (2012BAD37B01).

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