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# Preparation of Hydrophobic Drugs Cyclodextrin Complex by Lyophilization Monophase Solution

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**ABSTRACT** A novel method was evaluated for preparation of hydrophobic drugs cyclodextrin (CD) complex in this study. To obtain sterilized drug-CD complex lyophilized powder for injection or other purpose, the CD solution in water and the hydrophobic drug in tertiary butyl alcohol (TBA) were mixed in a suitable volume ratio, filtered through 0.22 μm millpores, and subsequently freeze-dried. A high drug concentration was obtained in the co-solvent due to the good solvency of TBA, which is miscible with water in any proportion, for hydrophobic drugs. Moreover, TBA could be removed rapidly and completely by freeze-drying because of its high vapor pressure and high melting point. The chemical stability of some labile active compounds was also improved in TBA-water co-solvent. Based on the data from differential scanning calormetry (DSC) and X-ray diffractometry (XRD), drug was amorphous in freeze-dried complex. The fourier transform infrared spectra indicated drug-CD interaction was present in drug-CD complex. An enhanced dissolution rate was also obtained in drug-CD complex. These results proved drug-CD complex had been formed after this technique. Thus, this report provided a simple, efficient, and economic technique for preparation of hydrophobic drugs CD complex, which may be useful practically in modifying hydrophobic drugs physicochemical properties and improving their absorption and pharmacodynamics.

**KEYWORDS** Cyclodextrin (CD), Hydrophobic drugs, Monophase solution, Lyophilization, Tertiary butyl alcohol (TBA)

## INTRODUCTION

Cyclodextrins (CDs) are well-known pharmaceutical excipients which can be used to improve the physicochemical and biopharmaceutical properties of drugs (e.g., solubility, stability, and bioavailability) (Miro et al., 2004; Dies et al., 2003; Fernandes et al., 2002). CDs are capable of forming inclusion complexes with enclaving a complete drug molecular or a part of it into

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their relatively hydrophobic cavity area. This molecular encapsulation will increase the solubility (Manolikar & Sawant, 2003), chemical stability (Vianna et al., 1998), and absorption (Jarho et al., 1996; Irwin et al., 1994) of the drug. The expected increase in bioavailability by CD complexes has been confirmed in many in vivo studies (Koester et al., 2004). Usually, different classes of methods can be used for the preparation of hydrophobic drugs CD complex. Grinding and slurry complexation are time-consuming, and solvent evaporation method requires excessive pharmaceutically unacceptable organic solvent usage and residual solvents need to be removed. Coprecipitation needs excessive CD and high temperature, and this is not suitable to heat unstable drugs. All these methods are difficult for industrial scaling up. Freeze-drying is an industrially applicable method especially for heat labile drugs and biopharmaceutical compounds, but tremendous amounts of water, if it was used as solvent, and excessive CD would be required because of the low solubility of hydrophobic drug in aqueous solution. This will make the preparation process time-consuming (Williams et al., 1998) and energy-wasting; excessive CD is also unbeneficial for humans. Some researchers have used organic solvent water co-solvent like acetone, ethanol (Gu et al., 2004) or methanol (Fan et al., 2005; Lo et al., 2004), and water to dissolve drug and CD, but freeze-drying was impractical because the melting point and vapor pressure of these organic solvents are very low. These organic solvents will not freeze even in liquid nitrogen. The melting point of dimethyl sulphoxide (DMSO) (Tesconi et al., 1999) is high but its vapor pressure is extremely low which will lead to high organic solvent residual. The difficulties for industrial scaling up and risk for body restrict the application of these kinds of co-solvent.

Tertiary butyl alcohol (TBA) has a good solvency for lipophilic drugs and merits to improve the stability of water labile drugs. Tertiary butyl alcohol (TBA) possesses a high vapor pressure (26.8 mmHg at 20°C) and a high melting point (24°C) (Teagarden & Baker, 2002). Adding TBA to water results in the formation of larger needle-shaped ice crystals with a larger surface area (13 fold increase) than round ice crystals which can facilitate sublimation. Furthmore, a more porous and lower resistance dry matrix can be formed with the presence of TBA and further accelerates the sublimation rate (Kasraian & Deluca, 1995). The sublimation of TBA takes heat away and prevents the product from reaching its collapse temperature. On the other hand, TBA also has low toxicity and is miscible with water in any proportion. All these contribute it as an ideal freeze-drying medium. In the previous study, Teagarden and Baker (2002) introduced TBA-water co-solvent as the solvent of Prostaglandin E1 (PGE1) lactose lyophilized powder preparation which improved the chemical stability of PGE1 evidently in both solution and dry powder. Prostaglandin E1 (PGE1) lactose lyophilized powder for injection prepared by lyophilization TBA-water co-solvent has been on the market (Teagarden et al., 1998). Tertiary butyl alcohol (TBA) water co-solvent has been progressively used in the pharmaceutical field, and liposomes (Li & Deng, 2004), solid dispersion (Van Drooge et al., 2004), and nanoparticles (Fournier et al., 2004) have been successfully prepared using this co-solvent. However, to the best of our knowledge, TBA-water co-solvent has not been used in preparation of CD complex yet.

In this study, TBA was introduced for preparation of hydrophobic drugs CD complex by lyophilization. The procedure was based on the initial formation of a clear homogeneous solution by dissolving hydrophobic drug and CD in TBA/water co-solvent system. Lyophilization monophase solution of TBA-water co-solvent that has a high melting point should contribute to easier and more economical processing. Two hydrophobic drugs, a topically active glucocorticosteroid budesonide and a long-acting β2 agonist salmeterol, were selected as model drugs. The interaction between drug and βCD or 2-hydroxypropyl derivative was studied in solution by the phase solubility method. The additional information on the complexing efficacies of the two CDs toward hydrophobic drugs was obtained by analysis with DSC, FTIR, and powder XRD. Based on these results, drug CD interaction was revealed and drug was indicated to be amorphous in CD complex, which suggested that the low solubility of hydrophobic drugs can be well circumvented by using this technology.

## MATERIALS AND METHODS

### Materials

Budesonide (Shanghai Instituite of Pharmaceutical Industry, Shanghai, China), Salmeterol xinafoate (Haerbin Pharmaceutical Group Corporation, Haerbin,

China), Pharmaceutical grade βCD (MW = 1135) containing 12% by weight of water, and HPβCD were supplied by Shanxi Liquan Corporation (Xian, China); HPLC grade methanol and acetonitrile were obtained from Tianjin Concord Technology Company (Tianjin, China). All other chemicals and solvents used were of analytical grade.

## Phase Solubility Studies

An excess amount of budesonide or salmeterol xinafoate was added to 10.0 mL of βCD or HPβCD aqueous solution with concentrations of 0, 3.0, 6.0, 9.0, 12.0, 15.0, and $18.0 \times 10^{-3}$ M. The suspensions were sealed and shaken at 37°C for 72 h. After equilibrium had been reached, the samples were centrifuged and filtered through 0.45 μm cellulose acetate membrane and suitably diluted. Each experiment was carried out in triplicate [coefficient of variation (CV) < 3%]. Salmeterol and budesonide concentration were determined by HPLC at 225 nm and 240 nm, respectively. The apparent 1:1 stability constants of the complexes Ks were calculated from the phase solubility diagrams according to the following equation: Ks = slope/$s_0$ (1-slope), where $s_0$ is solubility in the absence of βCD or HPβCD.

## Preparation of Solid Inclusion Complexes by Lyophilization Monophase Solution Method

### *Preparation of Monophase Solution*

Budesonide or salmeterol was dissolved in TBA (10 mg/mL); the required stoichiometric amount of βCD or HPβCD was dissolved in water and mixed with drug TBA solution. The drug concentration in co-solvent was 5 mg/mL. A homogenous CD and drug co-solvent system was the first step of this technology. For budesonide and salmeterol, drug CD complex was prepared in the molar ratio of 1:1 and 1:3, respectively. The co-solvent system was subsequently filtered through polycarbonate membrane with pore size of 0.22 μm. Finally, the solution was filled into each 5 mL vials.

### *Freeze-drying of Monophase Solution*

The co-solvent system was freeze-dried in 5 mL vials. The vials were freeze-dried according to the following procedure: The shelves were frozen at −40°C, the samples on the shelves were frozen for 4 h, vacuum was applied, and the samples were subjected to lyophilization (FD-1, Beijing Bioking Technology Company, Beijing, China) for 24 h with 40 mbar vacuum in a freeze-dryer, secondary drying at 25°C for 8 h. Finally, the vials were closed by pressing the seal. The hydrophobic drug CD complex lyophilized powder was attained.

## Preparation of Physical Mixtures

Physical mixtures (PMs) were obtained by homogeneously blending an accurately weighed (1:1 and 1:3 molar ratio for budesonide and salmeterol respectively) amount of drug and CD in a mortar.

## Differential Scanning Calorimetry (DSC)

A Shimadz differential scanning calorimeter (DSC-60) with a thermal analyzer was used for recording DSC thermograms of pure drug, βCD, HPβCD, and inclusion complex as well as their PM. Samples were placed in sealed aluminum pans and heated at 10°C/min under a nitrogen atmosphere (folw rate 15 mL/min) in the 30–400°C range. An empty aluminum pan was used as reference. The equipment was periodically calibrated with indium.

## Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) spectra were obtained using a Bruker IFS 55 FTIR spectrometer. The samples were mixed with dry KBr at about 1:100 (sample:KBr) ratio and pressed to form pellets. For each sample, the range of spectra was from 400–4000 $cm^{-1}$.

## Powder X-ray Diffractometry (XRD)

X-ray diffractometry (XRD) was carried out using a D/MAX 2400 model diffractometer with Co as anode material and a graphite monochromator, over a 2-theta range of 2° to 45 °, Cu $K_{\alpha 1+2}$ radiation, operated at a voltage of 40 kV and a current of 30 mA, scan step size was 0.02° (2θ). The analysis was carried out at room temperature under ambient conditions.

## Solubility and Dissolution Studies

Excessive drug, CD PM, or CD complex was added to screw capped vials containing 5 mL of phosphate buffered saline pH 7.4. These vials were shaken at 37°C, 60 rpm for 24 h. Then the samples were filtered through 0.22 μm cellulose acetate membrane and the concentration of drug in each sample was analyzed with HPLC.

The dissolution was evaluated using USP XXIV rotating paddle apparatus with a stirring speed of 100 ± 2 rpm. The dissolution medium was pH 7.4 phosphate buffer and the medium thermostated at 37.0 ± 0.5°C. Drug raw material, CD complex, or PM, containing the same amount of drug (30 mg and 100 mg for budesonide and salmeterol, respectively) was introduced into 200 mL dissolution medium. At settled time intervals for a period of 240 min, 1 mL sample was withdrawn and immediately replaced with fresh medium; the sample was filtered and drug concentration was analyzed by HPLC. All experiments were carried out in triplicate and average values were calculated. The cumulative amount of drug released at each time point was plotted.

# RESULTS AND DISCUSSION

## Phase Solubility Studies

The phase solubility profiles obtained for budesonide-CD and salmeterol-CD systems were presented in Fig. 1. According to Higuchi and Connors (1965) classification (Higuch & Connors, 1965), the shape of the diagrams of budesonide obtained followed an $A_L$-type system. The guest solubility increased linearly with CD concentration because of a significant contribution of drug and CD interaction. Thus, we assumed that a 1:1 complex was formed, and the value of Ks for budesonide βCD (674.4 $M^{-1}$) was higher than that for HPβCD (260.0 $M^{-1}$). For salmeterol, the solubility of salmeterol increased linearly with CD concentration when βCD or HPβCD was at low concentration. We speculated the phenyl was inserted into the CD cavity. The chemical structure of salmeterol was shown in Fig. 2 (Michael, 2000). The stability constant (Ks) was calculated from the initial portion of the phase solubility diagram, βCD (1080.0 $M^{-1}$) > HPβCD (782.8 $M^{-1}$), assuming that a 1:1 stoichiometric ratio complex was formed at the initial step. At middle βCD or HPβCD concentration, the solubility of salmeterol was not changed. Perhaps 1-hydroxy-2-naphthoic acid was encapsulated by CD because naphthalin could be encapsulated by CD and hydrogen bond was formed between 1-hydroxy-2-naphthoic acid and CD. With the continual increase of the concentration of CD, the solubility studies showed that CD improved the aqueous solubility of salmeterol further because the salicyl alcohol was entraped by CD. Additionally, the solubility studies showed that HPβCD was weaker in improving the solubility of budesonide and salmeterol which was attributable to the stereoeffect.

## Preparation of Hydrophobic Drug CD Complex by Lyophilization Monophase Solution Method

Tertiary butyl alcohol (TBA) is an ideal freeze-drying medium because of its high melting point and high vapor pressure which cause rapid and complete sublima-

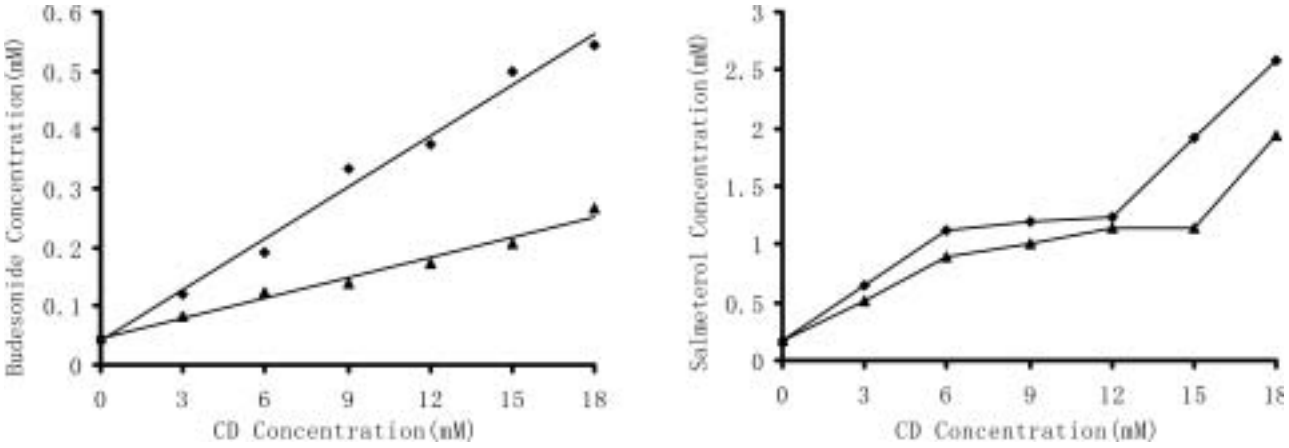


**FIGURE 1** **The phase solubility diagram of Budesonide and Salmeterol with βCD (◆) and HPβCD (▲).**

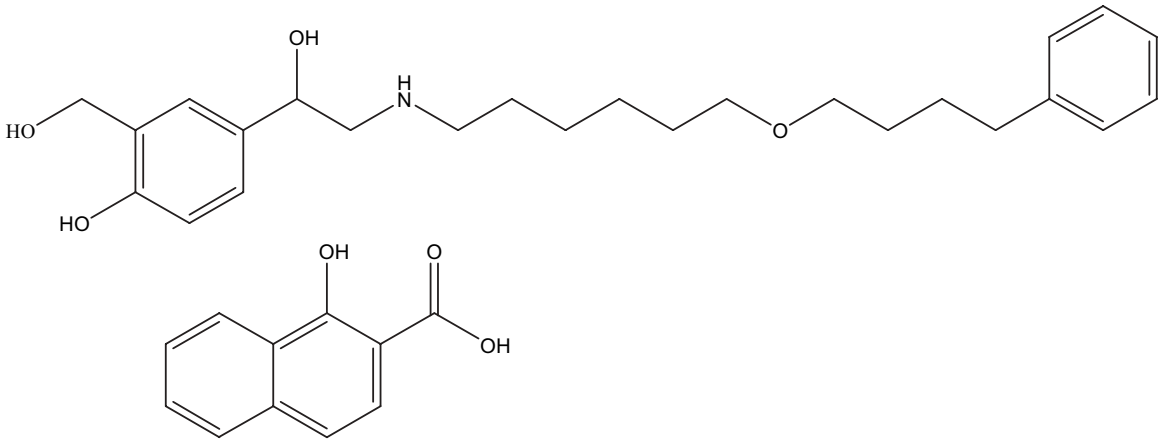


**FIGURE 2** **The chemical structure of salmeterol xinafoate.**

tion. Tertiary butyl alcohol (TBA) causes needle shaped ice crystals with high surface area formation, and the resistance of the dried powder layer was decreased by one order of magnitude. This accelerated sublimation rate and decreased the freeze-dried cycle.

To produce isotropic solution of hydrophobic drug and CD, the optimal TBA/water ratio should be found to guarantee physical stability of drug and CD in the TBA/water mixture which is necessary for drug CD complex formation. Different drugs have different optimal TBA/water ratios. The more hydrophobic the drug, the more TBA ratio in co-solvent was needed for preparing clear system. The TBA/water ratio was also correlative with the drug and CD concentration. In this experiment, the volume of TBA in co-solvent system should not be lower than 35% for budesonide to prepare clear solution while 40% was needed for salmeterol. So, we could make different phase diagrams for different drugs. The drug concentration in CD complex powder in this study was about 26% and 15% (w/w) for budesonide and salmeterol, respectively. The ratio of TBA/water in this study was 1/1 (v/v).

In the TBA/water co-solvent system, hydrophobic drug and CD were in molecule form. The hydrophobic drug was homogeneously dispersed in CD solution. CDs have an exterior hydrophilic surface and interior hydrophobic cavity which provides a favorable environment for lipophilic drug molecules. Hydrophobic drug inserted into the cavity of CD due to hydrophobic, van der Waals and hydrogen bond interactions, and drug CD inclusion complex was formed. After the solvent was removed by lyophilization, the inclusion complex could exist as a dry product which was beneficial for the chemical stability of drug.

Tertiary butyl alcohol (TBA) is a good solvent for hydrophobic drugs, so less volume of co-solvent is needed in hydrophobic drug CD complex preparation. The ratio for collision of drug molecule with CD molecule is high because of the high concentration of drug and drug CD complex form rapidly. Kinnarinen and his coworkers (2003) prepared budesonide CD complex by aqueous precipitation method. Excessive drug and excessive CD were added to aqueous solution. The budesonide concentration in CD solution was 0.8 mg/mL and the solution of drug and CD stirred for 6 days. The concentration of budesonide in co-solvent was 5 mg/mL in preparation of budesonide CD complex by lyophilization TBA-water co-solvent system. High drug concentration was beneficial for complex forming and energy-saving.

The residual TBA controlling was needed though TBA is a low toxic organic solvent and has little detriment to human body. The residual amount in samples was correlated with the formulation, initial TBA content, freezing rate, and additional technology. Amorphous excipient would not crystallize completely and was not beneficial for lyophilization. The residual TBA content would be higher when the TBA content in co-solvent was lower than 2%. Rapid freezing could lead to TBA encapsulated in ice crystal, incomplete crystallization, and then cause high TBA residual. On the other hand, annealing was a good method for reducing residual TBA because the TBA could nucleate at low temperature followed by crystal growth at a temperature higher than Tg' (Wittaya & Nail, 1998).

## Differential Scanning Calorimetry (DSC)

As shown in Fig. 3, budesonide exhibited a typical highly crystalline compound and showed an endothermic peak at 257°C corresponding to its melting point that was in accordance with a previous report (Bandi et al., 2004). The thermogram of βCD exhibited a very broad endothermic peak around 83°C corresponding to the loss of water, and its melting peak was observed at 325°C. In contrast, no signal could be observed in HPβCD during the same temperature range. The thermal analysis of budesonide CD PM revealed that the melting peak described for drug decreased markedly, especially for budesonide βCD PM. The reasons may be as follows: (i) the inclusion could be formed during the course of heating or (ii) the drug corresponded to only 25% of the mixture total mass and the CD might mask the detection of the endothermic peak of drug. The melting peak of budesonide disappeared entirely in its CD complex indicating the drug was in amorphous form after freeze-drying.

For salmeterol, the DSC thermogram showed an endothermic peak at 142°C due to its melting point indicating that salmeterol existed in a crystalline form. The salmeterol melting peak was observed in the PM of salmeterol with βCD or HPβCD, though the △H values were

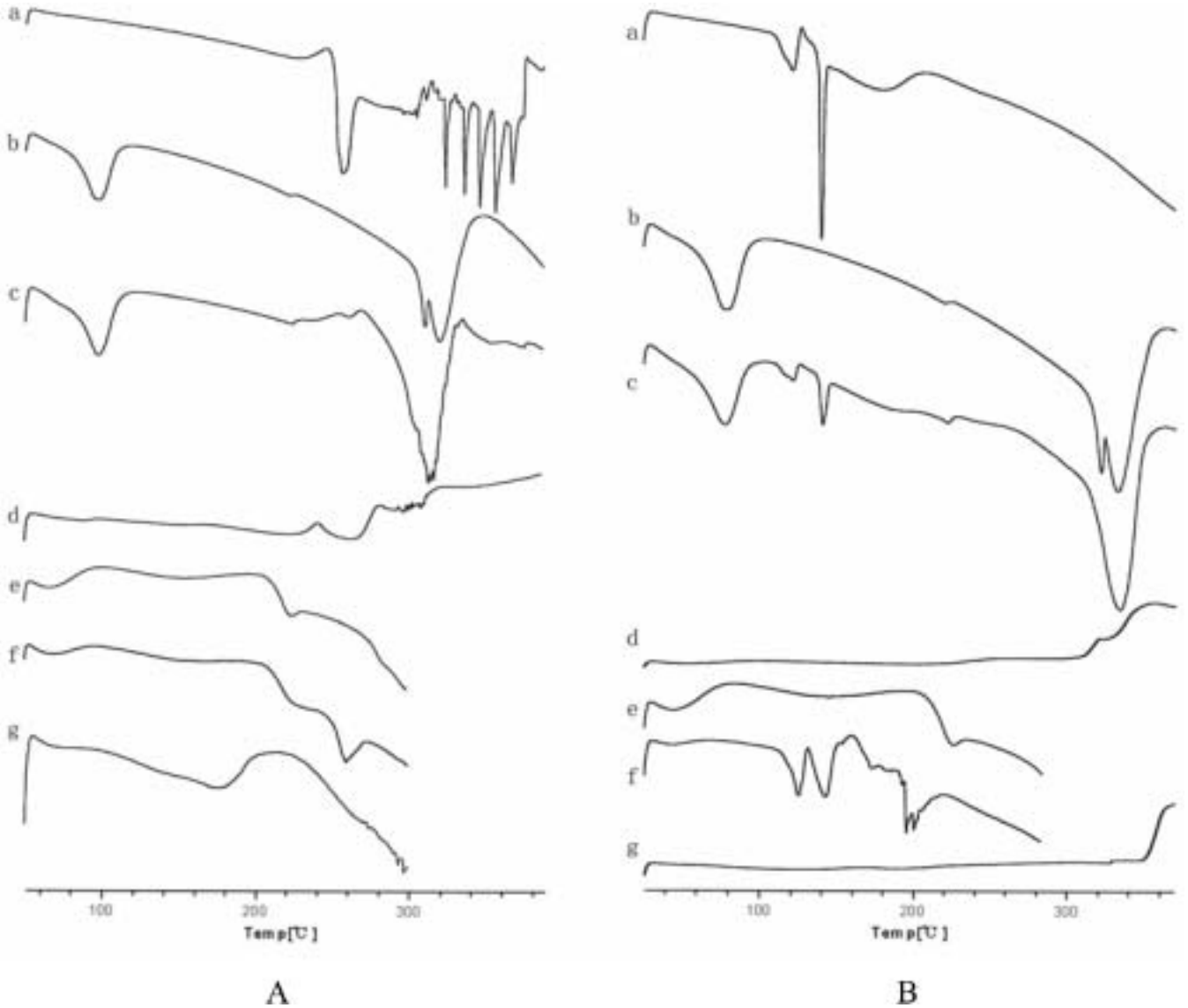


**FIGURE 3** DSC curves of Budesonide (A) and Salmeterol (B) (a) Pure drug (b) βCD (c) Drug βCD PM (d) Drug βCD complex (e) HPβCD (f) Drug HPβCD PM (g) Drug HPβCD complex.

lower than that of the pure drug. The dehydration peak and melting peak of βCD were observed also which indicated an absence of interaction between drug and CD in such systems. The DSC thermograms of salmeterol βCD and HPβCD inclusion complexes prepared by lyophilization monophase solution method were shown in Fig. 3d and g, respectively. No endothermal peak existed suggesting the amorphous nature of drug in freeze-dried complex.

## Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of drug-CD complexes were compared with that of the PMs and pure drugs. Budesonide indicated carbonyl stretching bands at 1718 and 1659 $cm^{-1}$ (Fig. 4) which were attributable to the stretch of nonconjugated acetyl carbonyl and conjugated dihydrobenzoquinone carbonyl groups, respectively. For the budesonide-βCD complex, the characteristic carbonyl stretching vibration at 1659 $cm^{-1}$ shifted to 1651 $cm^{-1}$. The stretching vibration at 1718 and 1659 $cm^{-1}$ of budesonide evidently reduced in its CD complexes, suggesting a change in the environment of carbonyl of budesonide as a consequence of the interaction with CD. A very small absorption peak characteristic of the C-H stretching in the range of 2800 to 3000 $cm^{-1}$ was displayed in CD complexes compared with PMs. For salmeterol-CD complexes, differences were found in the 1480–1600 $cm^{-1}$ regions attributed to the skeleton vibrations of the C = C bonds in the

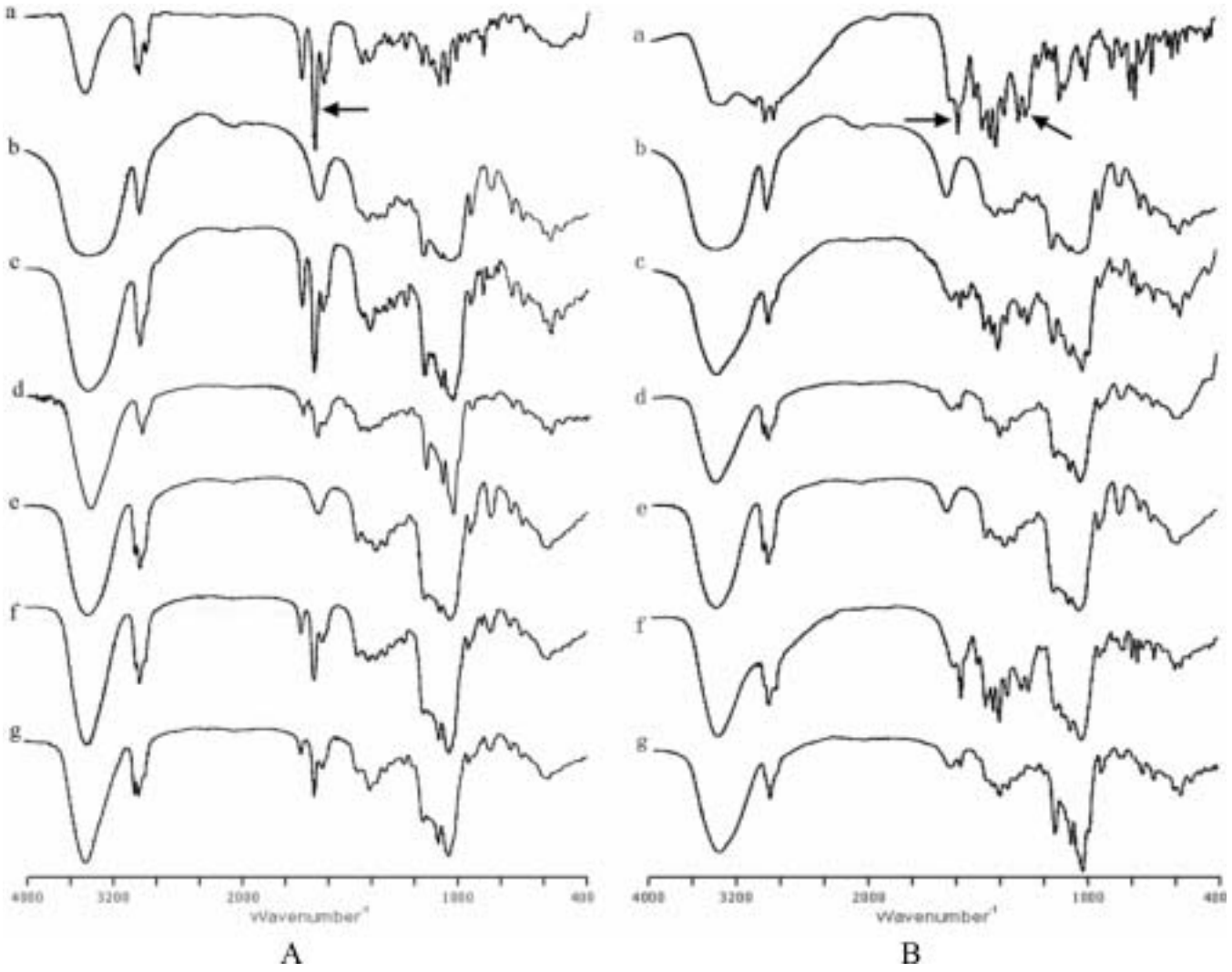


**FIGURE 4** FTIR spectra of Budesonide (A) and Salmeterol (B) (a) Pure drug (b) βCD (c) Drug βCD PM (d) Drug βCD complex (e) HPβCD (f) Drug HPβCD PM (g) Drug HPβCD complex.

aromatic ring. The shapes of these bands changed dramatically for the inclusion compounds as compared to those for PMs. The vibration band of C = C bond at 1580 $cm^{-1}$ and hydroxy in aromatic at 1273 $cm^{-1}$ also appeared in the PMs but were not found in the complex systems. These indicated that the vibrating and bending of the guest molecule were restricted due to the formation of an inclusion complex.

## Powder X-ray Diffractometry (XRD)

The powder XRD pattern analyze gave further support for the formation of a supramolecular compound between drug and CD. The XRD patterns of pure drugs, CDs, the PMs, and drug-CD inclusion compounds were shown in Fig. 5. The powder XRD patterns of budesonide, salmeterol, and βCD exhibited a series of sharp peaks characteristic of crystalline compounds. In

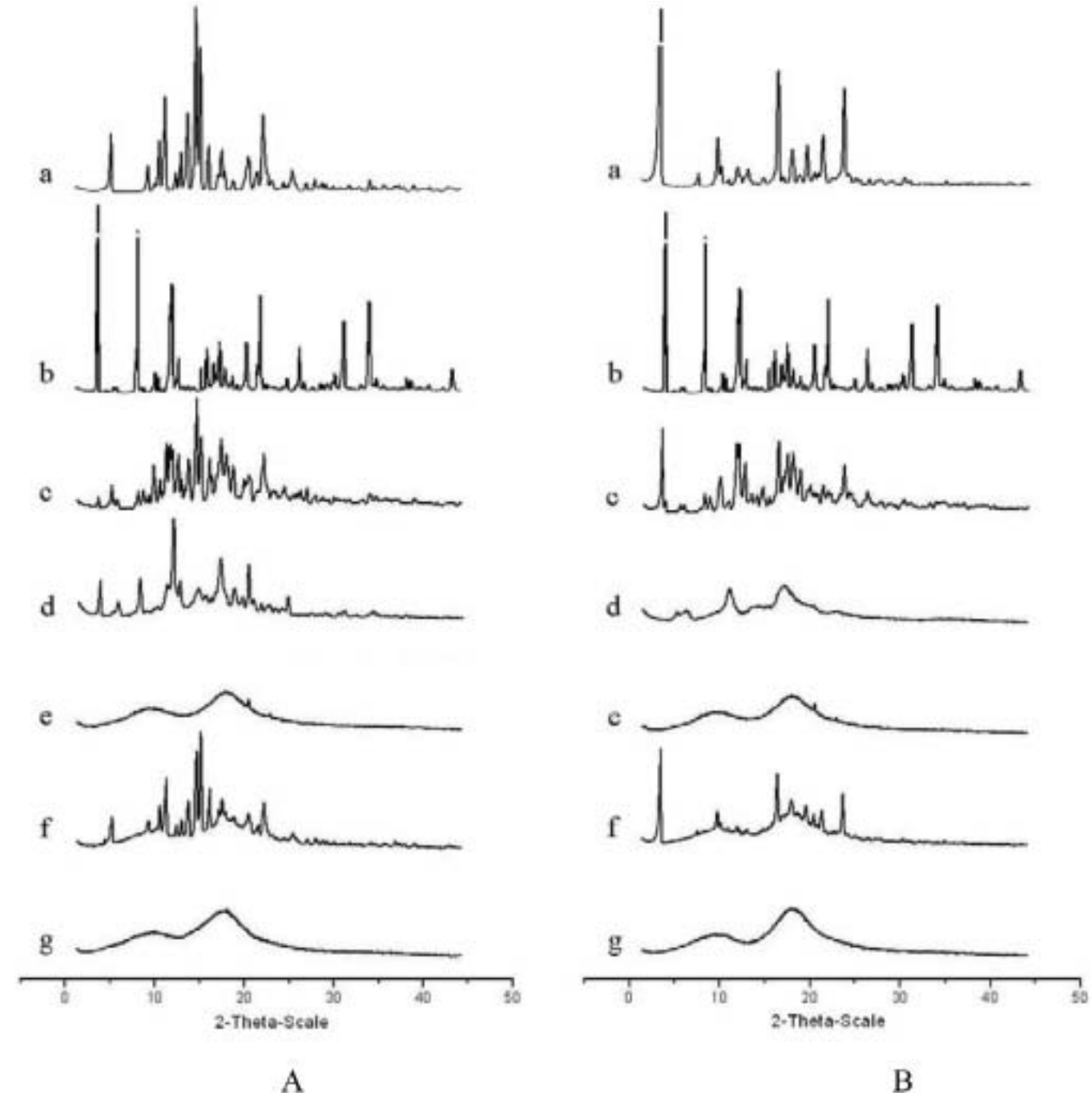


**FIGURE 5** powder X-ray diffraction patterns spectra of Budesonide (A) and Salmeterol (B) (a) Pure drug (b) βCD (c) Drug βCD PM (d) Drug βCD complex (e) HPβCD (f) Drug HPβCD PM (g) Drug HPβCD complex.

contrast, HPβCD was amorphous. The XRD patterns of the PM were the simple superposition of each component and the crystalline peaks of drug presented indicating no formation of new structure. For the CD complex, the XRD patterns indicated a loss of crystalline of drugs which revealed their amorphousness.

## Aqueous Solubility and Dissolution Studies

The solubility of pure drugs and binary systems were also studied. As shown in Table 1, the solubility of budesonide was increased unremarkably in its CD PM. Freeze-dried product showed 6.5 and 4.3 fold increase of the solubility from βCD and HPβCD complex, respectively. The solubility of salmeterol in its βCD and HPβCD PM was improved 2.5 and 2.4 fold, respectively. However, the drug solubility in its βCD and HPβCD complexes was raised 22.8 and 19.2 fold, respectively. This result suggested that the drug was encapsulated inside the host cavity.

As shown in Fig. 6, the drugs used in this study exhibited low solubility and for this kind of drugs, their absorption is dissolution rate limited. The dissolution rate of budesonide was very low and only 14.6% was released at the end of the dissolution study whereas 95.6% and 65.4% were released from βCD and HPβCD complexes separately. The PMs of budesonide with βCD and HPβCD showed a modest dissolution rate, 21.2% and 18.5% of the initial content were released, respectively. Evidently the dissolution rate of CD complex was much higher than the drug material or PM of drug with CD. Budesonide in CD complex dissolved very rapidly in the first minute and then decreased due to the recrystallization of drug in medium.

For salmeterol, 97.5% and 96.6% of drug were dissolved from the βCD complex and HPβCD complex, respectively, at 2 min. However, only 5.1% and 7.5% from the each PM and 0.3% from the pure drug were released at the same time point. The dissolution was incomplete even after four hours for pure drug and βCD or HPβCD PM.

The dissolution results carried out in pH 7.4 PBS showed that the presence of CD led to an improvement in the dissolution rate of budesonide and salmeterol. The PM showed a slight increase of the dissolution rate which was associated with the solubilizing effect and improving drug wettability of CD. The dissolution rate from CD complex was clearly increased compared with that from the PM. These results may be explained as follows: 1) the formation of inclusion complex in the solid state; 2) the amorphous state of drug and no energy was needed to break up the crystal lattice; 3) the drug particles were minished and specific surface area was enlarged; 4) CD or HPβCD was a solubilizer; and 5) the reduction of interfacial tension between water and hydrophobic drug. The dissolution rates were in the order: hydrophobic drug < hydrophobic drug HPβCD PM < hydrophobic drug βCD PM < hydrophobic drug HPβCD complex < hydrophobic drug βCD complex. The dissolution rates of budesonide and salmeterol in their βCD complexes were higher than in their HPβCD complexes. The same result was acquired with their PMs. This result was in accordance with higher stability constant of βCD complex revealed in phase solubility studies.

## CONCLUSIONS

In this investigation, a novel procedure to prepare hydrophobic drug CD inclusion complex was described, and freeze-drying TBA/water co-solvent was shown as a versatile technique for the production of sterile and pyrogen-free hydrophobic drug CD complex. Hydrophobic drugs were indicated in an amorphous form by using DSC and XRD. In this high energy state, drug released rapidly in dissolution medium, and the aqueous solubility of drugs could be improved markedly. Thus, the present study provided a maneuverable, energy saving and easily scaling up technique for preparation hydrophobic drugs CD complex.

**TABLE 1** **Aqueous Solubility of Budesonide and Salmeterol ($\mu g.mL^{-1}$)**

| | pure drug | drug βCD PM | drug βCD complex | drug HPβCD PM | drug HPβCD complex |
|---|---|---|---|---|---|
| Budesonide | 23.21 | 33.62 | 151.59 | 29.39 | 98.96 |
| Salmeterol | 87.65 | 220.82 | 1994.36 | 206.28 | 1686.32 |

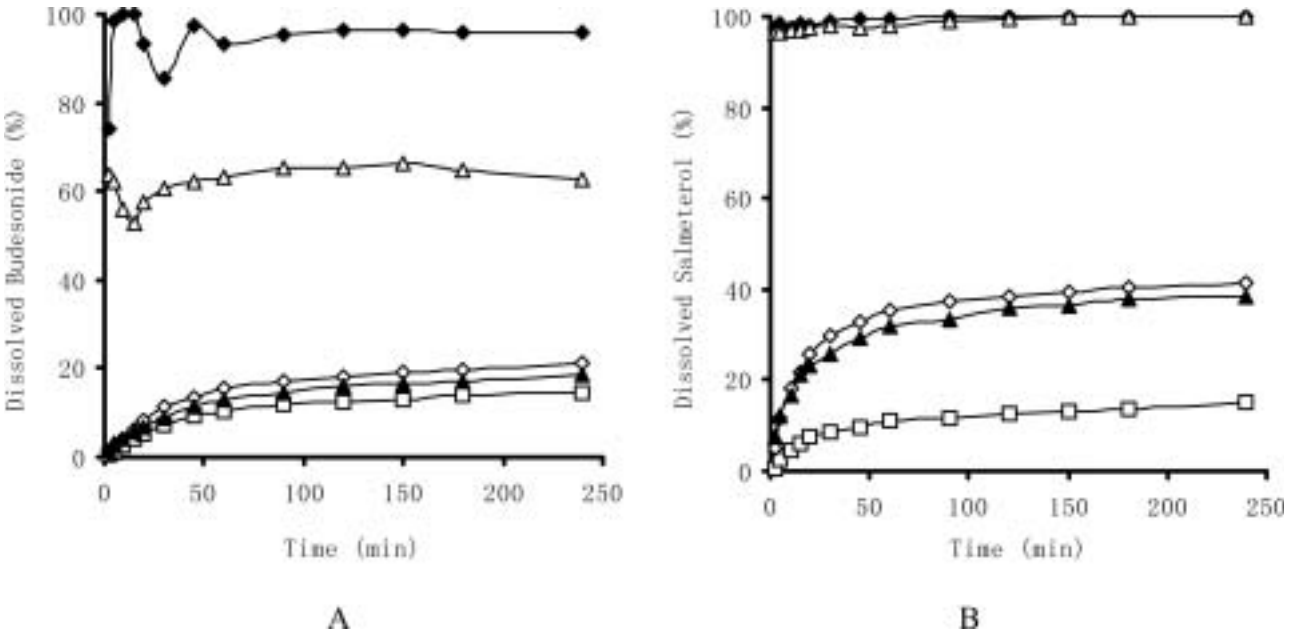


**FIGURE 6** **Dissolution curves of Budesonide (A) and Salmeterol (B) (□) Pure drug (◇) Drug βCD PM (◆) Drug βCD complex (▲) Drug HPβCD PM (△) Drug HPβCD complex.**

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## REFERENCES

Bandi, N., Wei, W., Roberts, C. B., Kotra, L. P., & Kompella, U. B. (2004). Preparation of budesonide and indomethacin hydroxypropyl-β-cyclodextrin(HPBCD) complexes using a single-step, organic-solvent-free supercritical fluid process. *Eur. J. Pharm. Sci.*, *23*, 159–168.

Dias, M. M. R., Raghavan, S. L., Pellett, M. A., & Hadgraft, J. (2003). The effect of β-cyclodextrins on the permeation of diclofenac from supersaturated solutions. *Int. J. Pharm.*, *263*, 173–181.

Fan, Y. X., Li, J. F., & Dong, C. (2005). Preparation and study on the inclusion complexes of two tanshinone compounds with β-cyclodextrin. *Spectrochim Acta A.*, *61*, 135–140.

Fernandes, C. M., Teresa Vieira, M., & Veiga, F. J. B. (2002). Physicochemical characterization and in vitro dissolution behavior of nicardipine-cyclodextrins inclusion compounds. *Eur. J. Pharm. Sci.*, *15*, 79–88.

Fournier, E., Dufresne, M. H., Smith, D. C., Ranger, M., & Leroux, J. C. (2004). A novel one-step drug-loading procedure for water-soluble amphiphilic nanocarriers. *Pharm. Res.*, *21*, 962–968.

Gu, F. G., Cui, F. D., & Gao, Y. L. (2004). Effect of complexation with hydroxylpropyl-β-cyclodextrin on solubility, dissolution rate and chemical stability of prostaglandin E1. *J. Chin. Pharm. Sci.*, *13*(3), 158–165.

Higuchi, T., & Connors, K. A. (1965). Phase-Solubility Techniques. In *Adv. Anal. Chem. Instrum*, Reilly, C. N., Ed.; New York: John Wiley & Sons, Inc., 117–212.

Irwin, W. J., Dwivedi, A. K., Holbrook, P. A., & Dey, M. J. (1994). The effect of cyclodextrins on the stability of peptides in nasal enzymic systems. *Pharm. Res.*, *11*(12), 1698–1703.

Jarho, P., Urtti, A., Pate, D. W., Suhonen, P., & Jarvinen, T. (1996). Increase in aqueous solubility, stability and in vitro corneal permeability of anandamide by hydroxypropyl-β-cyclodextrin. *Int. J. Pharm.*, *137*, 209–216.

Kasraian, K., & Deluca, P. P. (1995). The effect of tertiary butyl alcohol on the resistance of the dry product layer during primary drying. *Pharm. Res.*, *12*(4), 491–495.

Kinnarinen, T., Jarho, P., Jarvinen, K., & Jarvinen, T. (2003). Pulmonary deposition of a budesonide/cyclodextrin complex in vitro. *J. Control. Release*, *90*, 197–205.

Koester, L. S., Bertuol, J. B., Groch, K. R., Xavier, C. R., Moellerke, R., Mayorga, P., Costa, T. D., & Bassani, V. L. (2004). Bioavailability of carbamazepine: β-cyclodextrin complex in beagle dog from hydroxypropylmethylcellulose matrix tablets. *Eur. J. Pharm. Sci.*, *22*, 201–207.

Li, C. L., & Deng, Y. J. (2004). A novel method for the preparation of liposomes: freeze-drying of monophase solutions. *J. Pharm. Sci.*, *93*(6), 1403–1414.

Lu, W. L., Zhang, Q., Zheng, L., Wang, H., Li, R. Y., Zhang, L. F., Shen, W. B., & Tu, X. D. (2004). Antipyretic, analgesic and anti-inflammatory activities of ketoprofen beta-cyclodextrin inclusion complexes in animals. *Biol Pharm Bull.*, *27*, 1515–1520.

Manolikar, M. K., & Sawant, M. R. (2003). Study of solubility of isoproturon by its complexation with β-cyclodextrin. *Chemosphere.*, *51*, 811–816.

Michael, Y., Chowdhry, B. Z., Ashurst, I. C., Snowden, M. J., Davies-Cutting, C., & Gray, S. (2000). The physico-chemical properties of salmeterol and fluticasone propionate in different solvent environments. *Int. J. Pharm.*, *200*, 279–288.

Miro, A., Quaglia, F., Sorrentino, U., La Rotonda, M. I., D'Emmanuele, D. V., Bianca, R., & Sorrentino, R. (2004). Improvement of gliquidone hypoglycaemic effect in rats by cyclodextrin formulations. *Eur. J. Pharm. Sci.*, *23*, 57–64.

Teagarden, D. L., & Baker, D. S. (2002). Practical aspects of lyophilization using non-aqueous co-solvent systems. *Eur. J. Pharm. Sci.*, *15*, 115–133.

Teagarden, D. L., Peter, W. J., & Gold, P. M. (April 21, 1998). Stabilized Prostaglandin $E_1$. *United States Patent 5*, *741*, 523.

Tesconi, M. S., Sepassi, K., & Yalkowsky, S. H. (1999). Freeze-Drying above Room Temperature. *J. Pharm. Sci.*, *88*(5), 501–506.

Van Drooge, D. J., Hinrich, W. L. J., & Frijlink, H. W. (2004). Incorporation of lipophilic drugs in suger glasses by lyophilization using a

mixture of water and tertiary butyl alcohol as solvent. *J. Pharm. Sci.*, *93*(3), 713–725.

Van Drooge, D. J., Hinrichs, W. L. J., Wegman, K. A. M., Visser, M. R., Eissens, A. C., & Frijlink, H. W. (2004). Solid dispersions based on inulin for the stabilization and formulation of 9-tetrahydrocannabinol. *Eur. J. Pharm. Sci.*, *21*, 511–518.

Vianna, R. F. L., Bentley, M. V. L. B., Ribeiro, G., Carvalho, F. S., Neto, A. F., Oliveira, D. C. R., & Collett, J. H. (1998). Formation of cyclodextrin inclusion complexes with corticosteroids: their characterization and stability. *Int. J. Pharm.*, *167*, 205–213.

Williams, R. O., III, Mahaguna, V., & Sriwongjanya, M. (1998). Characterization of an inclusion complex of cholesterol and hydroxypropyl-β-cyclodextrin. *Eur. J. Pharm. Biopharm.*, *46*, 355–360.

Wittaya, A. S., & Nail, S. L. (1998). Freeze-drying of tert-butyl alcohol/water co-solvent systems: effect of formulation and process variables on residual solvents. *J. Pharm. Sci.*, *87*(4), 491–495.