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ORIGINAL ARTICLE

Enhancement of cyclosporine aqueous solubility using α- and hydroxypropyl β-cyclodextrin mixtures

B. Malaekeh-Nikouei · H. Nassirli · N. Davies

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Abstract Cyclodextrins (CDs) are cyclic oligosaccharides that form inclusion complexes with lipophilic molecules through their hydrophobic central cavity. In this study, the effect of α-CD, hydroxylpropyl-β-CD (HP-β-CD) and mixtures of these two CDs on the aqueous solubility of cyclosporine A (CyA) was investigated. Infrared spectroscopy and thermal analysis were used to confirm CyA-CD complex formation. CyA aqueous solubility was increased by 10 and 80 fold in the presence of α-CD and HP β-CD, respectively. The phase-solubility profile for HP-β-CD was linear while that for α-CD had positive deviation from linearity. In the presence of constant concentration of α-CD (15% w/v), aqueous solubility of CyA was further increased upon addition of HP-β-CD up to a concentration of 20% w/v. At higher HP-β-CD concentrations, aqueous solubility of CyA was observed to decrease. Addition of sodium acetate (up to 5% w/v) to aqueous solutions containing 20% w/v HP-β-CD and increasing concentrations of α-CD resulted in a significant reduction in CyA solubility. Complex formation between CyA and both α-CD and HP-β-CD was confirmed by differential scanning calorimetry (DSC). No significant changes were observed in the IR spectra of either CyA or CD following complex formation suggesting chemical interaction between CyA and the CD was unlikely. Phase-solubility studies showed that α-CD had a much greater effect on the solubility of CyA than HP-β-CD. Addition of HP-β-CD to aqueous solutions of α-CD affected the solubility of CyA in these systems. A mixture of 15% w/v α-CD and 20% w/v HP-β-CD was optimal for increasing aqueous solubility of CyA.

Keywords Cyclosporin A · Cyclodextrin · Aqueous solubility · Phase solubility

B. Malaekeh-Nikouei (✉)
Pharmaceutics Department, Faculty of Pharmacy and Pharmaceutical Research Centre, Mashhad University of Medical Sciences, Buali Sq., P.O. Box 9196773117, Mashhad, Iran
e-mail: bmalaekeh@yahoo.com

H. Nassirli
Analysis Laboratory, Pharmaceutical Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran

N. Davies
School of Pharmacy, University of Queensland, Brisbane, Australia

Introduction

Cyclosporine A (CyA) is a poorly water-soluble, cyclic undecapeptide having immunosuppressive properties [1, 2]. It is used to prevent rejection of transplanted organs such as kidney, liver and bone marrow, and in the treatment of selected autoimmune disorders such as uveitis, rheumatoid arthritis and early treatment of type I diabetes [3, 4]. Due to its relatively high molecular weight, lipophilicity and poor aqueous solubility, oral absorption of CyA is low and highly variable [5, 6]. As such, the current formulation of CyA for oral administration (Neoral®) is one which spontaneously forms a microemulsion in an aqueous environment [7]. For the injectible formulation (Sandimmune®), Cremophor EL is used for solubilising CyA which has the associated side effects of nephrotoxicity, anaphylactic hypersensitivity reactions, hyperlipidaemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy [8].

A family of compounds which have been extensively investigated and used to increase the aqueous solubility of

poorly water-soluble compounds are the cyclodextrins. Cyclodextrins (CDs) are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic central cavity. The most common naturally occurring CDs are α-CD, β-CD and γ-CD, consisting of six (hexamer), seven (heptamer) and eight (octomer) (α-1,4)-linked α-D-glucopyranose units, respectively [9–12]. Apart from these naturally occurring CDs, many CD derivatives have been synthesized such as hydroxypropyl-β-CD as a more water-soluble derivative of β-CD [11, 12]. Cyclodextrins are able to form inclusion complexes with many compounds by taking up the drug molecule in whole or part into the cavity [9, 10]. Complex formation is influenced by both the chemical structure and the physicochemical properties of the compound as well as the type of CD used [9].

Complexation of drug affects many physicochemical properties of a drug. The aqueous solubility of a drug-CD complex in particular, can be dramatically different to that of the free drug and as such CD complexation can be used to increase the oral bioavailability of poorly water-soluble compounds or in the formulation of aqueous-based injectibles [10]. Complexation can also be used to protect a drug against both chemical and enzymatic degradation [10, 13]. As no primary bonds are formed between the CD and the complexed molecule, the complex can dissociate upon dilution, or the molecule can by displaced by a more suitable guest or transferred to a matrix for which it has a higher affinity, such as a biological membrane [14].

The aim of this study was to evaluate the effect of α-CD, HP-β-CD and a mixture of these two CDs on the aqueous solubility of CyA. Phase solubility studies were conducted in the absence and presence of sodium acetate as a competing guest to determine inclusion or non-inclusion effects [15]. Complementary studies were also conducted using infrared (IR) spectroscopy and differential scanning calorimetry (DSC) to investigate CyA and CD interactions.

Materials and methods

Materials

CyA was purchased from LC laboratories (USA). α-CD, HP-β-CD and sodium acetate were obtained from American Maize-Products Company (USA), Roquette (France) and Sigma (USA), respectively. All materials were of analytical grade unless otherwise stated.

Phase-solubility analysis

For phase-solubility analysis, solutions of α-, HP-β- and mixtures of α- and HP-β-CD with different concentrations (0–20% w/v) in water or in the presence of sodium acetate (1, 2, 5% w/v) were prepared and an excess amount of CyA powder added. The vials were placed on a longitudinal rotator (Rotek, Australia) and rotated at room temperature for 7 days. Following this equilibration period, the systems were filtered through a 0.45 μm membrane filter and the concentration of CyA in the filtrate was analysed using high-pressure liquid chromatography (HPLC). In all cases, solubility determinations were carried out in triplicate.

HPLC assay

Quantitative determinations were performed by HPLC (Shimadzu, Japan) using a C18 column (Alltech, USA) and measured at a detection wavelength of 214 nm. The mobile phase composed of 90% methanol and 10% water and was used at a flow rate of 1.5 mL/min. The injection volume was 20 μL.

DSC studies

DSC was carried out using a DSC apparatus equipped with STARE software (METTLER TOLEDO SW7.01, Switzerland). Thermogram of different samples (CyA, freeze dried α-CD, HP β-CD and CD complexes) were collected using 5–10 mg of samples placed in sealed aluminium crucibles and heated from 25 to 250 °C at 10 °C/min under a nitrogen atmosphere. Empty 40 μL crucibles were used as reference.

IR studies

IR spectra of different samples (CyA, freeze dried α-CD, HP β-CD and CD complexes in potassium bromide) were recorded using a Unicom SP 1100 spectrometer (England) equipped with ABZARDADEH software. Spectra were collected over the range 500–4000 cm^{-1}.

Results

Phase solubility analysis

Figure 1 shows that the solubility of CyA in water increased linearly with increasing concentration of HP-β-CD. However, the relation between α-CD concentration and aqueous solubility of CyA was non-linear with the profile showing positive curvature. α-CD was more effective than HP β-CD in increasing the aqueous solubility of CyA and the solubility enhancing effect was observed up to a concentration of 15% α-CD beyond which no further increases in solubility were observed. The profile for a system containing increasing concentrations of α-CD and 20% w/v HP-β-CD was similar to that for α-CD except that the

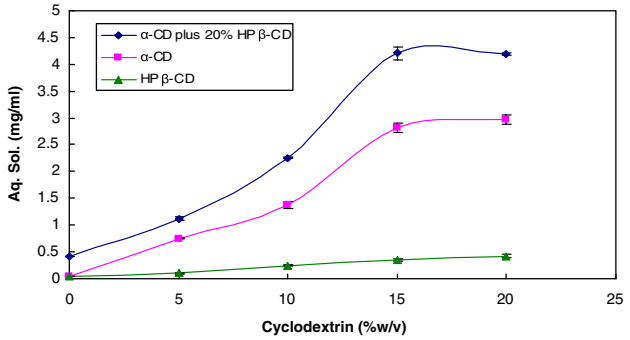

Fig. 1 Effect of different concentrations of α-CD, HP-β-CD and mixture of CDs on aqueous solubility of CyA (Mean ± SD, *n*=3)

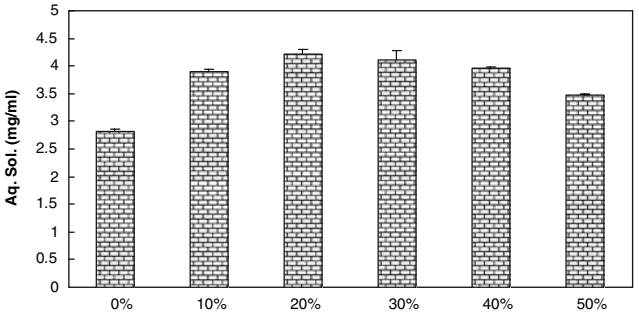

Fig. 2 Effect of different concentrations of HP-β-CD on aqueous solubility of CyA in the presence of 15% W/V α-CD (Mean ± SD, *n*=3)

aqueous solubility of CyA in the mixture was in all cases greater than that in the presence of α-CD alone.

The aqueous solubility of CyA in systems containing increasing concentrations of α-CD together with either 10% or 20% w/v of HP-β-CD are reported in Table 1. In all cases, solubility of CyA was greater in systems containing 20% w/v HP-β-CD ($p < 0.05$). Figure 2 shows the aqueous solubility of CyA in a system containing 15% w/v α-CD and various concentrations of HP-β-CD (the concentration of α-CD was chosen based on the results reported in Table 1). Solubility of CyA was observed to increase up to a HP-β-CD concentration of 20% w/v after which solubility of CyA decreased. Aqueous solubility of CyA was 3.895 ± 0.039, 4.209 ± 0.090, 4.114 ± 0.169, 3.956 ± 0.018 and 3.478 ± 0.011 mg/mL in systems containing 15% w/v α-CD and 10, 20, 30, 40, 50% HP-β-CD, respectively. No statistical difference was observed in the systems containing either 20 or 30% HP-β-CD ($p > 0.05$).

As reported in Table 1 and Fig. 2, the highest aqueous solubility of CyA was observed in systems containing 15% α-CD and 20% HP-β-CD. In this case, aqueous solubility of CyA increased from 0.042 ± 0.001 mg/mL in the absence of CD to 4.203 ± 0.126 mg/mL representing a 100-fold increase.

As shown in Figs. 3 and Fig. 4, the aqueous solubility of CyA in the presence of either α-CD or mixtures with HP-β-CD was decreased significantly upon addition of sodium acetate to the systems. The magnitude of the effect was related to the concentration of sodium acetate added (either 1 or 5% w/v). For example, the aqueous solubility of CyA in the presence of 15% α-CD and 20% HP-β-CD was

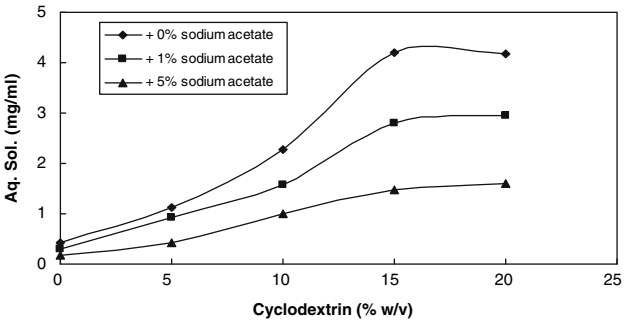

Fig. 3 Effect of different concentrations of α-CD on aqueous solubility of CyA in the presence of 20% w/v HP-β-CD and different concentrations of sodium acetate (Mean ± SD, *n*=3)

decreased from 4.3 mg/mL to 2.7 and 1.7 mg/mL by adding 1 and 5% w/v sodium acetate respectively. In all cases, the effect of sodium acetate on aqueous solubility was significant ($p < 0.05$).

DSC studies

Thermograms of CyA, α-CD, HP-β-CD and their complexes are reported in Fig. 5 and Fig. 6. CyA showed an endothermic peak at 130 °C. α-CD showed endothermic events at 78, 110 and 130 °C while HP-β-CD showed a broad peak between 30 and 120 °C. The isolated α-CD-CyA and HP-β-CD-CyA complexes did not show any significant thermal events over the temperature range studied confirming complexation between CyA and the two cyclodextrins.

Table 1 Effect of α-CD and HP-β-CD mixtures on aqueous solubility of CyA (Mean ± SD, *n* = 3)

α- + 10% HP-β-CD	Aq. sol. (mg/mL)	α- + 20% HP-β-CD	Aq. sol. (mg/mL)
5%	0.997 ± 0.076	5%	1.119 ± 0.026
10%	1.900 ± 0.235	10%	2.268 ± 0.019
15%	3.895 ± 0.039	15%	4.209 ± 0.090
20%	3.950 ± 0.025	20%	4.166 ± 0.050

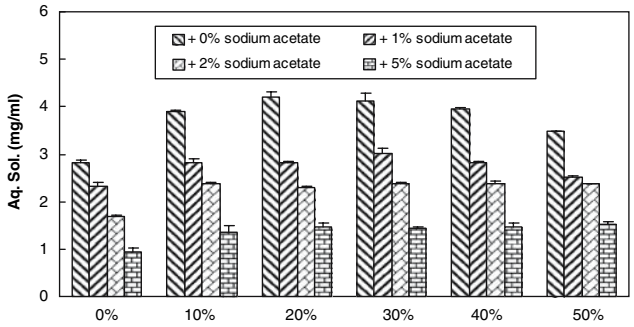

Fig. 4 Effect of different concentrations of HP-β-CD on aqueous solubility of CyA in the presence of 15% w/v α-CD and different concentrations of sodium acetate (Mean ± SD, n=3)

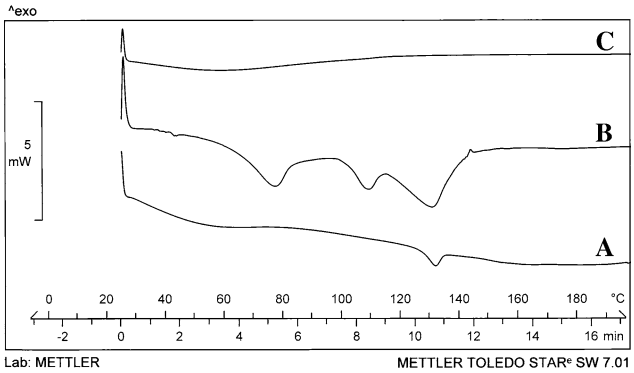

Fig. 5 DSC thermograms of (**A**) CyA, (**B**) α-CD, (**C**) α-CD- CyA complex

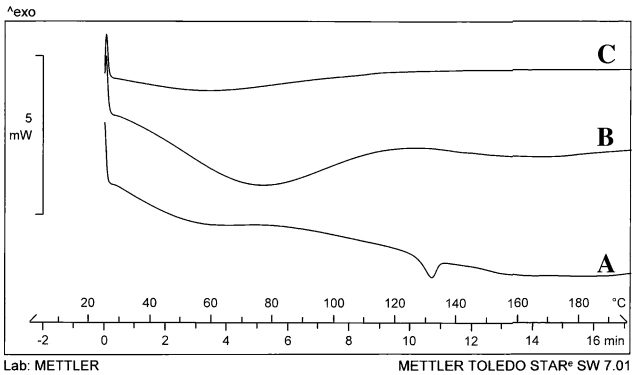

Fig. 6 DSC thermograms of (**A**) CyA, (**B**) HP-β-CD, (**C**) HP-β-CD-CyA complex

IR studies

The infrared spectrum of CyA showed intense amide and carbonyl bands at 1627 cm^{-1} and at 2930 cm^{-1}. α-CD had two bands at 1630 cm^{-1} (C–O band) and 2915 cm^{-1} (aliphatic C–H band) and a broad band between 3200–3600 cm^{-1}. The α-CD-CyA complex showed similar bands to α-CD at 1635 and 2925 cm^{-1}. The spectra of HP-β-CD and HP-β-CD-CyA complex showed bands at 1627 cm^{-1} (C–O band) and 2922 cm^{-1} (aliphatic CH), and 1633 and 2920 cm^{-1}, respectively. Thus no significant shifts in the bands were observed between the free compounds (CyA or CD) and the isolated complexes (data not shown).

Discussion

α-CD was found to be more effective than HP-β-CD in increasing the aqueous solubility of CyA. The cavity of α-CD (4.7–5.3 Å) is smaller than that of HP-β-CD (6.0–6.5 Å) [12, 16]. Considering that CyA has a cyclic structure with pendant aliphatic groups including methyl, propyl and butyl, the smaller cavity of α-CD may be better suited for interaction with these small aliphatic groups resulting in higher order complexes with regards to CD as suggested by the positive deviation from linearity as observed in the phase solubility profile for CyA and α-CD [12, 17, 18]. In contrast, a linear phase solubility profile was observed for CyA and HP-β-CD (at least up to 20% w/v cyclodextrin) suggesting the formation of 1:1 inclusion complex or at least complexes that are first order with respect to HP-β-CD. It is possible that HP-β-CD either forms weaker inclusion complexes with the small pendent aliphatic groups of CyA or interacts at a different site, for example the C6 pendant chain. A synergistic effect on the aqueous solubility of CyA was noted for mixtures of α-CD and HP-β-CD. The synergistic rather than simply additive effects noted for the mixture would suggest a more complex mechanism for their combined interaction over competitive complexation of the small aliphatic groups or complexation of additional groups by HP-β-CD.

The phase solubility profile for α-CD was observed to plateau out at 2.96 mg/mL at concentrations of greater than 15% w/v CD. This represents the solubility limit of the α-CD/CyA complex and is similar to the aqueous solubility of α-CD being 14.6% w/v [12]. A similar plateau at an equivalent α-CD was observed in the presence of 20% HP-β-CD. Interestingly, the solubility of CyA decreased in systems containing concentrations of HP-β-CD greater than 20%. This may result from the formation of CD aggregates at higher concentrations which may affect their ability to interact with the hydrophobic pendant groups of the CyA [19].

The results for the affect of α-CD and HP-β-CD observed in the present study are slightly different to those which have been previously reported. For example, the aqueous solubility of CyA in the presence of 10% w/v α-CD and 10% w/v HP-β-CD was reported to be 1.88 mg/mL [20] and 0.09 mg/mL [15], respectively. In this study, the observed solubilities at this concentration of CD were

1.37 and 0.24 mg/mL respectively. The difference between observations is likely to be due to the different method of preparation of the complexes.

Although it is well documented that CDs increase the solubility of poorly water-soluble compounds by the formation of inclusion complexes [21, 22], recent studies have also demonstrated that CDs can increase solubility by non-inclusion effects by the formation of micellar structures [15, 23]. For example, in a study by Loftsson et al. to evaluate the effect of 10% w/v HP-β-CD solution on aqueous solubility of CyA, it was reported that the aqueous solubility of CyA was increased in this system by addition of the competitive guest cholesterol. They proposed that association of cholesterol/HP-β-CD complexes occurred forming water soluble microaggregates which could then solubilise the CyA through non-inclusion complexation [15]. It has also been reported that organic cations and anions like acetate or hydroxy acids (such as citric acid) can also increase the solubility of drug-CD complexes by formation of ternary drug-CD-acid or base complexes [9, 15].

In the present study, the addition of sodium acetate to systems containing increasing concentration of α-CD and HP-β-CD resulted in a decrease in the solubility of the CyA with the shape of the phase solubility profile changing from positive deviation from linearity to a more linear profile. The positive deviation from linearity of the phase solubility profile is related to higher order complexes formed between α-CD and probably the many small (C1–C3) aliphatic pendant groups present on the cyclic CyA. Considering that the acetate ion also has a methyl group then the decreased solubility of CyA observed for systems containing α-CD is likely to be due to competitive complexation by the acetate ion. This is in contrast to the work reported by Loftsson in 2003 where the solubility of hydrocortisone was increased in the presence of 1% sodium acetate by what was described as non-inclusion effects [15]. It is possible that the small size of the methyl group present in the acetate ion is able to form complexes with the smaller internal cavity of α-CD but is not suitable for complex formation with the larger cavity of β-CD. Hence, when α-CD increases the solubility of compounds by complexing with small alkyl groups then it can be expected that solubility of the complex will decrease in the presence of competitive organic ions such as the acetate. The non-inclusion effects on enhancing solubility for acetate ions observed in systems containing the larger β-CD may even exist in the systems containing HP-β-CD and reported in the present manuscript. Inspection of Fig. 3 shows the phase solubility profile approaching linearity as was observed for systems containing HP-β-CD alone. It is proposed therefore that the results presented in Fig. 3 are a result of a combination of effects with a decrease in solubility resulting from competitive competition of the acetate ion for the α-CD (dominant effect) and an increase in the solubility of the CyA/ HP-β-CD complex as a result of non-inclusion effects (less dominant).

DSC is a powerful way to evaluate physicochemical properties of drug and CD complexation [23]. The endothermic peak of CyA at 130 °C is due to the semi-crystalline structure of the drug while the endothermic peak of α-CD and HP-β-CD is due to water loss [24, 25]. These peaks were not observed in isolated CyA/CD complexes. Disappearance of these endothermic peaks is strong evidence that inclusion complexes are formed between the CyA and both CDs [23, 26, 27]. IR studies indicated no significant spectral shifts for CyA-CD complexes suggesting that there is no chemical interaction between CyA and the CDs.

In conclusion, phase-solubility studies showed that α-CD had a much greater effect on the solubility of CyA than HP β-CD. Addition of HP-β-CD to aqueous solutions of α-CD affected the solubility of CyA in these systems. A mixture of 15% w/v α-CD and 20% w/v HP-β-CD was optimal for increasing aqueous solubility of CyA.

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References

1. Allison, A.C.: Immunosuppressive drugs: The first 50 years and a glance forward. Immunopharmacology **47**, 63–83 (2000)
2. Kawabata, T.T., Munson, A.E.: Immunopharmacology. In: Brody, T.M., Larner, J., Minneman, K.P. (eds.) Human pharmacology, molecular to clinical, 3rd edn. pp. 599–637. Mosby, USA (1998)
3. Lake, D.F., Akporiaye, E.T., Hersh, E.M.: Immunopharmacology. In: Katzung, B.G. (ed.) Basic & clinical pharmacology, 8th edn. pp. 959–986. The McGraw-Hill Companies, USA (2001)
4. Ptachcinski, R.J., Venkataramanan, R., Burckart G.J.: Clinical pharmacokinetics of cyclosporine. Clin. Pharmacokinet. **11**(2), 107–132 (1986)
5. Lee, E., Choi, H., Lee, S., Kim, C.: Bioavailability of cyclosporine A dispersed in sodium lauryl sulfate dextrin based Solid microspheresInt. J. Pharm. **218**, 125–131 (2001)
6. Guo, J., Chen, Y., Ping, Q.: 6) Pharmacokinetic behavior of cyclosporine A in rabbits by oral administration of lecithin and Sandimmun Neoral. Int. J. Pharm. **216**, 17–21 (2001)
7. Al-Meshal, M.A., Khadir, S.H., Bayomi, M.A., Al-Angary, A.A.: Oral administration of liposomes containing cyclosporine: A pharmacokinetic studyInt. J. Pharm. **168**, 163–168 (1998)
8. Gelderblom, H., Verweij, J., Nooter, K., Sparreboom, A.: 8) Cremophor EL: The drawbacks and advantages of vehicle selection for drug formulation. Eur. J. Cancer **37**, 1590–1598 (2001)
9. Loftsson, T., O'Fee, R.: Cyclodextrins in solid dosage forms. Business Briefing: Pharmatech. 176–180 (2003)
10. Davis, M.E., Brewster, M.E.:Cyclodextrin-based pharmaceutics: Past, present and future. Nature Rev. **3**, 1023–1035 (2004)
11. Loftsson, T., Masson, M.: Cyclodextrins in topical drug formulations: Theory and practice. Int. J. Pharm. **225**, 15–30 (2001)

12. Martin Del Valle, E.M.: Cyclodextrins and their uses: A review. Process Biochem. **39**, 1033–1046 (2004)
13. Filipovic-Grcic, J., Voinovich, D., Moneghini, M., Becirevic-Lacan, M., Magarotto, L., Jalsenjak, I.: Chitosan microspheres with hydrocortisone and hydrocortisone–hydroxypropyl-β-cyclodextrin inclusion complex. Eur. J. Pharm. Biopharm. **9**, 373–379 (2000)
14. Filipovic-Grcic, J., Becirevic-Lacan, M., Skalko, N., Jalsenjak, I.: Chitosan microspheres of nifedipine and nifedipine-cyclodextrin inclusion complexes. Int. J. Pharm. **135**, 183–190 (1996)
15. Loftsson, T., Matthiasson, K., Masson, M.: The effect of organic salts on cyclodextrin solubilization of drugs. Int. J. Pharm. **262**, 101–107 (2003)
16. Ran, Y., Zhao, L., Xu, Q., Yalkowsky, S.H.: 16) Solubilization of cyclosporine A. AAPS PharmSciTech **20**(1), article 2 (2001)
17. Loftsson, T., Magnudottir, A., Masson, M., Sigurjonsdottir, J.F.: Self-association and cyclodextrin solubilization of drugs. J. Pharm. Sci. **91**(11), 2307–2316 (2002)
18. Fromming, K., Szejtli, J.: CDs in Pharmacy. Kluwer Academic Publishers, Netherlands (1994) pp. 1–32
19. Suzuki, M., Tasutsui, M., Ohamori, H.: NMR study of the self-assembly of an azo dye-cyclomaltooctanose (γ-cyclodextrin) complex. Carbohyd. Res. **264**, 233–230 (1994)
20. Fukaya, H., Iimura, A., Hoshiko, K., Fuyumuro, T., Noji, S., Nabeshima, T.: A cyclosporin A/maltosyl-α-cyclodextrin complex for inhalation therapy of asthma. Eur. Respir. J. **22**(2), 213–219 (2003)
21. Zhang, A., Liu, W., Wang L., Wen Y.: Characterization of inclusion complexation between fenoxaprop-p-ethyl and cyclodextrin. J. Agr. Food Chem. **53**(18), 7193–7197 (2005)
22. Liu, L., Zhu, S.: 22) Preparation and characterization of inclusion complexes of prazosin hydrochloride with beta-cyclodextrin and hydroxypropyl-beta-cyclodextrin. J. Pharm. Biomed. Anal. **40**(1), 122–127 (2006)
23. Anselmi, C., Centini, M., Ricci, M., Buonocore, A., Granata, P., Tsuno, T., Facino, R.M.: 23) Analytical characterization of a ferulic acid/γ-cyclodextrin inclusion complex. J. Pharm. Biomed. Anal. **40**(4), 875–881 (2006)
24. Zerrouk, N., Gine's Dorado, J.M., Arnaud, P., Chemtob, C.: 24) Physical characteristics of inclusion compounds of 5-ASA in α and β cyclodextrins. Int. J. Pharm. **171**, 19–29 (1998)
25. Calabrò, M.L., Tommasini, S., Donato, P., Raneri, D., Stancanelli, R., Ficarra, P., Costa, C., Catania, S., Rustichelli, C., Gamberini, G.: 25) Effects of α- and β-cyclodextrin complexation on the physicochemical properties and antioxidant activity of some 3-hydroxyflavones. J. Pharm. Biomed. Anal. **35**, 365–377 (2004)
26. Luciana, M.A, Fraceto, L.F., Santana, M.H.A., Pertinhez, T.A., Junior, S.O., de Paula, E.: 26) Physico-chemical characterization of benzocaine-β-cyclodextrin inclusion complexes. J. Pharm. Biomed. Anal. **39**(5), 956–963 (2005)
27. Li, N., Zhang, Y., Wu, Y., Xiong, X., Zhang, Y.: 27) Inclusion complex of trimethoprim with β-cyclodextrin. J. Pharm. Biomed. Anal. **39**(3–4), 824–829 (2005)