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ORIGINAL ARTICLE

The effect of cyclodextrin mixtures on aqueous solubility of beclomethasone dipropionate

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Abstract The aim of present study was to evaluate the effect of natural, synthetic cyclodextrins (CDs) and CD mixtures on aqueous solubility of beclomethasone dipropionate (BDP). The phase solubility studies were done in the presence of 6 CDs. Furthermore, aqueous solubility of BDP was tested in the presence of CD mixtures. The solubility of BDP in water was increased by 30, 77, 155 and 30 folds in the solution containing 20% w/v α-CD, hydroxylpropyl β-CD (HP-β-CD), hydroxypropyl γ-CD (HP-γ-CD) and sulphobutylether β-CD (SBE-β-CD), respectively. CD mixtures had remarkable effect on the aqueous solubility of BDP so that solubility in water increased between 200 and 1,500 times in the presence of different CD mixtures. Further addition of sodium acetate to the solubilisation medium reduced the aqueous solubility. In conclusion, CD complexation was able to improve the aqueous solubility of BDP. The synergistic effect of cyclodextrin mixture was observed.

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Introduction

Cyclodextrins (CDs) are oligosaccharides with a truncated cone structure. The interior of the cone is relatively apolar and creates a hydrophobic micro-environment but because of the large number of hydroxyl groups on CD structure, they are water-soluble [1–4]. These structures are able to form inclusion complexes with many drugs by encapsulation of the whole drug molecule, or a lipophilic part of the molecule [4, 5]. One or more CD molecules contain one or more entrapped molecules. Most frequently the host (CD):guest (drug) ratio is 1:1, 1:2 and 2:1 [6, 7].

Different types of behaviors can be identified in phase solubility relationships. In A-type profiles, the apparent solubility of the substrate increases as a function of CD concentration but B-type profiles are indicative of the formation of complexes with limited water solubility [7, 8]. The ability of a CD to form an inclusion complex with a guest molecule depends on the relative size of the CD to the size of the guest molecule or certain functional groups within the guest [4, 5]. Another important factor is the thermodynamic interactions between the different components of the system (CD, guest, solvent) [9]. Recently, it has shown that CD and drug interaction is not limited to the inclusion complexation. Non-inclusion interactions exist in aqueous CD solution [7, 10, 11].

In the present study, we investigated the effect of natural, synthetic CDs and CD mixtures on aqueous solubility of beclomethasone dipropionate (BDP). The results showed that the mixture of 20% α-CD and 20% HP-γ-CD was optimal for increasing aqueous solubility of BDP.

Previously, complex formation between this drug and selected CDs has been investigated. The affinity order of these CDs for BDP was as follows: γ-CD > dimethyl-β-cyclodextrin (DM-β-CD) > HP-β-CD > β-CD [12, 13]. The improvement in the percutaneous absorption of BDP after using of this drug as inclusion complex with γ-CD was evaluated by Uekama et al. [14]. Also, Cabral-Marques and Almeida reported the effect different variables on spray-drying process of BDP/γ-CD complex to obtain suitable powder for lung delivery [15].

Materials and methods

Materials

Beclomethasone dipropionate (BDP) was supplied by Jai Radhe Sales (India). α-CD, β-CD, hydroxylpropyl β-CD (HP-β-CD) (molar substitution = 0.7), sulphobutylether β-CD (SBE-β-CD) (degree of substitution = 6.28) and γ-CD were purchased from Seebio (China) and hydroxypropyl γ-CD (HP-γ-CD) (molar substitution = 0.6), was obtained from Aldrich (USA). Sodium acetate and acetonitrile were from Merck (Germany). All materials were analytical grade unless otherwise stated.

Phase solubility studies

For phase solubility analysis, aqueous CD solutions with different concentrations (0–15% w/v for β-CD and 0–20% w/v for other CDs) were prepared and excess amounts of BDP powder were added to each vial. The vials were placed on a rotator (Labinco, Netherlands) and rotated at room temperature (23–25 °C) for 24 h. Following the equilibration period, the solution was filtered through a 0.45 μm membrane filter and the concentration of BDP in the filtrate was analyzed using high-pressure liquid chromatography (HPLC). In the next step, phase solubility studies were performed in the presence of CD mixtures. Also, the intrinsic solubility of BDP in water was determined. In all cases, solubility determinations were carried out in triplicate. An apparent stability constant was calculated from the initial straight line portion of phase-solubility diagrams. Solubility diagram type was determined according to the Higuchi and Connors [16].

HPLC assay

Quantitative determinations were performed by HPLC (Shimadzu, Japan) using a C18 column and measured at a detection wavelength of 254 nm. The mobile phase composed of water:acetonitrile (2:3) was used at a flow rate of 1 mL/min with the injection volume of 20 μL. The HPLC method was linear to a concentration of 0.00001–0.5 mg/mL.

Results

The aqueous solubility of BDP increased linearly in the presence of α-CD, β-CD, SBE-β-CD, HP-β-CD and HP-γ-CD but the phase solubility diagram of BDP was B-type with γ-CD (Fig. 1). Among different CDs, HP-γ-CD had the best effect on the drug aqueous solubility. The aqueous solubility of BDP was 2.38 ± 1.38 μg/mL and increased to 356.54 ± 73.45 μg/mL in the presence of 20% w/v of HP-γ-CD. The solubility of this steroid increased until the γ-CD concentration reached 5%. After this point, the solubility of drug decreased. The effect of α-CD and HP-β-CD

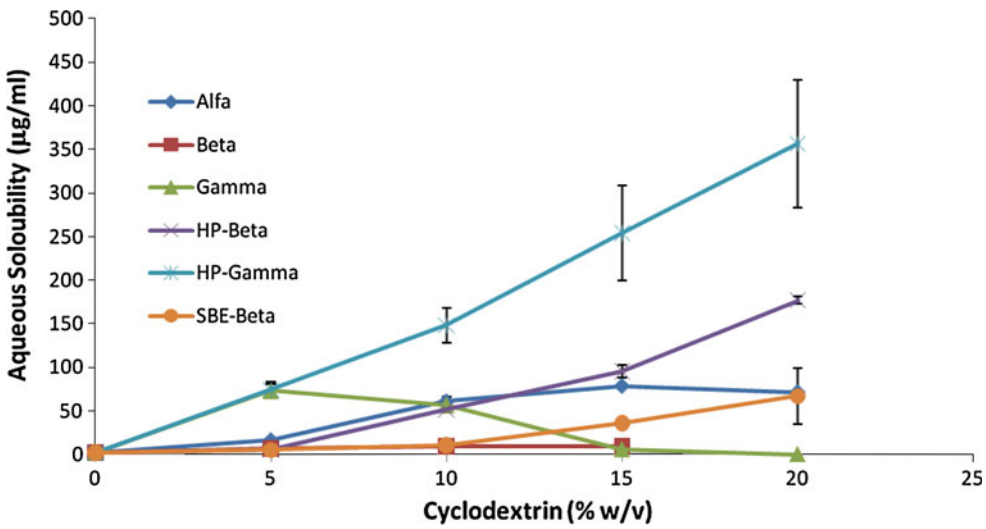

Fig. 1 Phase-solubility diagrams of beclomethasone-CD complex at room temperature (mean ± SD, $n = 3$)

Table 1 Type of phase solubility diagram of beclomethasone-CD inclusion complex, apparent inclusion complex stability constant and complexation efficiency (CE) value

Cyclodextrin	Diagram type[a]	$K_{1:1}$ (M^{-1})[b]	R^2	Complexation efficiency (CE)[c]
α-CD	A_L	226.74	0.958	0.001
β-CD	A_L	22.65	0.837	0.0001
SBE-β-CD	A_L	385.74	0.928	0.0017
HP-β-CD	A_L	681.61	0.975	0.003
HP-γ-CD	A_L	1229.85	0.991	0.0054
γ-CD	B_S	Not determined	Not determined	Not determined

[a] According to the Higuchi and Connors [16]

[b] Apparent stability constant

[c] Calculated according to the Ref. [7]

Table 2 Aqueous solubility of beclomethasone in the presence of cyclodextrin mixtures (mean ± SD, $n = 3$)

Cyclodextrin mixtures	Cyclodextrin concentration			
	Aqueous solubility (μg/mL)			
	5%	10%	15%	20%
α-CD	16.8 ± 1.4	61.1 ± 1.8	78.8 ± 7.1	71.8 ± 4.4
β-CD	7.1 ± 1.4	9.2 ± 0.9	9.4 ± 2.5	–
γ-CD	73.2 ± 7.6	57.1 ± 8.9	5.9 ± 1.7	0.3 ± 0.0
HP-β-CD	5.9 ± 1.2	51.8 ± 8.8	95.6 ± 4.2	177.2 ± 3.2
SBE-β-CD	5.3 ± 0.9	10.9 ± 2.7	35.9 ± 2.1	67.1 ± 32.1
HP-γ-CD	74.4 ± 9.3	148.2 ± 20.5	254.1 ± 54.4	356.5 ± 73.4
α-CD + HP-β-CD	140.5 ± 8.8	550.6 ± 14.5	1396.6 ± 25.9	1899.6 ± 14.9
α-CD + SBE-β-CD	101.6 ± 1.9	235.6 ± 15.3	432.9 ± 3.3	513.2 ± 7.3
HP-β-CD + HP-γ-CD	141.9 ± 3.8	439.1 ± 30.1	647.3 ± 44.1	1116.8 ± 105.7
α-CD + HP-γ-CD	320.7 ± 23.1	1130.7 ± 44.1	2624.1 ± 483.3	3399 ± 297.1
α-CD + HP-γ-CD + 1% sodium acetate	330.5 ± 29.2	779.0 ± 75.0	1398.7 ± 45.9	1886.3 ± 30.2
α-CD + HP-γ-CD + 5% sodium acetate	264.2 ± 6.7	870.9 ± 42.1	1580.4 ± 40.5	2375.3 ± 115.7
α-CD + HP-γ-CD + HP-β-CD	275.2 ± 5.9	1132.5 ± 21.6	2209.3 ± 13.3	2282.8 ± 29.2

on the solubilisation of beclomethasone was almost similar in the presence of 5–15% of CD. Complexation coefficient and complexation efficiency of BDP with different CDs were summarized in Table 1.

The effect of CD mixtures on aqueous solubility of BDP was tested. In all cases, the synergistic effect was observed by using of CD mixtures. As it was presented in Table 2, the best effect on solubility was achieved by α-CD + HP-γ-CD mixture. In these cases, solubility in water increased between 200 and 1,500 times in the presence of different CD mixtures. Also, to highlight the role of non-inclusion complexation, the effect of α-CD + HP-γ-CD mixture on aqueous solubility was evaluated in the presence of different concentrations of sodium acetate. In both concentrations of 1–5%, aqueous solubility was decreased by addition of sodium acetate to the complexation medium (Table 2).

Discussion

BDP typically formed B-type diagrams with γ-CD. The similar relationship was achieved by other steroids such as fluorometholone [17], prednisolone [10] and 6α-methyl prednisolone [10]. Other diagrams were of A_L type, indicating that the drug-CD complexes are of first-order with respect to CD. Phase-solubility diagrams are used to indicate order of drug/CD inclusion complexes. Linear curve shows that complexes are first-order with respect to CD while positive deviation from linearity is due to higher-order inclusion complex formation at higher concentration of CD. These higher-order complexes are formed by association of additional CD molecules to the 1:1 complex as it is observed by using of CD mixtures [5, 18].

In the present study, HP-γ-CD showed the highest association constant among the different CDs while

β-CD-prednisolone complex, γ-CD-6α-methyl prednisolone complex and SBE-β-CD-fluorometholone complex had the highest association constant in the previous studies [10, 17]. The better effect of HP-γ-CD suggests that the larger cavity of this CD is more appropriate for the complexation of BDP. It has mentioned that the A-ring in steroid structure is the major site for drug-CD complexation [2, 7]. Uekama et al. showed that the A-ring of hydrocortisone is able to enter inside the β-CD and γ-CD cavity [19]. NMR spectroscopy results in the study of Larsen et al. showed that the A-ring of prednisolone and 6α-methyl prednisolone was the predominant site of interaction with β-CD and only in the case of 6α-methyl prednisolone significant interactions with the D-ring were observed [10].

It has been reported that modification in the D-ring only influenced the drug binding ability to a minor degree compared to the A-ring but in the structure of BDP, presence of (propionyloxy) acetyl and propionyloxy groups on D-ring, provide another binding site for α-CD complexation. The potential guest list for CD encapsulation is quite varied and includes different compounds such as straight or branched chain aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, hydrocarbons, halogens, amines, amino acids and aromatics [9, 20]. As it was suggested that hydrocortisone cannot enter α-CD through A-ring [19] but the existence of these gropus on D-ring may explain the better effect of α-CD. Totally, synthetic CDs had better impact on aqueous solubility compared to natural ones. Derivatization of CDs may change the properties and dimensions of the CD hydrophobic cavity and provide additional binding sites [7].

CD mixtures had synergistic effects on aqueous solubility of BDP. These results are suggesting formation of drug complexes with different CDs. CDs increase the solubility by two manners, inclusion and non-inclusion complexes. The inclusion complexes are formed by taking some lipophilic moiety of the drug molecule into the central cavity of CD [4, 5]. A variety of non-covalent forces, such as van der Waals forces, hydrogen bonding, hydrophobic interactions and other forces are responsible for the formation of inclusion complex between drug and CD [21]. As it was mentioned before, the ability of a CD to form an inclusion complex with a guest molecule is a function of two key factors, steric and thermodynamic interactions. The first one depends on the relative size of the CD to the size of the guest molecule or certain functional groups within the guest [4, 9]. According to this parameter, α-CD can typically complex low molecular weight drugs or aliphatic side chains of compound, β-CD will complex heterocyclic structures and aromatics, and γ-CD can accommodate larger molecules such as macrocycles, antibiotics and steroids [4, 9]. The second critical factor is the thermodynamic interactions between CD, guest and solvent. For complex formation, a favourable net energetic driving force that pushes the guest into the CD is necessary [9].

On the other hand, it has been shown that CD and CD complexes are self-associate to form water-soluble aggregates or micelles or micelle-like structures. These structures are consisting of two to several hundred CD molecules and/or CD complexes [7]. Formation of aggregates composed over 50 drug/HP-β-CD complexes was reported by Messner et al. [22].

The role of non-inclusion complexation on increasing solubility was evaluated using water soluble polymers such as hydroxypropyl methylcellulose (HPMC) and anionic and cationic compound such as sodium acetate and benzalkonium choloride [11, 23]. The aqueous solubility of hydrocortisone-β-CD complexes was increased in the presence of HPMC (0.25% w/v) and sodium acetate (1% w/v) [24] but in our previous study, aqueous solubility of Cyclosporine A was decreased by increasing sodium acetate concentration [25]. However, in the present study, addition of sodium acetate had negative effect on aqueous solubility of BDP. It can be due to medium effect such as increased ionic strength. Because it has been reported that different mechanisms such as dilution, competitive displacement, protein binding, drug uptake by tissue, changing in ionic strength and temperature play important role in drug release from drug–CD complexes [3, 21]. Reduction in complexation efficiency by increasing in the ionic strength has been shown.

Conclusion

The results of present study showed that it is possible to improve the aqueous solubility of BDP using CD complexation. Phase-solubility studies showed that HP-γ-CD had a much greater effect on the solubility of BDP than other CDs. The synergistic effect of CD mixture on BDP aqueous solubility was observed.

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Conflict of interests None

References

1. Eastburn, S.D., Tao, B.Y.: Applications of modified cyclodextrins. Biotech. Adv. **12**, 325–339 (1994)
2. Hirayama, F., Uekama, K.: Cyclodextrin-based controlled drug release system. Adv. Drug. Deliv. Rev. **36**, 125–141 (1999)

3. Loftsson, T., Masson, M.: Cyclodextrins in topical drug formulations: theory and practice. Int. J. Pharm. **225**, 15–30 (2001)
4. Loftsson, T., Duchene D.: Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. **329**, 1–11 (2007)
5. Davis, M.E., Brewster, M.E.: Cyclodextrin-based pharmaceutics: past, present and future. Nat. Rev. Drug. Discov. **3**, 1023–1035 (2004)
6. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998)
7. Kurkov, S.V., Ukhatskaya, E.V., Loftsson, T.: Drug/cyclodextrin: beyond inclusion complexation. J. Incl. Phenom. Macrocycl. Chem. **69**, 297–301 (2011)
8. Brewster, M.E., Loftsson, T.: Cyclodextrins as pharmaceutical solubilizers. Adv. Drug. Deliv. Rev. **59**, 645–666 (2007)
9. Martin Del Valle, E.M.: Cyclodextrins and their uses: a review. Process. Biochem. **39**, 1033–1046 (2004)
10. Larsen, K.L., Aachmann, F.L., Wimmer, R., Stella, V.J., Kjolner, U.M.: Phase solubility and structure of the inclusion complexes of prednisolone and 6α-methyl prednisolone with various cyclodextrins. J. Pharm. Sci. **94**, 507–515 (2005)
11. Loftsson, T., Matthiasson, K., Masson, M.: The effects of organic salts on the cyclodextrin solubilization of drugs. Int. J. Pharm. **262**, 101–107 (2003)
12. Almeida, R., Cabral Marques, H., Pulmonary administration of beclomethasone: α-cyclodextrin complex. In: Duchêne, D. (ed.), Proceedings of the 12th International Cyclodextrin Symposium, de Santé, pp. 889–892. APGI Publishing, Montpellier (2004)
13. Leite Pinto, J.M.C., Cabral Marques, H.M.: Beclomethasone–cyclodextrin inclusion complex for dry powder inhalation. STP. Pharma. **9**(3), 253–256 (1999)
14. Uekama, K., Otagiri, M., Sakai, A., Irie, T., Matsuo, N., Matsuoka, Y.: Improvement in the percutaneous absorption of beclomethasone dipropionate by gamma-cyclodextrin complexation. J. Pharm. Pharmacol. **37**(8), 532–535 (1985)
15. Cabral-Marques, H., Almeida, R.: Optimisation of spray-drying process variables for dry powder inhalation (DPI) formulations of corticosteroid/cyclodextrin inclusion complexes. Eur. J. Pharm. Biopharm. **73**, 121–129 (2009)
16. Higuchi, T., Connors, K.A.: Phase solubility techniques. Adv. Anal. Chem. Instr. **4**, 117–212 (1965)
17. Malaekeh-Nikouei, B., Sajadi Tabassi, S.A., Ashari, H., Gholamzadeh, A.: Evaluation the effect of cyclodextrin complexation on aqueous solubility of fluorometholone to achieve ophthalmic solution. J. Incl. Phenom. Macrocycl. Chem. **65**, 335–340 (2009)
18. Loftsson, T., Magnudottir, A., Masson, M., Sigurjonsdottir, J.F.: Self-association and cyclodextrin solubilization of drugs. J. Pharm. Sci. **91**, 2307–2316 (2002)
19. Uekama, K., Fujinaga, T., Otagiri, M., Yamasaki, M.: Inclusion complexations of steroid hormones with cyclodextrins in water and in solid phase. Int. J. Pharm. **10**, 1–15 (1982)
20. Rekharsky, M.V., Inoue, Y.: Complexation thermodynamics of cyclodextrins. Chem. Rev. **98**, 1875–1917 (1998)
21. Shimpi, S., Chauhan, B., Shimpi, P.: Cyclodextrins: application in different routes of drug administration. Acta. Pharm. **55**, 139–156 (2005)
22. Messner, M., Kurkov, S.V., Loftsson, T.: Self-assembled cyclodextrin aggregates and nanoparticles. Int. J. Pharm. **387**(1–2), 199–208 (2010)
23. Loftsson, T.: Increasing the cyclodextrin complexation of drugs and drug bioavailability through the addition water soluble polymers. Pharmazie **53**, 733–740 (1998)
24. Loftsson, T., Frioriksdottir, H.: The effect of water-soluble polymer on the aqueous solubility and complexing abilities of β-cyclodextrin. Int. J. Pharm. **163**, 115–121 (1998)
25. Malaekeh-Nikouei, B., Nassirli, H., Davies, N.: Enhancement of cyclosporine aqueous solubility using α-and hydroxypropyl β-cyclodextrin mixtures. J. Incl. Phenom. Macrocycl. Chem. **59**, 245–250 (2007)