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The novel application of tertiary butyl alcohol in the preparation of hydrophobic drug–HPβCD complex

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Abstract

This report describes a novel application of tertiary butyl alcohol (TBA) in the preparation of hydrophobic drug–hydroxypropyl β-cyclodextrin (HPβCD) complex. The straightforward, economic preparation procedure consists of dissolving both the hydrophobic drug and HPβCD in TBA, which is subsequently freeze-dried to give the hydrophobic drug–HPβCD complex in the form of a porous powder. TBA was selected as the medium due to it being a good solvent for hydrophobic drug and HPβCD; in addition, it is also a versatile lyophilization medium and is widely used in pharmaceutical processes. In this study, ketoprofen and nitrendipine were used as model drugs and their HPβCD complexes were prepared by lyophilization of the TBA system. Based on the data from differential scanning calorimetry (DSC) and X-ray diffractometry (XRD), the drugs were amorphous in freeze-dried samples. The infra-red (IR) spectrum indicated that a drug–HPβCD interaction took place in the freeze-dried complex. Dissolution experiments showed that the hydrophobic drug dissolved rapidly from the HPβCD complex in both simulated gastric juice and simulated intestinal fluid. These results confirmed that this technique produced a hydrophobic drug–HPβCD complex. TBA was found to be a suitable freeze-drying medium for the preparation of hydrophobic drug–HPβCD complex. This approach is versatile, energy-conserving and can easily be scaled up. It is expected to have further application in modifying the physicochemical characteristics of hydrophobic drugs and improving their absorption and pharmacodynamic properties.

Introduction

Tertiary butyl alcohol (TBA) is an excellent freeze-drying medium. It possesses a very high vapour pressure (26.8 mmHg at 20°C), it melts around room temperature (24°C) and has a low toxicity (Teagarden & Baker 2002). During freeze-drying, TBA forms needle-shaped crystals with a high surface area and low surface resistance, and besides, the sublimation of TBA allows a low product temperature to be maintained and prevents the product from reaching the collapse temperature. All these properties are beneficial for acceleration of the sublimation rate and allowing the formation of a porous dry powder (Kasraian & DeLuca 1995).

TBA has been progressively used in many pharmaceutical processes. In recent years, there has been an increasing number of reports describing the preparation of solid dispersions, liposomes and nanoparticles using TBA as the solvent for hydrophobic drugs (Fournier et al 2004; Li & Deng 2004; Van Drooge et al 2004a, b). TBA is also an excellent nonaqueous solvent for the lyophilization of highly water-sensitive drugs (Van Drooge et al 2004c). Pure TBA has been used as a neat vehicle for lyophilization of the water-labile antitumour agent SarCNO, the chemical stability of which was greatly improved in TBA, thereby allowing the preparation of a freeze-dried SarCNO powder (Ni et al 2001). However, to the best of our knowledge, there is presently little information available in the literature about the use of TBA as a solvent for the preparation of cyclodextrin complexes.

In theory, hydroxypropyl β-cyclodextrin (HPβCD) should be soluble in TBA as a result of hydrogen bond formation between the oxygen, hydroxyl groups of HPβCD and the hydroxyl of TBA, and this has been demonstrated by our experiments. This is the first report about the solubility of HPβCD in TBA. Using TBA as a medium for the preparation of a hydrophobic drug–HPβCD complex, a high drug concentration can be achieved due to the excellent solubility of the hydrophobic drug and HPβCD in TBA, which helps accelerate

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complex formation and shorten the lyophilization cycle. In a TBA solution of the hydrophobic drug and HPβCD, the drug and HPβCD were molecularly dispersed in TBA, the hydrophobic drug molecule, or part of its structure, can insert itself into the cavity of HPβCD on account of hydrophobic, van der Waals or hydrogen-bonding interactions. Removal of the solvent by lyophilization results in the hydrophobic drug–HPβCD complex being obtained in powder form. Using TBA as a solvent means that many problems, such as the residual organic solvent, excessive HPβCD and extreme pH associated with other preparation methods, can be avoided.

In this study, two hydrophobic drugs – a nonsteroidal anti-inflammatory drug, ketoprofen, and a dihydropyridine calcium-channel antagonist, nitrendipine – were selected as model drugs. Both these drugs have limited aqueous solubility. Solid binary inclusion systems were prepared at a 1:1 molar ratio by lyophilization of the TBA system. The interactions between the drugs and HPβCD were investigated by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and X-ray diffractometry (XRD). The dissolution rates of ketoprofen and nitrendipine in simulated gastric juice and in simulated intestinal fluid were markedly increased when they were in the form of HPβCD complexes. This suggests that lyophilization of hydrophobic drug and HPβCD in TBA solution is a simple, economic and convenient method for the preparation of hydrophobic drug–HPβCD complex.

Materials and Methods

Materials

Ketoprofen was purchased from Xinan Pharmaceutical Factory (Chongqing, China); nitrendipine was obtained from Nanjing Pharmaceutical Factory (Nanjing, China); HPβCD was purchased from Shanxi Liquan Corporation (Xian, China). All other chemicals and reagents were of analytical grade.

Preparation of ketoprofen– and nitrendipine–HPβCD complexes by lyophilization of a TBA system

Ketoprofen or nitrendipine and the stoichiometric amount of HPβCD (in a 1:1 molar ratio) were dissolved in TBA. The monophase solution was sterilized by filtration through a 0.22-μm membrane filter, and then the isotropic solution was transferred to 5-mL vials. Freeze-drying was done in a laboratory freeze-drier (FD-1; Beijing Bioking Technology Company, Beijing, China). The samples were cooled on a shelf to a temperature of −45°C and held at that temperature for 4 h. Primary drying was performed at a shelf temperature of −40°C for 24 h with a chamber pressure of 100 mbar, followed by secondary drying at a shelf temperature of 20°C for 6 h. The vials were fitted with lids and sterilized drug–HPβCD complex powder was obtained. In this study, the concentration of ketoprofen and nitrendipine in TBA solution was 15 mg mL^{-1} and 18 mg mL^{-1}, respectively. The drug content in the final powder was about 16% and 22% (w/w), respectively. Physical mixtures were prepared by homogeneous blending of quantificational drug and HPβCD in a mortar.

Differential scanning calorimetry (DSC)

A Shimadzu DSC-60 differential scanning calorimeter was used for recording DSC thermograms of drug materials and physical mixtures, as well as inclusion complexes. All accurately weighed samples were placed in sealed aluminium pans and heated at a rate of 10°C min^{-1} under nitrogen (15 mL min^{-1}) from 30°C to 300°C, and an empty aluminium pan was used as a reference.

Fourier transform infrared spectroscopy (FTIR)

Infrared spectra were obtained using a Bruker IFS55 FTIR spectrometer. The samples were ground and mixed uniformly with potassium bromide, then the powders were compressed at a pressure of 10 T cm^{-2} for 5 min to form pellets. The scanning range was 400–4000 cm^{-1}.

X-ray diffractometry (XRD)

The X-ray diffractometry patterns were recorded on a D/MAX 2400 model X-ray diffractometer fitted with a monochromator, Cu Kα radiation was at a voltage 50 kV and a 150-mA current was maintained over the 3–45° diffraction angle (2θ) range, and scanning was performed in steps of 0.02° (2θ). The analysis was carried out at room temperature.

Dissolution studies

Dissolution studies were carried out according to the paddle method using the USP dissolution apparatus; simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8 phosphate-buffered saline (PBS)) were used as dissolution media. Drug, or an equivalent quantity of HPβCD complex or a physical mixture was added to the medium. The dissolution medium was maintained at 37 ± 0.5°C and a rotation speed of 100 ± 2 rev min^{-1}. Samples were taken at selected time intervals, filtered, and the volume removed was replaced with fresh medium. The concentration of ketoprofen and nitrendipine was measured by UV and HPLC, respectively. All experiments were performed six times. The cumulative amount of drug released at each time point was plotted and the profiles of drug release from the raw material, physical mixture and HPβCD complex were presented.

Statistical methods

The physicochemical characteristics of drugs and binary systems, including DSC, FTIR and XRD, were analysed in triplicate. Data in dissolution studies are expressed as mean ± s.d. of results from six experiments. In Figure 1, the Tg and enthalpy difference of the drug as pure material or binary systems was examined using the Kruskal–Wallis test with non-parametric post-hoc test. In Figure 4, the percentage of drug released from the HPβCD complex, physical mixture or pure drug material at each time point was examined using a

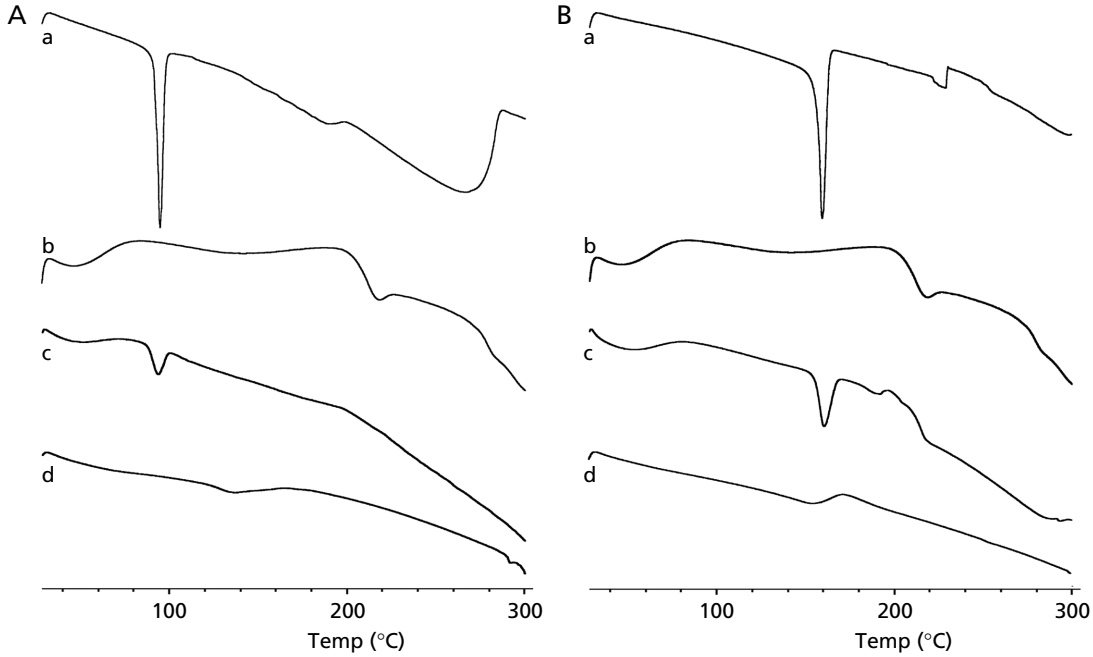

Figure 1 DSC curves of ketoprofen (A) and nitrendipine (B), showing pure drug (a), HPβCD (b), drug–HPβCD physical mixture (c) and drug–HPβCD complex (d).

one-way analysis of variance. A post-hoc comparison of the individual groups was performed using Tukey's Honestly Significant Difference test.

Results and Discussion

Preparation of ketoprofen– and nitrendipine–HPβCD complexes by lyophilization of a TBA system

Mura et al (1998) prepared a ketoprofen–HPβCD complex by lyophilization of an equimolar ketoprofen and HPβCD aqueous solution. One gram of the physical mixture could be dissolved in 500 mL water, even in ammonia solution. The pK_a of ketoprofen is 4.5 and, accordingly, the drug is dissociated in ammonia solution. It is well known that drug in ionized state is not advantageous for stable cyclodextrin complex formation. Using TBA as a solvent, 10 mL is enough to dissolve 1 g ketoprofen and HPβCD physical mixture, and this makes handling simple and energy-efficient. Choi et al (2003) prepared nitrendipine–HPβCD complex by the solvent evaporation method. They added 0.18 g nitrendipine and a stoichiometric amount of HPβCD to 500 mL 50% ethanol, then the solvent ethanol was removed in a rotary evaporator under vacuum at about 40°C and dried in a vacuum for 24 h. In our study, a similar amount of nitrendipine and HPβCD were dissolved in 10 mL TBA and the solvent was removed by freeze-drying. The solvent required for the preparation was markedly reduced and the process of lyophilization of the TBA system was easy, efficient and convenient, so that it was possible to scale up the procedure.

This method is different from conventional methods for we selected a menstruum TBA, which is an excellent solvent for hydrophobic drugs and HPβCD. The solubility of HPβCD in TBA is over 50% (w/v) and less TBA is needed to dissolve a hydrophobic drug and HPβCD. The drug and HPβCD are homogeneously dispersed in TBA solution, and the isotropic solution is lyophilized after sterilization by passage through a 0.22-μm filter, then the sterilized drug inclusion complex was obtained in the form of a loose dry powder. This method could be applied to the preparation of hydrophobic drug–HPβCD complex for injection, inhalation or other forms of administration.

Differential scanning calorimetry

The thermograms of pure drug and corresponding binary systems are shown in Figure 1. Ketoprofen and nitrendipine showed sharp endothermic peaks at 97.2°C and 162.0°C, respectively, corresponding to the fusion of anhydrous crystalline drug. HPβCD showed a broad endothermal peak at around 80°C, which was associated with water loss from amorphous HPβCD. The drug endothermal peaks disappeared completely in the freeze-dried complexes, while they were present in their physical mixtures. The Tg of the complex was significantly different from that of the drug material ($P < 0.001$) and the physical mixture ($P < 0.001$). The enthalpy difference of the complex was significantly reduced compared with the pure drug ($P < 0.001$) and the physical mixture ($P < 0.001$). This phenomenon indicated that the drug was amorphous and the drug–HPβCD inclusion complex had been formed. Drug nucleation was prevented due to its molecular dispersion in the amorphous carrier HPβCD.

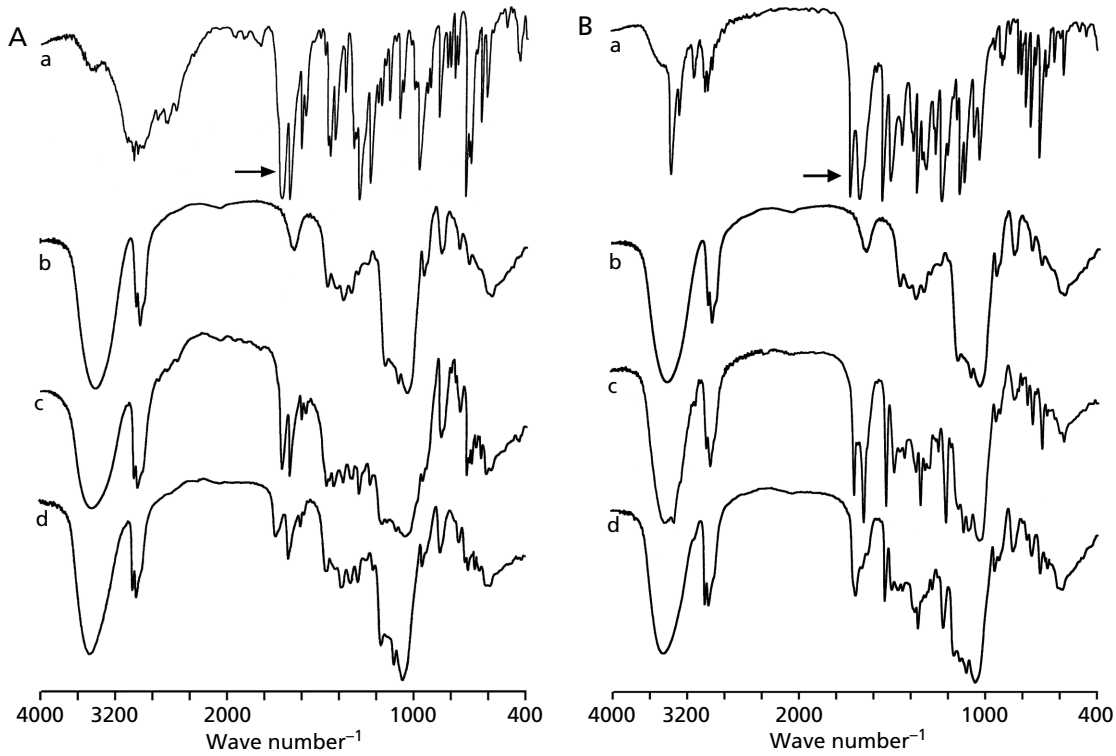

Figure 2 FTIR spectra of ketoprofen (A) and nitrendipine (B), showing pure drug (a), HPβCD (b), drug–HPβCD physical mixture (c) and drug–HPβCD complex (d).

Infrared spectroscopy

Figure 2 shows the infrared spectra of ketoprofen, nitrendipine and their binary systems with HPβCD. Ketoprofen shows a carbonyl stretching band at 1697 cm^{-1} and in its HPβCD complex, the intensity of the carbonyl stretching band was reduced markedly and shifted to a higher frequency at 1728 cm^{-1}, indicating hydrogen bond formation between ketoprofen and HPβCD. For nitrendipine, strong absorption peaks were seen at 1701 cm^{-1} and 1650 cm^{-1}, which were attributed to the stretching vibration of the carbonyl group. The band shape was broader and shifted to a single peak at wavenumber 1685 cm^{-1} suggesting that, after the formation of the 1:1 complex, intermolecular hydrogen bonds were formed. For the physical mixture of nitrendipine and HPβCD, the spectrum was that resulting from the simple superposition of each component.

X-ray diffractometry

The powder X-ray diffraction patterns of pure drug and various binary solid systems are shown in Figure 3. Ketoprofen and nitrendipine exhibited characteristic peaks, showing that ketoprofen and nitrendipine existed in crystalline form. In contrast, HPβCD was amorphous. The diffraction patterns of the physical mixtures were simply a superposition of the patterns of the components and the crystalline drug peaks appeared on the diffuse background of the amorphous carrier. In contrast, a flat, typical amorphous state was observed for the freeze-dried product due to the drug being dispersed molecularly within the HPβCD. The diffraction patterns of the HPβCD complex differed markedly from that of the physical mixture, confirming the formation of the inclusion complex.

Dissolution studies

The dissolution results are presented in Figure 4. The dissolution rate of ketoprofen from pure drug powder was very slow. A markedly increased dissolution rate was observed from its HPβCD physical mixture ($P < 0.001$ vs pure drug) as HPβCD dissolved immediately when added to the dissolution medium and increased the drug wettability. The dissolution rate of ketoprofen from the HPβCD complex was enhanced significantly and drug dissolved rapidly during the first few minutes ($P < 0.001$ vs pure drug, $P < 0.001$ vs physical mixture). At 5 min, 90% of the drug had been released from the ketoprofen–HPβCD complex in simulated gastric juice, while only 1.43% and 14.06% was released from the pure drug and its physical mixture, respectively. The increased dissolution rate and solubility was advantageous for improving its absorption and decreasing its gastric irritation.

For nitrendipine, the improvement in the dissolution rate obtained with the physical mixture was not apparent during the first 15 min ($P > 0.05$), but then the dissolution rate improved markedly due to the increased wettability and decreased surface tension between the drug and aqueous medium ($P < 0.01$ vs pure drug). The solubility of nitrendipine was significantly improved by complexation with HPβCD ($P < 0.001$ vs pure drug, $P < 0.001$ vs physical mixture). The amount of drug released from the HPβCD complex was 459.2 fold and 440.2 fold compared with the pure drug

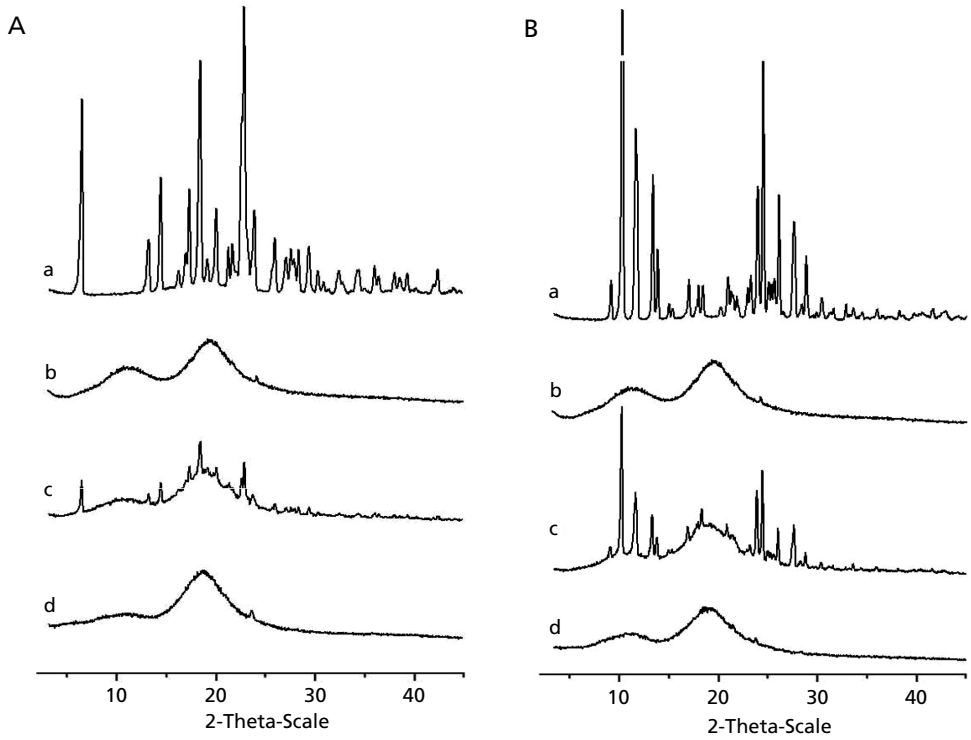

Figure 3 X-ray powder diffraction patterns of ketoprofen (A) and nitrendipine (B), showing pure drug (a), HPβCD (b), drug–HPβCD physical mixture (c) and drug–HPβCD complex (d).

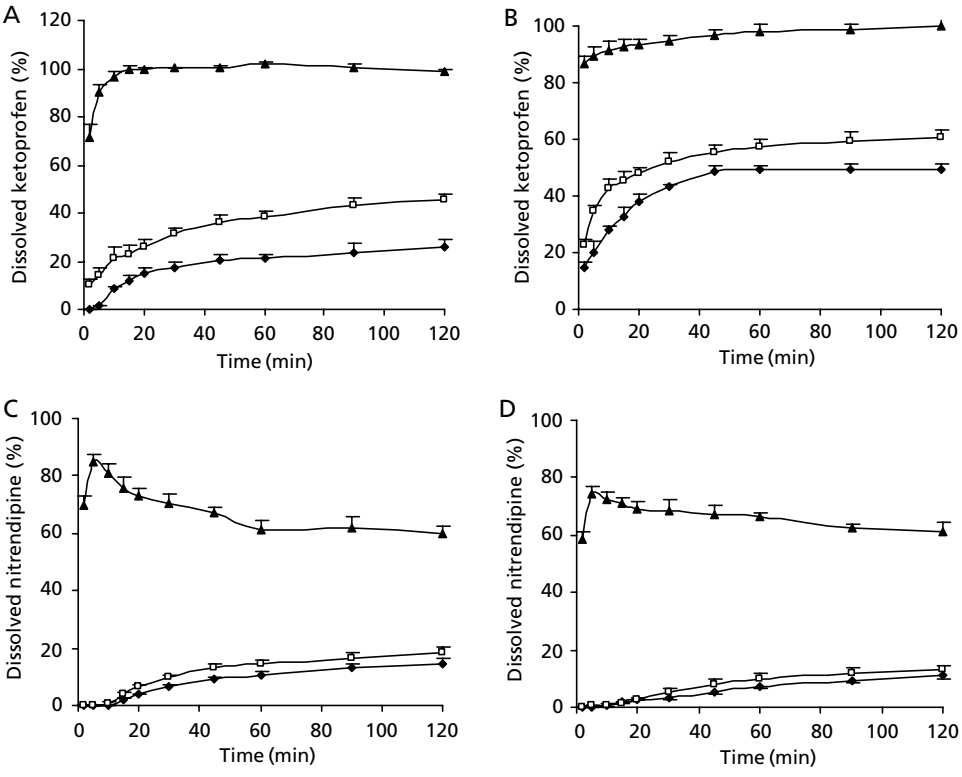

Figure 4 Dissolution curves of ketoprofen and nitrendipine in simulated gastric juice (A, C) and in simulated intestinal fluid (B, D), showing pure drug (♦), drug–HPβCD physical mixture (□) and drug–HPβCD complex (▲). Data are means ± s.d., n = 6.

and physical mixture in simulated gastric juice at 5 min. In addition, in simulated intestinal fluid, 74.3% of the drug was released from the HPβCD complex versus 0.2% and 0.67% released from the pure drug and drug–HPβCD physical mixture at the same time. Nitrendipine is relatively insoluble in water, which results in dissolution being the rate-limiting step for drug absorption. Accordingly, it would be expected that formation of the nitrendipine–HPβCD complex would greatly accelerate its dissolution and improve its absorption.

At each time point, the percentage of drug released from the HPβCD complex was improved markedly compared with the drug material ($P < 0.001$) or the physical mixture ($P < 0.001$). The significant improvement in the dissolution rate of the two hydrophobic drug–HPβCD complexes could be attributed to the following reasons: firstly, the high-energy amorphous state of the drug, which reduced the energy needed to destroy the crystal lattice during dissolution in the medium; secondly, the formation of an inclusion complex; thirdly, the size of the drug particles were reduced and the specific surface area was increased; fourthly, use of a hydrophilic solubilizer HPβCD as carrier; finally, a reduction in interfacial tension between the drug and the aqueous medium.

Conclusions

This study describes a novel application of TBA as a medium for the preparation of hydrophobic drug–HPβCD complex. Sterilized drug–HPβCD complex was obtained in powder form by freeze-drying the TBA system, and drug complexation with HPβCD resulted in a significant improvement in the drug dissolution properties. Thus, this method offers a simple and economic technique for preparation of hydrophobic drug–HPβCD complex, which can easily be scaled up. It may be of practical use in modifying the physicochemical characteristics and improving the absorption of hydrophobic drugs.

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