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ORIGINAL ARTICLE

Complexation of hydrophobic drugs with hydroxypropyl-β-cyclodextrin by lyophilization using a tertiary butyl alcohol system

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Abstract In this study, a novel method is presented for the preparation of a hydrophobic drug hydroxypropyl-β-cyclodextrin (HPβCD) complex. Ketoprofen and nitrendipine were used as model drugs and their HPβCD complexes were prepared by lyophilization of a tertiary butyl alcohol (TBA) system. The preparation procedure is as follows: both hydrophobic drugs and HPβCD are dissolved in TBA and subsequently passed through a 0.22 μm millpore filter. Then the solvent is removed by lyophilization to give a hydrophobic drug HPβCD complex in porous powder form. Based on the data obtained from FTIR, a hydrogen bond is formed between the drug and HPβCD. DSC, SEM and XRD results show that the drugs are amorphous in freeze-dried samples. The solubility of the hydrophobic drugs in simulated gastric juice and simulated intestinal fluid was increased markedly compared with pure drug. An in vitro release experiment showed that the dissolution rate of drug from the HPβCD complex was markedly enhanced compared with the pure drug and the physical mixture. This method is versatile, economic and easily scaled up. It is suitable for heat- and water-labile drugs and is expected to have a wide application in modifying the physicochemical characteristics of hydrophobic drugs.

Keywords Lyophilization · Tertiary butyl alcohol (TBA) · Hydroxypropyl-β-cyclodextrin (HPβCD) · Hydrophobic drugs · Complexation

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Introduction

The usefulness of many drugs is limited due to their low aqueous solubility and chemical instability. The low absorption rate of hydrophobic drugs is the main obstacle in designing a dosage system. Various techniques have been investigated to increase the solubility and improve the chemical stability of hydrophobic drugs. Among them, the cyclodextrin (CD) complex has attracted the interest of many pharmaceutical researchers and its efficiency has been confirmed in improving the solubility, stability and bioavailability of a number of lipophilic active compounds [1–3]. βCD is the most common CD used in pharmaceutical formulations for modification of the physicochemical properties and improving the bioavailability of hydrophobic drugs. However, its low intrinsic solubility in water (1.85 g/100 ml) and nephrotoxicity have restricted its application. Attempts have been made to improve CDs by chemical derivatization. HPβCD, the condensation product of βCD with propylene oxide, has a higher water solubility (50-fold improvement), it has a low toxicity when given parenterally [4], it is highly biocompatible and pharmacologically inactive [5, 6],

so that it can be administered parenterally, orally, ophthalmically and by inhalation. Many pharmaceutical researchers have used HPβCD as a safe and effective material to improve the absorption and bioavailability of hydrophobic drugs [7, 8]. The itraconazole HPβCD complex for injection was marketed in 1999 after being approved by the FDA. Due to the low aqueous solubility of hydrophobic drugs, organic solvent (methanol, ethanol or acetone), excessive CD, solubilizers or an extreme pH are often needed for the preparation of hydrophobic drug- CD complexes [9]. However, many additives, residual organic solvent and excessive CD are unacceptable for human use.

In this report, a novel medium tertiary butyl alcohol (TBA) was introduced and evaluated for the preparation of hydrophobic drug HPβCD complexes. The procedures used for the preparation of hydrophobic drug HPβCD complexes are as follows: the hydrophobic drug and a stoichiometric amount of HPβCD are solubilized in TBA and passed through a 0.22 μm millpore filter, then the TBA is removed by lyophilization to give a sterilized hydrophobic drug HPβCD complex in porous powder form. TBA is a versatile lyophilization medium since it has a very high vapor pressure (26.8 mmHg at 20°C) and a high freezing point (24°C) [10]. During freeze drying, needle shaped crystals with a high surface area are formed, the sublimation of TBA takes heat away and this results in the maintenance of a low product temperature which prevents the product from reaching the collapse temperature. All these are beneficial for acceleration of the sublimation rate and porous powder formation [11]. Another merit of freeze-drying of TBA is that it is an excellent nonaqueous solvent for the lyophilization of highly water-sensitive drugs [12]. Other volatile organic solvents, such as methanol and ethanol, which have been used for lyophilization have a very low freezing point, which would affect the drying rate, cake quality and leave a lot of residual solvent.

Experimental

Materials

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

Preparation of ketoprofen and nitrendipine HPβCD complexes by lyophilization of TBA solution

Ketoprofen or nitrendipine and the stoichiometric amount of HPβCD (in 1:1 molar ratio) were dissolved in TBA. The drug concentration in TBA solution was 15 mg/ml. An isotropic hydrophobic drug and HPβCD solution was the first step of this technique. The homogeneous solution was sterilized by filtration through 0.22 μm polyvinylidene difluoride membrane filter (Shanghai Xingya Material Factory, Shanghai, China), then transferred to 5 ml vials. Freeze-drying was carried out using a laboratory freeze-drier (FD-1, Beijing Bioking Technology Company, Beijing, China). The samples were cooled on the shelves to a temperature of –55°C and maintained at that temperature for 6 h, then vacuum was applied and the samples were subjected to lyophilization at a shelf temperature of –55°C for 24 h with a chamber pressure of 100 mbar, followed by secondary drying at a shelf temperature of 20°C for 8 h. Finally, the vials were fitted with lids and a sterile complex powder was obtained.

Characteristics of ketoprofen and nitrendipine HPβCD complexes

Differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and X-ray diffractometry (XRD) were used to identify the state of the drug present in the HPβCD complexes.

Scanning electron microscopy (SEM)

A Shimadzu SSX-550 Scanning Electron Microscope was used to examine the raw materials and binary systems. The powders were fixed on a brass stub and then were made electrically conductive by coating with a thin layer of gold in a vacuum for 150 s at 17 mA and 8 Pa. The pictures were taken at an excitation voltage of 15 kV.

Physical mixtures (PMs) were prepared by homogeneous blending of drug and HPβCD in a mortar.

Solubility and dissolution studies

Excessive pure drug, physical mixture as well as HPβCD complex were added to simulated gastric juice (pH1.2) and simulated intestinal fluid (pH6.8 PBS), thermostated at 37 ± 0.5°C and shaken at 60 rpm for 24 h. After equilibrium had been reached, the samples were passed through 0.22 μm polycarbonate pore

filters and the drug concentrations were determined by UV for ketoprofen and HPLC for nitrendipine.

Dissolution studies were carried out according to the paddle method using the USP dissolution apparatus, and pH6.8 PBS was used as the dissolution medium. Then, 200 mg ketoprofen or 10 mg nitrendipine, and an equivalent quantity of HPβCD complex or physical mixture were added to the medium. The dissolution medium was thermostated at 37 ± 0.5°C and the rotation speed was 100 ± 2 rpm. Samples were taken at selected intervals and filtered, and replaced with fresh medium at the same time. The drug concentrations were analyzed by UV and HPLC respectively. All experiments were carried out in triplicate. The cumulative amount of drug released at each time point was plotted.

Results and discussion

Preparation of ketoprofen and nitrendipine HPβCD complexes by lyophilization of TBA solution

TBA is a good solvent for hydrophobic drugs and HPβCD, and the solvent volume needed for the preparation of hydrophobic drug HPβCD complexes was reduced. Both hydrophobic drug and HPβCD were homogeneously dispersed in TBA solution. Hydrophobic drug molecules could be inserted into the cavity of HPβCD due to hydrophobic, Van der Waals and hydrogen bond interactions. This isotropic solution was passed through 0.22 μm pore filters, and TBA was transferred by lyophilization.

Lyophilization of TBA solution is a novel method for the preparation of hydrophobic drug HPβCD complexes. TBA is an excellent solvent for hydrophobic drugs and HPβCD, and this is beneficial for drug molecules interacting with HPβCD molecules, and the hydrophobic drug HPβCD complex is formed rapidly. TBA is suitable for freeze-drying because it has a high vapor pressure and a high melting point, and it is also an excellent solvent for lyophilization of highly water-sensitive drugs. Using TBA as a solvent, many problems associated with aqueous media can be avoided. 1-(2-Chloroethyl)-3-sarcosinamide-1-nitrosourea (SarCNU) is sufficiently soluble in water but is highly unstable. Its T_{90} in aqueous solution at room temperature is <6 h, which has hindered its application. Ni et al. introduced TBA as the medium for SarCNU and prepared SarCNU lyophilized power for injection successfully. The stability of SarCNU in both solution and lyophilized powder was improved markedly [13].

Despite the fact that TBA has a low toxicity and a minimal detrimental effect on health, the residual amount needs to be controlled. The residual amount of TBA is related to the formulation, freezing rate, cake thickness and technology used [14]. The physical state of the excipient will affect the residual amount of TBA and amorphous additive is infaust for crystalline matrix formation. The freezing rate will affect the crystallization of the solute. A lower freezing rate is better than a higher one because fast freezing will lead to TBA being entrapped in solute matrix which will result in a high level of residual TBA. Annealing could facilitate complete TBA crystallization during freezing, which is beneficial for sublimation and low residual content. In this study, no endothermal peaks were seen at the TBA boiling point of 82°C in the drug complex DSC thermograms.

Characteristics of hydrophobic drug HPβCD complexes

Differential scanning calorimetry, and powder X-ray diffraction studies showed that drugs were in an amorphous state in lyophilized samples, indicating the interaction of drug and the HPβCD cavity. Drug nucleation was prevented as a result of its molecular dispersion in the amorphous carrier HPβCD. FTIR spectra showed that a hydrogen bond had been formed between the hydrophobic drug and HPβCD and the supermolecular structure was formed after lyophilization of the hydrophobic drug HPβCD TBA solution.

Scanning electron microscopy

The scanning electron microphotographs of ketoprofen, nitrendipine and their binary systems are shown in Fig. 1. Ketoprofen appeared as irregular-shaped crystals and nitrendipine was in the form of prism-shaped crystals. HPβCD was observed as amorphous spheres with concaves shapes. The PM showed particles of HPβCD with embedded ketoprofen or nitrendipine particles. In the PMs, the drug crystals only adhered to the surface of HPβCD showing that no interaction had taken place between drug and carrier. In contrast, the shape and morphology of the freeze-dried complexes changed distinctly, the samples appeared as irregular sheet agglomerates and the particle size was reduced, the original morphology of both components disappeared, and the drug HPβCD complex had a high surface area. The microphotographs confirmed that a new solid phase was formed after freeze-drying of the hydrophobic drug HPβCD TBA solution.

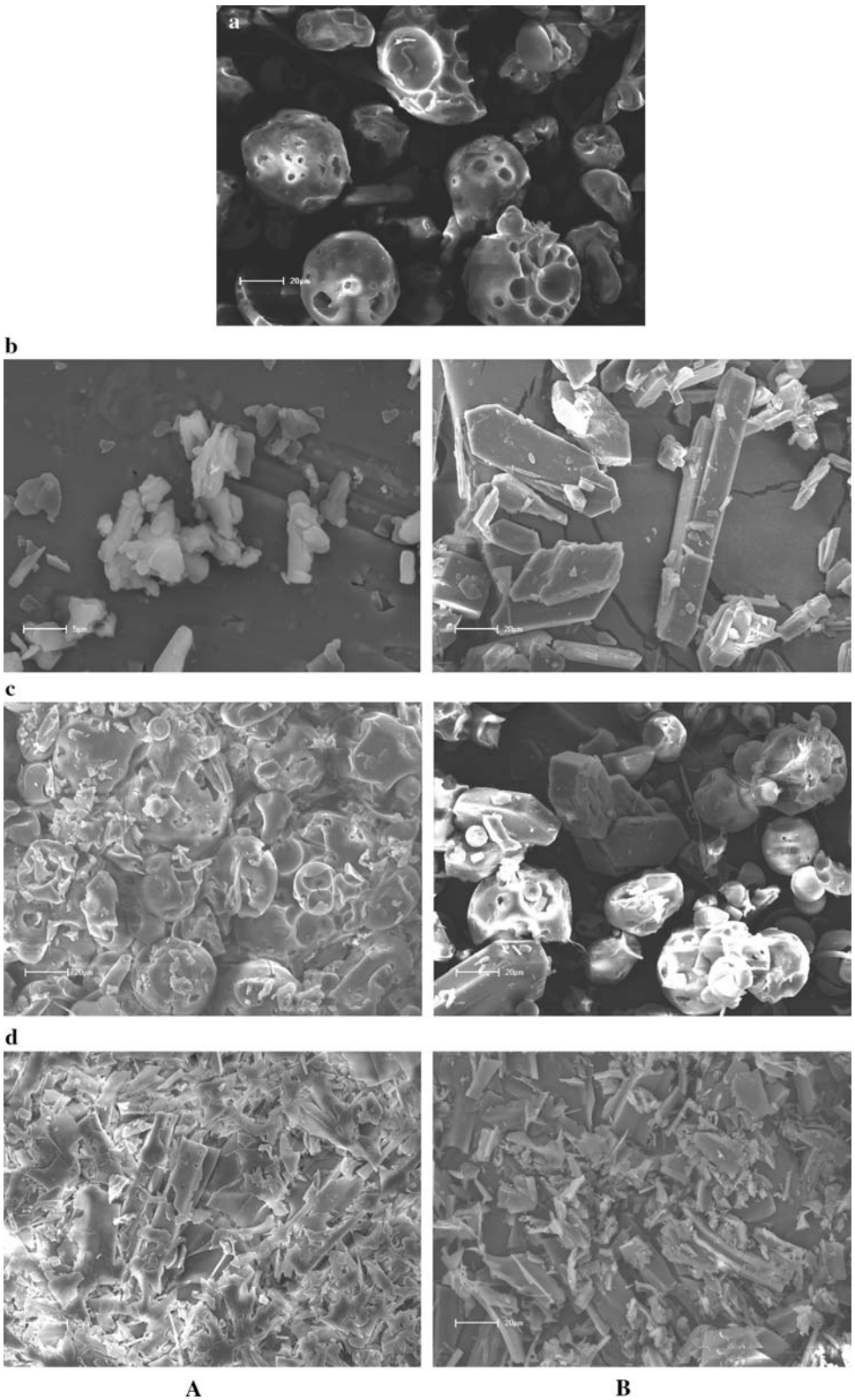

Fig. 1 Scanning electron microphotographs of ketoprofen (A) and nitrendipine (B) (**a**) HPβCD (**b**) Pure drug (**c**) drug HPβCD PM (**d**) drug HPβCD complex

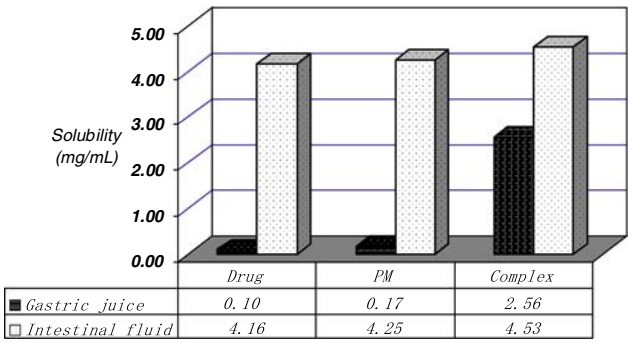

Fig. 2 The solubility results of ketoprofen

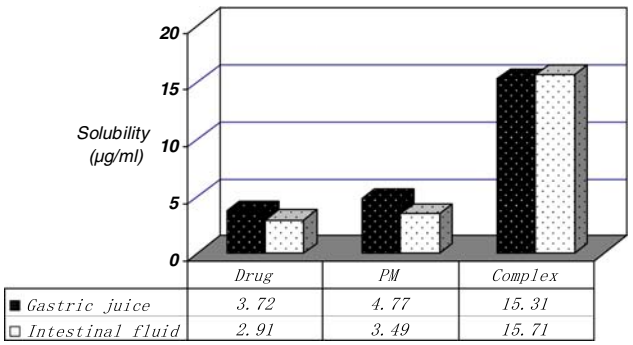

Fig. 3 The solubility results of nitrendipine

Solubility and dissolution studies

The solubility results of ketoprofen and nitrendipine are presented in Figs. 2, 3. As can be seen, the solubility of the drugs was not significantly increased in their HPβCD PMs. While, in the case of their HPβCD complexes prepared by lyophilization of the TBA system, the solubility of the two drugs was markedly enhanced. The solubility of ketoprofen in simulated gastric juice was increased 25.6-fold and this is advantageous for absorption and reducing the gastric irritation of ketoprofen. Nitrendipine is relatively insoluble in aqueous medium. The solubility of nitrendipine in its HPβCD complex was increased 4.11- and 5.39-fold in simulated gastric juice and intestinal fluid, respectively.

The dissolution results are presented in Fig. 4. The dissolution rate of ketoprofen as pure drug powder was very low. An increased dissolution rate was observed from its HPβCD physical mixture which was associated with the solubilizing effect and the improved drug wettability of HPβCD. The dissolution rate of ketoprofen from the HPβCD complex was significantly increased compared with that from the physical mixture and the drug dissolved rapidly during the first few minutes.

Nitrendipine is a class 2 drug with a low solubility and high permeability according to the Biopharmaceutic Drug Classification System, and its dissolution in vivo is the rate-controlling step for drug absorption. There was no apparent improvement in the dissolution rate obtained with the physical mixture. The dissolution rate of nitrendipine from the HPβCD complex was enhanced markedly and the drug dissolved much more rapidly in the first few minutes. Nitrendipine in the HPβCD complex dissolved very rapidly in the first few minutes and then slowed down due to the recrystallization occurring in the dissolution medium. The significant improvement in the dissolution rate of the two hydrophobic drug HPβCD complexes could be attributed to the following reasons: (1) the amorphous state of drug presented in lyophilized powder; (2) the drug particles are reduced in size and the specific surface area is increased as can be seen from the SEM; (3) HPβCD is a solubilizer; (4) a reduction in interfacial tension between the aqueous medium and the hydrophobic drugs.

Conclusions

In this study, a novel method for the preparation of hydrophobic drug HPβCD complexes was introduced. Because both the hydrophobic drug and HPβCD are readily soluble in TBA, a high drug concentration could be attained and many problems associated with aqueous solutions, such as the large amount of solvent

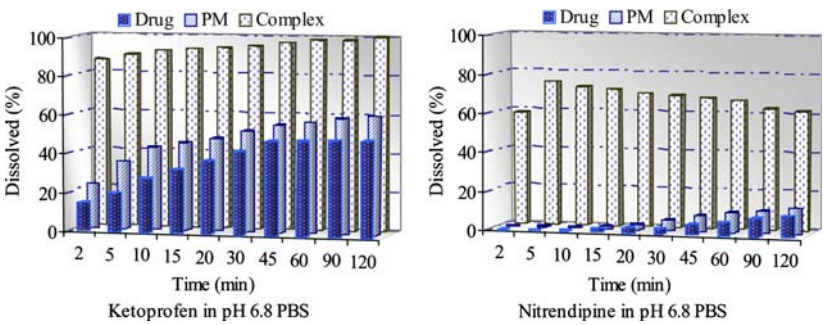

Fig. 4 Dissolution curves of ketoprofen and nitrendipine in pH6.8 PBS

needed or drug hydrolysis, could be avoided. Two hydrophobic drug HPβCD complexes were successfully prepared using this technique and confirmed by DSC, FTIR, XRD, and SEM. The solubility and dissolution rate of the hydrophobic drugs were improved markedly in aqueous medium. Thus, the present study provides a simple, more economic and easy to scale up technique for the preparation hydrophobic drug HPβCD complexes.

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