Carbohydrate Polymers 95 (2013) 733–739

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation and characterization of inclusion complexes formed between baicalein and cyclodextrins

Qiuna Zhou[a,b], Xiaohui Wei[a,b,*], Wei Dou[a,b], Guixin Chou[a,b], Zhengtao Wang[a,b]

[a] The MOE Key Laboratory for Standardization of Chinese Medicines and the SATCM Key Laboratory for New Resources and Quality Evaluation of Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201210, China
[b] Shanghai R&D Center for Standardization of Traditional Chinese Medicines, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 12 November 2012
Received in revised form
18 December 2012
Accepted 21 February 2013
Available online 1 March 2013

Keywords:
Baicalein
Cyclodextrin
Dissolubility
Inclusion complex
Phase solubility
Stability

ABSTRACT

This study investigates the solubility of baicalein (Ba) with the addition of modified cyclodextrins using phase solubility method. The solubility of Ba in the presence of natural (α-, β-, and γ-) cyclodextrins and its derivatives, namely, hydroxypropyl-β-cyclodextrin (HP-β-CD) and (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), was higher than that of free Ba. In particular, the stability constant of inclusion complex with DM-β-CD was 13672.671 mol^{-1}, which was the highest among the examined cyclodextrins. The inclusion complexes of Ba and DM-β-CD were prepared via freeze-drying method, which were both characterized in the solution and solid state by UV–vis spectroscopy, differential scanning calorimetry (DSC), proton nuclear magnetic resonance (^{1}H NMR), scanning electron microscopy (SEM), and X-ray powder diffractometry (XRPD). The UV–vis, DSC, ^{1}H NMR, SEM, and XRPD results proved the formation of inclusion complex between Ba and DM-β-CD. Furthermore, the dissolution rate and thermal stability of the inclusion complex were significantly enhanced compared with the pure drug. Therefore, using DM-β-CD can effectively improve the solubility and thermal stability of free Ba, which is a promising approach to promote its clinical application.

1. Introduction

Baicalein (Ba) is an active ingredient of the herbal medicine extracted from *Scutellaria baicalensis* (Fig. 1). Ba is a flavonoid that accounts for 5.41% of the dried medicinal herb (Gao, Gu, Jiang, & Guo, 2010). Related studies have shown that Ba is a material basis of pharmacodynamics compared with baicalin (Yim et al., 2004). Ba has attracted considerable attention in the recent years primarily because of its various interesting activities, such as anti-inflammatory (Shen, Chiou, Chou, & Chen, 2003), anti-HIV (Wu, Attele, Zhang, & Yuan, 2001), anti-tumor (Lee, Leung, Lai, & Wu, 2005), anti-fibrosis (Shimizu, 2000), anti-anaphylaxis (Kimata et al., 2000), protecting neurons (Kim et al., 2007; Lebeau, Esclaire, Rostène, & Pélaprat, 2001), scavenging free radical and anti-oxidation (Gao, Huang, Yang, & Xu, 1999), and so on. However, the clinical application of Ba has been seriously influenced by its low solubility and instability. Therefore, finding an effective workaround to improve the apparent solubility of Ba to facilitate its clinical application is necessary.

Cyclodextrins (CDs) are a family of macrocyclic oligosaccharides known as α-, β-, and γ-CD, which are composed of six, seven, or eight α-(1,4) linked glycosyl units, respectively (Loftson & Brewster, 1996; Saenger, 1980). CDs have the ability to encapsulate guest hydrophobic molecules of opportune polarity and dimension inside their cavities because of their special molecular structure: hydrophobic internal cavity and hydrophilic external surface. As a result of the complex formation, the characteristic properties of the included substance, such as solubility, chemical reactivity, and spectral property, will be changed. Thus, CDs are frequently utilized as complexing carriers to increase the solubility, dissolution rate, and stability of poorly soluble and astable drugs (Peggy, Maria, & Beatriz, 2010; Uekama, Hirayama, & Irie, 1998; Wang & Cai, 2008). This study investigated the possibility of overcoming the unfavorable properties of Ba via cyclodextrin complexation.

The function of a series of CDs, both natural CDs (α-, β-, and γ-CD) and their derivatives (hydroxypropyl-β-cyclodextrin (HP-β-CD) and (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD)) was investigated to assess the role of both cyclodextrin cavity size and the presence and type of substituent on their ability to generate effective interactions with the drug. Equimolar solid systems of the drug with selected CDs were prepared via freeze-drying method. Drug-CD interactions in the solution and solid state were characterized by phase-solubility analysis, UV–vis spectroscopy, DSC, ^{1}H NMR, SEM, and XRPD.

* Corresponding author at: Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201210, China. Tel.: +86 21 51322637; fax: +86 21 51322519.
E-mail address: xhweixh@hotmail.com (X. Wei).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.02.038

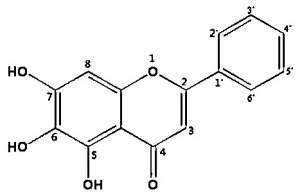

Fig. 1. The structure of Ba.

2. Materials and methods

2.1. Materials

Ba (purity: 98%), α-CD, β-CD, γ-CD, HP-β-CD (DS = 6.0), and DM-β-CD were purchased from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China). All reagents were of analytical grade. Double distilled water was used throughout the experiment.

2.2. Phase solubility studies

Phase solubility studies were investigated in water at 25 °C ± 0.5 °C in the dark based on the method reported by Higuchi and Connors (1965). An excess amount of Ba was added to 10 ml of double distilled water containing increasing concentrations of CDs, ranging from 0 mmol l^{-1} to 13.28 mmol l^{-1}, in 50 ml Erlenmeyer flask. The resulting suspensions were then equilibrated in a thermostatic shaking water bath for 72 h at 25 °C ± 0.5 °C protected from light. After equilibrium was achieved, the supernatant were filtered through 0.22 μm polytetrafluoroethylene (PTFE) filter (Shanghai ANPEL Scientific Instrument Co. Ltd., China). The concentration of dissolved Ba was assayed by HPLC (Waters, USA). The result showed that CDs have no effect on the retention time and peak area of Ba in the aqueous solution. Each experiment was carried out in triplicate. Phase solubility diagram was plotted using CD concentration as the *X*-axis and Ba concentration as the *Y*-axis. The stability constant (K_s), representing a dynamic balance between the free and associated drug molecules, is an important parameter in the complexation process (Wang & Zheng; Zarzycki & Lamparczyk, 1998). Generally speaking, the range of K_s between the CDs or their derivatives and the vast majority of drugs is 100–20000 l mol^{-1} (Stella & Rajewski, 1997). The higher value of K_s can make CDs complex better with the guest drug, showing an enhanced complexation and solubility (Banerjee, Chakraborty, & Sarkar, 2004). The stability constants were calculated from the phase solubility diagram using the Higuchi–Connors equation [Eq. (1)]:

$$K_s = \frac{solp}{S_0(1 - solp)} \quad (1)$$

where S_0 is the solubility of Ba in the absence of CDs, which can be obtained from the straight-line of the phase solubility diagram.

2.3. High-performance liquid chromatography (HPLC) analysis of Ba

Ba was analyzed using a Waters HPLC System (Waters, USA) with 254 nm photodiode array (PDA) detector. Reversed-phase HPLC column (Extend – C^{18} column, 4.6 mm × 250 mm, 5 μm) was applied in the study. The detection wavelength, column temperature, and mobile phase were 273 nm, 25 °C, and methanol: 0.05% phosphoric acid (65:35, v/v), respectively. The injection volume was 10 μl and the flow rate was 1.0 ml min^{-1} under our assay conditions. The calibration curve of Ba was linear over the concentration ranging from 0.2274 μg ml^{-1} to 75.8 μg ml^{-1}, fitting the regression equation: $y = 56486x - 2849.2$ ($r^2 = 1.0000$, $n = 7$), wherein y is the Ba peak area and x is the Ba concentration.

2.4. Preparation of the inclusion complex and physical mixture

An inclusion complex of Ba with the selected CD was prepared via the freeze-drying method. Ba and the selected CD were weighted accurately in a 1:1 molar ratio according to the results obtained from the pilot phase solubility studies. The drug and CD were dissolved in pure ethanol and double distilled water, respectively. The Ba ethanol solution was added slowly into the aqueous solution of CD, and the mixed solution was then ultrasonically handled for 6 h at 45 °C. The ethanol was entirely removed by rotary evaporation at 45–55 °C. The residue was redissolved in cold water and filtered through a 0.22 μm PTFE filter. Ba has a poor solubility in cold water, thus, the free Ba was left on the filter membrane. The clear monophase solution was frozen, and then lyophilized for 48 h.

A physical mixture was prepared by simply mixing Ba and the selected CD with a 1:1 molar ratio in a mortar for 5 min to obtain a homogeneous admixture.

Considering the stability of Ba, we investigated the stability of Ba at 45 °C for 12 h. The result indicated that the content of Ba was 98.24% after 12 h and the value of RSD% was 0.68%. It indicated that Ba was stable at 45 °C for 12 h.

2.5. Characterization of Ba/DM-β-CD inclusion complex

2.5.1. UV–vis spectroscopy

Inclusion complex formation between Ba and DM-β-CD in solution was studied using the spectral shift method. UV–vis absorption spectra were recorded by a TU-1901 double-beam UV–vis spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., China). Measurements were done at room temperature (25 ± 0.5 °C) in the spectral range of 220–400 nm. The concentration of Ba in the sample inclusion complex was 1.28×10^{-3} mmol l^{-1}, whereas the DM-β-CD concentration varied from 0 mmol l^{-1} to 16 mmol l^{-1}. All the mixtures were stirred for 4 h and the date represented the average of at least three determinations. For the control, absorbance peak of free Ba in aqueous solution was also obtained.

2.5.2. Differential scanning calorimetry (DSC)

DSC measurements were performed with a DSC – 822e system (METTLER-TOLEDO Greifensee, Switzerland). The weighed samples of approximately 2–4 mg were placed in flat-bottomed aluminum crucibles and heated at a scan rate of 10 °C min^{-1} in the range of 25–300 °C. An empty aluminum crucible served as reference, and indium was used to calibrate the temperature.

2.5.3. NMR spectroscopy

The ^{1}H-NMR spectra of free Ba, DM-β-CD, and Ba/DM-β-CD complex in 1:1 molar ratio were carried out on a Bruker AVANCE III spectrometer (Bruker, Switzerland) at 400 MHz and 298.3 K.

2.5.4. Scanning electron microscopy (SEM)

The particle shape/morphology and surface features of the samples (Ba, DM-β-CD, physical mixture, and the inclusion complex) were determined and photographed using SEM (HITACHI S-520, Japan). A small piece of double-sided adhesive tape was fixed onto an aluminum stub, and the powders were sprinkled and dispersed on the stub surface. Prior to examination, the samples were sputter coated with gold–palladium under argon atmosphere to render them electrically conductive.

2.5.5. X-ray powder diffractometry (XRPD)

The XRPD patterns of Ba, DM-β-CD, physical mixture, and the inclusion complex were obtained at ambient temperature using a

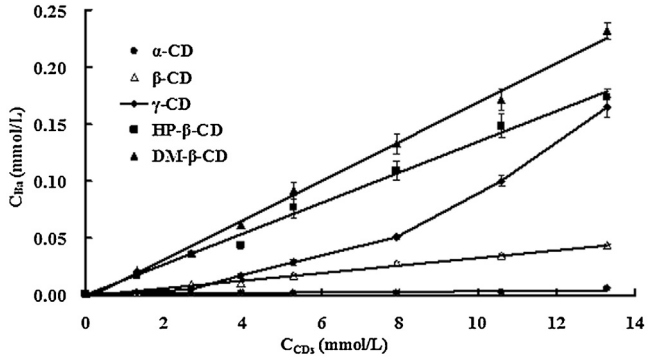

Fig. 2. Phase solubility diagrams of Ba with CDs, in aqueous solution at 25 ± 0.5 °C.

Shimadzu XRD-6000X (Shimadzu, Japan). The samples were irradiated with a Ni-filtered Cu K(α) radiation, at a voltage of 40.0 kV, and 40.0 mA current. The scanning rate was employed for 2° min^{-1} over a diffraction angle of 2θ ranging from 3° to 50°.

2.6. Dissolution rate studies

The in vitro dissolution rate studies of Ba and the inclusion complex were carried out in 0.6% sodium dodecyl sulfate (SDS) solution at 37 °C ± 0.5 °C according to ChP2010 using a dissolution apparatus (RCZ – 6C2, Shanghai Huanghai Instrument Co. Ltd., China) through the paddle method. Ba or the equivalent inclusion complex with DM-β-CD was added to 250 ml of 0.6% SDS solution. Suitable aliquots were withdrawn using a filter-syringe (pore size 0.22 μm) at specified times. The drug concentration was determined by HPLC at 273 nm. At the same time, the same volume of fresh medium was added to the beaker.

2.7. Stability studies

Comparative tests involving the stability of free Ba and the inclusion complex were carried out in the following conditions: (a) stored in brown glass bottles at a temperature of 40 °C for 10 days and (b) dissolved in aqueous solution for 24 h at room temperature using a water bath (Zeng, Ren, Zhou, Yu, & Chen, 2011). After a fixed period of time, the retention amount of free or complex Ba was measured to evaluate the embedding effect of the inclusion complex.

3. Results and discussion

3.1. Phase solubility studies

The phase solubility diagram is a widely accepted method for the assessment of the effect of CD complexation on drug solubility. Fig. 2 presents the phase solubility diagrams of Ba with CDs. The solubility of Ba linearly increased with increasing CDs concentration, except in the γ-CD diagram, indicating a feature of A_L-type phase-solubility diagrams with the formation of the 1:1 complex for α-CD, β-CD, HP-β-CD, and DM-β-CD. The solubility of Ba increased as the concentration of γ-CD increased from the phase solubility diagram of γ-CD, resulting in an A_P-type phase solubility diagram. The γ-CD curve displayed a positive deviation from the straight line of the A_P-type, indicating the formation of 1:1 and 1:2 stoichiometric ratios of Ba/γ-CD complexes (Higuchi & Connors, 1965). At higher concentrations of γ-CD, complexation between more than one γ-CD molecule and one guest molecule possibly occurred. Furthermore, the A_P-type phase-solubility diagram also indicated the formation of complex aggregates that can solubilize additional amount of the guest molecules through non-inclusion complexation or formation of micelle-like structures (Loftsson, Magnúsdóttir, Másson, & Sigurjónsdóttir, 2002; Loftsson, Másson, & Brewster, 2004). The stability constants of the complexes with the examined β-CD derivatives were distinctly higher than the parent CDs. The enhanced performance of the derivatives is attributed to the presence of hydroxypropyl and even more of the methyl substituents that expanded the hydrophobic region of the macromolecule by capping the edge of the cavity and the increased substrate binding via a hydrophobic effect. The stability constant values of the complexes with Ba were in the order DM-β-CD > HP-β-CD > γ-CD > β-CD > α-CD. Similar rank order was observed as for their solubilizing efficiency toward Ba (Table 1).

Table 1
Apparent stability constants of 1:1 complexes of Ba with the different examined CDs, and related solubilizing efficiency values.

CD type	K_s (1:1) ($l\,mol^{-1}$)	Solubilizing efficiency[a]
α-CD	312.63	6.05
β-CD	2586.66	33.13
γ-CD	1016.95	120.86
HP-β-CD	10771.49	137.27
DM-β-CD	13672.67	180.86

[a] Ratio between solubility of drug in the presence of 13.28 mmol CD and drug alone in deionized water at 25 °C.

Based on the results, DM-β-CD was selected for further studies as the best potential carrier for Ba.

3.2. Characterization of the inclusion complex of Ba-DM-β-CD

3.2.1. UV analysis

The inclusion complex composed of Ba and DM-β-CD in aqueous solution was characterized by UV spectroscopy. Fig. 3A illustrates the effects of DM-β-CD concentration on the spectra of Ba in the aqueous solution. Two absorption peaks of Ba alone in the aqueous solution were manifested at 273 and 320 nm, respectively. The absorbance of Ba was remarkably altered by the addition of DM-β-CD. Evident absorption changes of Ba have been observed with and without DM-β-CD. Increasing the concentration of DM-β-CD from 0 mmol l^{-1} to 16 mmol l^{-1} increased the absorbance of Ba without any shifts of λ_{max}. The observed hyperchromic shift may be attributed to the perturbation of the chromophore electrons of the drug because of the inclusion into the cyclodextrin cavity (Crupi et al., 2007; Stancanelli et al., 2008; Ventura et al., 2006). Therefore, Ba can form inclusion complex with DM-β-CD in aqueous solution.

3.2.2. DSC analysis

The DSC analysis provided additional evidence that inclusion complex were formed. When guest molecules were imbedded in CD cavities or crystal lattice, their melting, boiling, and sublimation points shifted to different temperatures or disappear (Marques, Hadgraft, & Kellaway, 1990). The thermograms of Ba, DM-β-CD, the inclusion complex, and the physical mixture are shown in Fig. 3B. The DSC thermogram of Ba was typical of a crystalline substance, exhibiting a sharp endothermic peak at 272.45 °C, corresponding to the melting point of the drug. In the Ba/DM-β-CD physical mixture, the characteristic thermal profile of Ba was shifted to lower temperatures in the region of 261.25 °C. The DM-β-CD and the inclusion complex did not show any sharp endothermic peak in the temperature range investigated, indicating the amorphous characteristic of the two samples. The thermogram of the physical mixture was similar to the superimposition of thermograms of individual Ba and DM-β-CD; thus, there may be absence of interaction between Ba and DM-β-CD in the physical mixture (Marques et al., 1990). The disappearance of the endothermic peak from the thermogram obtained for Ba compared with the thermogram obtained

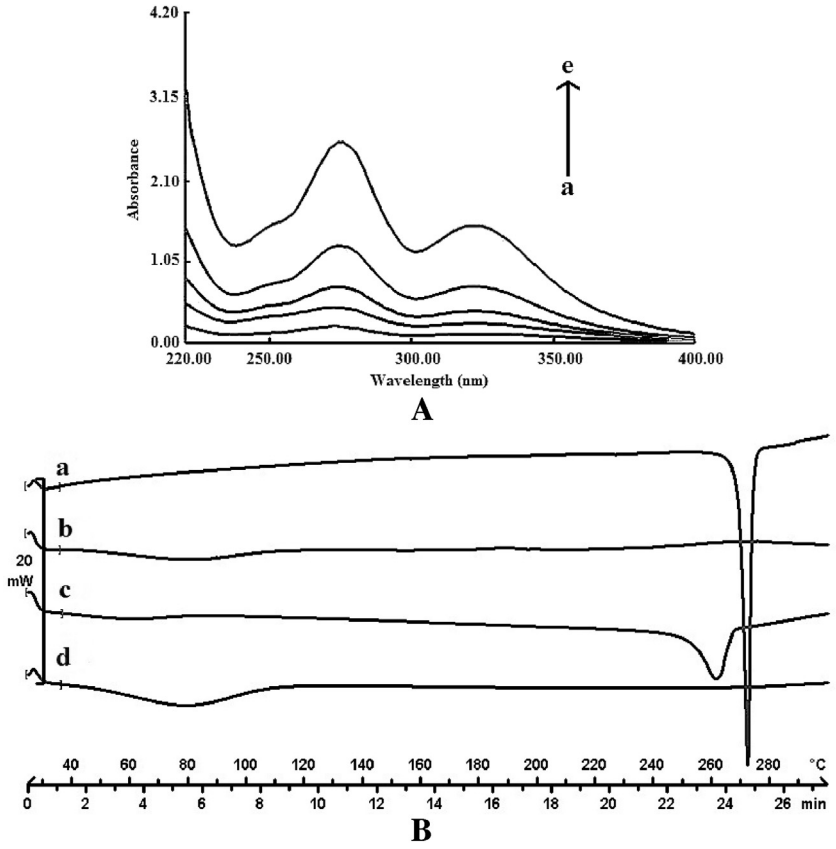

Fig. 3. (A) UV spectra of Ba (1.28×10^{-3} mM) in the presence of DM-β-CD, the concentration of DM-β-CD (from a to e): 0, 2, 4, 8 and 16 mM. (B) DSC thermograms: (a) Ba, (b) DM-β-CD, (c) physical mixture, and (d) inclusion complex.

for the complex may indicate the occurrence of an inclusion complex between Ba and DM-β-CD.

3.2.3. NMR analysis

Ba is soluble in organic solvents, such as methanol, ethanol, and DMSO, but hardly soluble in water. The ^{1}H-NMR spectrum (400 MHz, D_2O) for the Ba/DM-β-CD complex dissolved in D_2O shows direct evidence for the inclusion of a guest molecule inside the CD cavity. In the low-field region, a broad doublet at δ 7.80 (2H, brd, J = 8.5 Hz, H-2′ and 6′), a multiplet at δ 7.55–7.63 (3H, m, H-3′, 4′ and 5′) for ring B, two broad singlets at δ 6.75 (1H, brs, H-8) and 6.61 (1H, brs, H-3) for ring A and C, respectively, were clearly detected (Fig. 4A).

The formation of inclusion complexes can also be proven by the chemical shifts of Ba in ^{1}H NMR spectra (400 MHz, CD_3OD). Fig. 4B illustrated that the protons of Ba were influenced because of the presence of DM-β-CD. Table 2 lists the detailed variation of the chemical shifts of Ba before and after forming inclusion complexes with DM-β-CD. The broadening and downfield shifts of the all proton signals of Ba in the presence of DM-β-CD were observed. These results indicated that the B-ring, C-ring, and A-ring were included in the cavity of DM-β-CD.

Therefore, the structure of the Ba/DM-β-CD complex was shown as follows (Fig. 4C).

Table 2
The chemical shifts ($\Delta\delta$) of Ba and Ba/DM-β-CD and their complexation shifts ($\Delta\delta$).

No.	Ba (free)	Ba/DM-β-CD	$\Delta\delta$[a]
H-3	6.62 (1H, s)	6.65 (1H, brs)	0.03
H-8	6.73 (1H, s)	6.75 (1H, brs)	0.03
H-3′4′5′	7.53–7.58 (3H, m)[b]	7.60 (3H, brs)[b]	0.04
H-2′6′	7.98 (2H, dd. J = 8.0, 2.0)[b]	8.01 (2H, brd, J = 4.8)[b]	0.03

[a] $\Delta\delta = \delta$ (complex) − δ (free),
[b] Overlap.

3.2.4. SEM analysis

SEM is a qualitative method used to study the structural aspects of CDs and drugs, or the products obtained using different methods of preparation, such as physical mixture, coevaporation, and others (de Araujo et al., 2008; Duchene & Wouessidjewe, 1992). The SEM photographs of Ba, DM-β-CD, their physical mixture, and their inclusion complex are shown in Fig. 5A. Ba appeared as prismatic-shaped crystals and DM-β-CD was composed of spherical particles with an amorphous character. The SEM image of their physical mixture exhibited the characteristic Ba crystals, mixed with the cyclodextrin particles or adhered to their surface, thus confirming the presence of crystalline drug. However, the Ba/DM-β-CD inclusion complex appeared as a compact and homogeneous plate-like structure with crystal particles and was a drastic change in the morphology and shape of the drug particles. It was no longer possible to differentiate Ba and DM-β-CD, revealing an apparent interaction between Ba and DM-β-CD.

3.2.5. XRPD analysis

XRPD is a useful method for the judgment of cyclodextrin complexation in powder or microcrystalline states. The diffraction pattern of the complex should be obviously distinct from that of the superimposition of each of the components if a true inclusion complex was formed (Naidu et al., 2004). The XRPD patterns of free Ba, DM-β-CD, physical mixture, and the corresponding inclusion complex are shown in Fig. 5B. The Ba diffractogram displayed a series

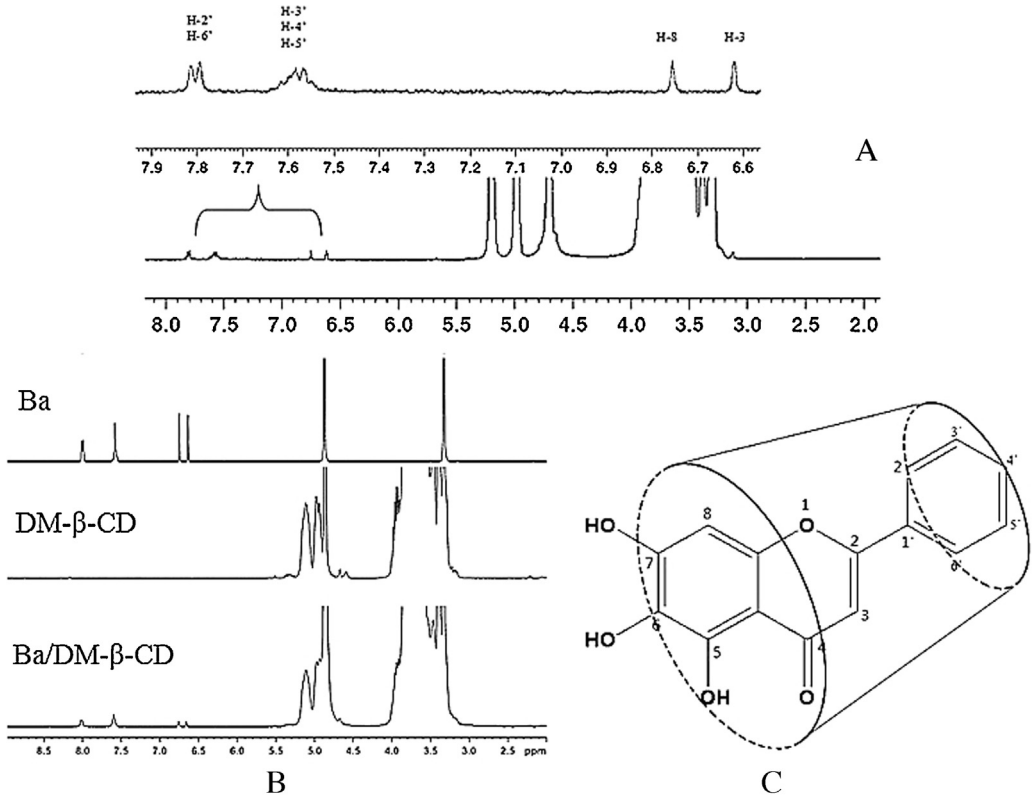

Fig. 4. ^{1}H NMR spectrum: (A) ^{1}H NMR spectrum of Ba/DM-β-CD complex in D_2O, (B) ^{1}H NMR spectrum of Ba, DM-β-CD, and Ba/DM-β-CD complex in CD_3OD, and (C) the structure of Ba/DM-β-CD complex.

of intense peaks, indicating its crystalline property. By contrast, the DM-β-CD pattern pointed out an amorphous structure that lack crystalline peaks. The XRPD pattern of the physical mixture exhibited the feature of amorphous cyclodextrins, whereas some crystallinity peaks of the drug were distinguished. Therefore, the pattern of the physical mixture was possibly to the superimposition of the patterns of Ba and DM-β-CD, suggesting that no formation of a new crystal form occurred. However, compared with the diffraction patterns of Ba and the physical mixture, the sharp peaks completely disappeared from the pattern of the inclusion complex, revealing its amorphousness. These results confirm the formation of Ba/DM-β-CD inclusion complex.

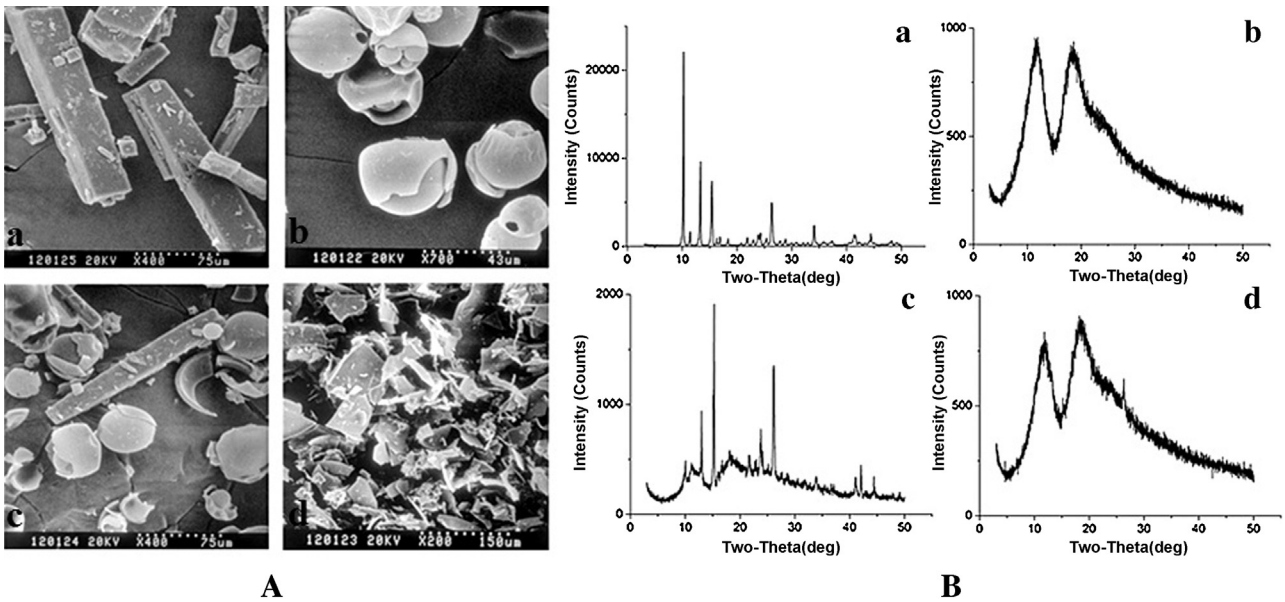

Fig. 5. (A) SEM photographs: (a) Ba, (b) DM-β-CD, (c) physical mixture, and (d) inclusion complex. (B) XRPD patterns: (a) Ba, (b) DM-β-CD, (c) physical mixture, and (d) inclusion complex.

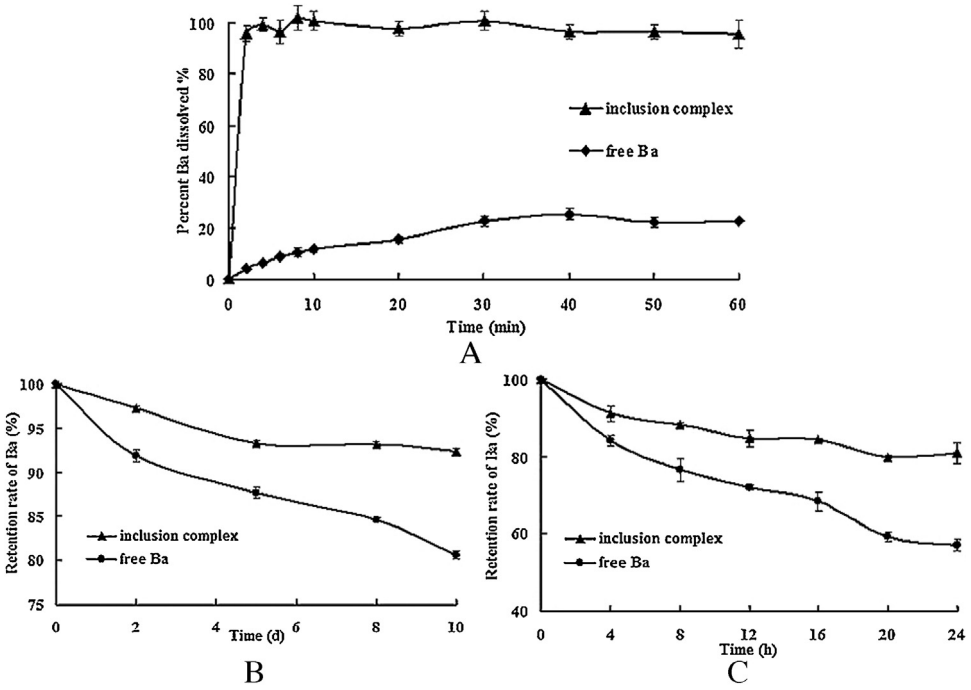

Fig. 6. (A) Dissolution profiles of Ba and its complex with DM-β-CD, (B) the stability of Ba and inclusion complex at a temperature of 40 °C, and (C) the stability of Ba and inclusion complex in the aqueous solution at room temperature.

3.3. Dissolution rate studies

The dissolution profiles of Ba and its complex with DM-β-CD are shown in Fig. 6A. The complex evidently displayed faster dissolution rate compared with free Ba. Only about 20% of the drug was dissolved in 60 min. For the Ba/DM-β-CD inclusion complex, the amount dissolved after 2 min was more than 95%. Thus, the Ba/DM-β-CD complex was the most effective in improving the drug dissolution behavior. The high increase of the drug dissolution rate for the inclusion complexes may be due to the following reasons: the formation of soluble inclusion complex, amorphization of the drug and consequent solubility increase, and the enhanced wettability and reduction of particle size (Veiga, Fernandes, & Maincent, 2001).

3.4. Stability studies

Fig. 6B exhibits the degradation profiles of free Ba and its complex with DM-β-CD at 40 °C. For free Ba, the remaining amount of Ba was 80.6% after 10 d, whereas a slight change of the inclusion complex was observed, with a decrease of 7.7%. The result suggested that the stability of Ba against heat was enhanced when inclusion complex was formed with DM-β-CD.

The stability of Ba in the aqueous solution is illustrated in Fig. 6C. The result demonstrated that the inclusion complex formation of Ba and DM-β-CD can significantly improve the stability of Ba in the aqueous solution, with the rate of 81.0% for the Ba detected in the inclusion complex solution, whereas 57.0% for the free Ba solution at room temperature after 24 h. Thus, the stability of Ba in aqueous solution was also enhanced when the inclusion complex between Ba and DM-β-CD was formed.

4. Conclusion

Cyclodextrin complexation was successful in improving Ba physicochemical properties, such as dissolution rate and thermal stability. DM-β-CD exhibited the best performance among the examined CDs, indicating that its cavity was the most suitable for accommodating the drug molecule. The inclusion complex of Ba and DM-β-CD was successfully prepared by freeze-drying method and characterized by UV–vis spectroscopy, DSC, ^{1}H NMR, SEM, and XRPD. Moreover, the stoichiometry of the complex between Ba and DM-β-CD was 1:1 based from the phase solubility analysis with the stability constant (K_s) 13672.67 l mol^{-1} at 25 °C ± 0.5 °C, and the solubilizing efficiency was stronger than other CDs.

Acknowledgement

This work was supported by grant no. 2011JW21 from the Shanghai Municipal Education Commission.

References

Banerjee, R., Chakraborty, H., & Sarkar, M. (2004). Host–guest complexation of oxicam NSAIDs with β-cyclodextrin. *Biopolymers*, *75*, 355–365.

Crupi, V., Ficarra, R., Guardo, M., Majolino, D., Stancanelli, R., & Venuti, V. (2007). UV–vis and FTIR–ATR spectroscopic techniques to study the inclusion complexes of genistein with β-cyclodextrins. *Journal of Pharmaceutical and Biomedical Analysis*, *4*, 110–117.

de Araujo, D. R., Tsuneda, S. S., Cereda, C. M. S., Carvalho, F. D. G. F., Preté, P. S. C., Fernandes, S. A., et al. (2008). Development and pharmacological evaluation of ropivacaine-2-hydroxypropyl-β-cyclodextrin inclusion complex. *European Journal of Pharmaceutical Sciences*, *33*, 60–71.

Duchene, D., & Wouessidjewe, D. (1992). Industrial uses of cyclodextrins and their derivatives. *Journal of Coordination Chemistry*, *27*, 223–236.

Gao, Y., Gu, Z. L., Jiang, X. G., & Guo, C. Y. (2010). A new progress on the baicalein pharmacological study. *Lishizhen Medicine and Materia Medica Research*, *21*, 1765–1767.

Gao, Z., Huang, K., Yang, X., & Xu, H. (1999). Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. *Bichimca et Biophysica Acta-General Subjects*, *1472*, 643–650.

Higuchi, T., & Connors, K. (1965). Phase solubility techniques. *Advances in Analytical Chemistry and Instrumentation*, *4*, 117–212.

Kimata, M., Shichijo, M., Miura, T., Serizawa, I., Inagaki, N., & Nagai, H. (2000). Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. *Clinical and Experimental Allergy*, *30*, 501–508.

Kim, D. H., Jeon, S. J., Son, K. H., Jung, J. W., Lee, S., Yoon, B. H., et al. (2007). The ameliorating effect of oroxylin A on scopolamine-induced memory impairment in mice. *Neurobiology Learning Memory*, *87*, 536–546.

Lebeau, A., Esclaire, F., Rostène, W., & Pélaprat, D. (2001). Baicalein protects cortical neurons from beta-amyloid (25–35) induced toxicity. *Neuroreport*, *12*, 2199–2201.

Lee, H. Z., Leung, H. W., Lai, M. Y., & Wu, Ch. H. (2005). Baicalein induced cell cycle arrest and apoptosis in human lung squamous carcinoma CH27 cells. *Anti-cancer Research*, *25*, 959–964.

Loftson, T., & Brewster, M. E. (1996). Pharmaceutical applications of cyclodextrins. I. Drug solubilization and stabilization. *Journal of Pharmaceutical Science*, *85*, 1017–1025.

Loftsson, T., Magnúsdóttir, A., Másson, M., & Sigurjónsdóttir, J. F. (2002). Self-association and cyclodextrin solubilization of drugs. *Journal Pharmaceutical Sciences*, *91*, 2307–2316.

Loftsson, T., Másson, M., & Brewster, M. E. (2004). Self-association of cyclodextrins and cyclodextrin complexes. *Journal Pharmaceutical Sciences*, *93*, 1091–1099.

Marques, H. M., Hadgraft, J., & Kellaway, I. W. (1990). Studies of cyclodextrin inclusion complexes, I. The salbutamol–cyclodextrin complex as studied by phase solubility and DSC. *International Journal of Pharmaceutics*, *63*, 259–266.

Naidu, N. B., Chowdary, K. P. R., Murthy, K. V. R., Satyanarayana, V., Hayman, A. R., & Becket, G. (2004). Physicochemical characterization and dissolution properties of meloxicam-cyclodextrin binary systems. *Journal Pharmaceutical and Biomedical Analysis*, *35*, 75–86.

Peggy, A. P., Maria, P. B., & Beatriz, E. E. (2010). Encapsulation of cinnamon and thyme essential oils components (cinnamaldehyde and thymol) in β-cyclodextrin: Effect of interactions with water on complex stability. *Journal of Food Engineering*, *99*, 70–75.

Saenger, W. (1980). Cyclodextrin inclusion compounds in research and industry. *Angewandte Chemie International Edition*, *19*, 344–362.

Shen, Y. C., Chiou, W. F., Chou, Y. Ch., & Chen, Ch. F. (2003). Mechanisms in mediating the anti-inflammatory effects of baicalin and baicalein in human leukocytes. *European Journal of Pharmacology*, *465*, 171–181.

Shimizu, I. (2000). Sho-saiko-to: Japanese herbal medicine for protection against hepatic fibrosis and carcinoma. *Journal of Gastroenterology and Hepatology*, *15*, 84–90.

Stancanelli, R., Ficarra, R., Cannavà, C., Guardo, M., Calabrò, M. L., Ficarra, P., et al. (2008). UV–vis and FTIR-ATR characterization of 9-fluorenon-2-carboxyester/(2-hydroxypropyl)-β-cyclodextrin inclusion complex. *Journal of Pharmaceutical and Biomedical Analysis*, *47*, 704–709.

Stella, V. J., & Rajewski, R. A. (1997). Cyclodextrins: Their future in drug formulation and delivery. *Pharmaceutical Research*, *14*, 556–567.

Uekama, K., Hirayama, F., & Irie, T. (1998). Cyclodextrin drug carrier systems. *Chemical Reviews*, *98*, 2045–2076.

Veiga, F., Fernandes, C., & Maincent, P. (2001). Influence of the preparation method on the physicochemical properties of tolbutamide/cyclodextrin binary system. *Drug Development and Industrial Pharmacy*, *27*, 523–532.

Ventura, C. A., Giannone, I., Musumeci, T., Pignatello, R., Lorella, R., Landolfi, C., et al. (2006). Physicochemical characterization of disoxaril–dimethyl-β-cyclodextrin inclusion complex and in vitro permeation studies. *European Journal of Medicinal Chemistry*, *41*, 233–240.

Wang, J., & Cai, Z. (2008). Investigation of inclusion complex of miconazole nitrate with β-cyclodextrin. *Carbohydrate Polymers*, *72*, 255–260.

Wang, L. L., & Zheng, W. S. (2006). Inclusion effects of β-CD and HP-β-CD on momobenzone. *Chinese Pharmaceutical Journal*, *41*, 529–531.

Wu, J. A., Attele, A. S., Zhang, L., & Yuan, Ch. S. (2001). Anti-HIV activity of medicinal herbs: Usage and potential development. *The American Journal of Chinese Medicine*, *29*, 69–81.

Yim, J. S., Kim, Y. S., Moon, S. K., Cho, K. H., Bae, H. S., Kim, J. J., et al. (2004). Metabolic activities of ginsenoside Rbl, baicalin, glycyrrhizin and geniposide to their bioactive compounds by human intestinal microflra. *Biological and Pharmaceutical Bulletin*, *27*, 1580–1583.

Zarzycki, P. K., & Lamparczyk, H. (1998). The equilibrium constant of β-cyclodextrin–phenolphtalein complex; influence of temperature and tetrahydrofuran addition. *Journal of pharmaceutical and biomedical analysis*, *18*, 165–170.

Zeng, J., Ren, Y., Zhou, Ch. L., Yu, Sh. Q., & Chen, W. H. (2011). Preparation and physicochemical characteristics of the complex of edaravone with hydroxypropyl-β-cyclodextrin. *Carbohydrate Polymers*, *83*, 1101–1105.