Drug Development and Industrial Pharmacy, 2012; 38(3): 331–340

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.604327

informa
healthcare

RESEARCH ARTICLE

Enhancement of dissolution rate and bioavailability of sulfamethoxazole by complexation with β-cyclodextrin

Nurten Özdemir and Jülide Erkin

Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ankara, Tandoğan-Ankara, Turkey

Abstract

The purpose of this study was to improve the solubility and dissolution rate of sulfamethoxazole (SMZ) with inclusion compound of β-cyclodextrin (β-CD). The interaction between SMZ and β-CD in solution was studied by the phase-solubility method. The phase-solubility studies revealed the formation of inclusion complexes with poor solubility with an inclusion complex of 1:1 molar ratio and a stability constant of 122.3 M^{-1}. The solid complexes of SMZ with β-CD were prepared by using kneading and coprecipitation methods. The physical mixture of these chemicals was also prepared for comparison. Inclusion complexation was confirmed by the results from the studies of infrared spectoroscopy (IR) and differential scanning calorimetry (DSC). The effect of water-soluble polymers i.e., polyethylene glycol 20000 and non-ionic surfactants i.e., polysorbate 20 on the complexation of SMZ with β-CD was also investigated by the same methods. The rates of release of the active material from the complexes were determined from dissolution studies using USP XXII paddle method. The formulation, that provided delivery of active material near to the target value in six healthy volunteers and *in vivo* tests, clearly revealed that the bioavailability of active material was found to be enhanced by preparing ternary mixtures.

Keywords: β–Cyclodextrin, sulfamethoxazole, bioavailability, kneading method, coprecipitation method

Introduction

Sulfamethoxazole (4-Amino-N-(5-methylisoxazol-3yl) benzenesulphonamide) is a member of the sulfonamide family of bacteriostatic antibiotics that inhibits normal bacterial utilization of para-aminobenzoic acid (PABA) for the synthesis of folic acid, an important metabolite in DNA synthesis[1,2].

Sulfamethoxazole is absorbed from gastro-intestinal area; however, its absorption and bioavailability are limited with its dissolution rate, due to its low solubility like all the sulfonamide groups. The aim of the study was to develop the inclusion complexes of sulfamethoxazole using cyclodextrins to enhance the solubility and oral bioavailability.

Cyclodextrins (CDs) were first isolated in 1891 as degradation products of starch and were characterized as cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic cavity in the center[3,4]. In aqueous solutions, CDs are able to form non-covalent inclusion complexes with various types of lipophilic drugs[5]. Encapsulation of a drug molecule is quite effective on many of its physicochemical properties and can result in increased aqueous solubility and stability[6,7]. Some additives such as water-soluble polymers or ion pairing agents also have a significant effect on the drug-cyclodextrin complex formation[8–11]. The purpose of this study was to investigate the effect of a commonly used water-soluble polymers, i.e., polyethylene glycol 20000 and non-ionic surfactants i.e., polysorbate 20, on the complexation of sulfamethoxazole with cyclodextrin.

The solid dispersions of active materials using cyclodextrins are prepared by coprecipitation method, kneading method, lyophilization method, physical mixtures etc.[12–15].

In this study, the solid dispersions were prepared by kneading and coprecipitation methods[16,17]. The physical mixtures were also prepared for comparison. The rates of release of the active material from the resulting

Address for Correspondence: Dr. Nurten Özdemir, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ankara, Tandoğan-Ankara, 06100 Turkey. Tel: +903122033151; Fax: +903122131081. E-mail: nozdemir@pharmacy.ankara.edu.tr

(Received 22 February 2011; revised 21 June 2011; accepted 05 July 2011)

complexes were determined from dissolution studies using USP XXII paddle method[25].

The cumulative amount of excreted drug and the rate of drug excretion from the urine can be useful in estimating the bioavailability for *in vivo* study[18]. Thus urinary excretion data were used in this study. The selected formulations were given orally to the six healthy volunteers and urinary excretion rate were followed for 24 hours. Urine analyses were carried out by using Bratton-Marshall method[19,20]. Powder form of active material was also studied in the same manner for comparison.

Materials and methods

Materials

Sulfamethoxazole (SMZ), β-cyclodextrin (β-CD), polyethylene glycol 20000 (PEG 20000) and polysorbate 20 (PS 20) were kindly supplied by Fako Pharmaceutical Co. Ltd., Fluka and Merck, respectively. All other chemicals were analytical grade.

Apparatus

For the experiments, an ultraviolet (UV) spectrophotometer (Shimadzu UV 1202), infrared (IR) spectrophotometer (Jasco FT/IR 420), differential scanning calorimetry (DSC) (Netzsch Geratebau DSC 204), and a dissolution tester (USP paddle method Aymes, Turkey) were used.

Phase-solubility studies

Solubility measurements were performed by the method of Higuchi and solutions containing various concentrations of β-CD ranging from 2×10^{-3} M to 15×10^{-3} M (The solubility of β-CD is 16.3×10^{-3} M) were shaken with 100 mg of sulfamethoxazole in sealed flasks in a thermostate water both at a constant temperature of 37°C[21]. After an equilibrium was attained (approximately three days), aliquots were withdrawn and filtered through 0.45 μm filters. A portion of the filtrate was then diluted with water and analyzed spectrophotometrically. The solubility constant and the ratio of SMZ/β-CD in the complexes were calculated from the phase solubility diagram[22].

The solubilizing effect of water-soluble polymers and nonionised surface active materials was also investigated with phase-solubility studies. For this purpose, an excess amount of the drug was added to aqueous solutions containing 0–15% of β-CD and 0.0–0.50% (w/v) of polymer and non-ionic surfactant (PEG 20000 and PS 20, respectively). After equilibration, at least three days took place at room temperature, the suspensions were filtered through at 0.45 μm membrane filter and analyzed by UV spectrophotometer. The type of complexation and the apparent stability constants (K_c) of the drug-cyclodextrin complexes were calculated from the slope of the phase-solubility diagrams.

Solubility studies

Solubilities of active material and complexes were studied at two different pH values of 4.5 and 7.0. An excess amount of active material (an amount of material, more than it could be dissolved) was added to a closed flask with either pH 4.5 or pH 7.0 and then mixed in a magnetic mixer at 37°C. Thereafter, the liquid phase was filtered through 0.45 μm filters and the amount of active material in this solution was determined. Solubility of active material was calculated by the point measured during formation of the equilibrium status.

Preparation of solid complexes

The solid complexes of SMZ with β-CD were prepared by using the three different methods.

Kneading method

In kneading method, aqueous ethanol solution (50%) was added to the mixture of SMZ/β-CD (1:1) until creamy homogenous product was obtained. This mixture was transferred to a mortar and kneaded for 30 min. Then it was dried in an oven under vacuum at 40°C and sieved.

Coprecipitation method

In coprecipitation method, SMZ (0.1 g) was dissolved in acetone and added to the aqueous solution of β-CD. The solvent was allowed to evaporate and then dried under vacuum at 40°C for 24 h and sieved.

Preparation of physical mixture

The calculated and exactly weighed (1:1 molar ratio) amounts of SMZ and β-CD were mixed in a ceramic mortar using the geometric dilution technique.

The solid complexes including solubility enhancer such as water-soluble polymer and surface active agent were also prepared with the same methods.

Characterization of complexes

The infrared spectroscopy (IR) of SMZ, β-CD and the inclusion complexes were measured with KBr discs. Amount of active material in each sample were kept constant in each measurement. Differential scanning calorimetry (DSC) was performed using a scanning rate of 10°C/min on a Netzsch Geratebau DSC 204. Samples were heated in a sealed aluminum pans from 50 to 300°C.

Dissolution studies

The USP XXII paddle method was used in the dissolution rate studies at 37.0 ± 0.5°C. The dissolution rate test was performed at 50 rpm in 900 mL at pH 4.5. All formulations were tested for 3 h. Samples taken at predetermined time intervals were measured spectrophotometrically.

In vivo studies

The formulations chosen for *in vivo* studies are given as below:

Formulation 1:
Sulfamethoxazole 100 mg

β-cyclodextrin 448 mg
Formulation 2:
Sulfamethoxazole 100 mg
β-cyclodextrin 448 mg
PEG 20000 35 mg

The *in vivo* tests were made on six healthy male volunteers whose ages were between 25–50 years. Every 15 days, the prepared formulations and pure SMZ as a control were given to the subjects on an empty stomach with 200 mL of water. Each subject took 100 mL of water hourly. No food was allowed for four hours. Urine samples were collected at hours 0, 1, 2, 3, 4, 5, 6, 7, 8–12 and 12–24. Urine analyses were carried out by using Bratton-Marshall method.

Results and discussion

Phase-solubility diagram

The cationic form of SMZ is the dominating form at pH below about 1.5, the nonionised form dominates between pH 2.0 and 6.0 and the anionic form dominates above pH about 7.0. β-CD is able to solubilize both nonionised and the anionic form of the drug but it has a small solubilizing effect on the cationic form. After taking into consideration of these results, the pHs of the medium were adopted as either 4.5 or 7.0.

The apparent stability constants (K_c) of the drug-β-CD complexes were also determined at room temperature with the presence of PEG 20000 and PS20.

The 1:1 stability constants (k) of the soluble complexes were calculated from the following equation:

$$k = S_t - S_o / [S_o(L_t - (S_t + S_o))] \quad (1)$$

where S_t is total concentration of dissolved SMZ, S_o is the equilibrium solubility of SMZ in the presence of β-CD and L_t is the total concentration of β-CD[22,23].

Phase-solubility diagrams are categorized into A and B types. A type curves indicate the formation of soluble inclusion complexes while B type suggests the formation of inclusion complexes with poor solubility. A B_S type response denotes complexes of limited solubility. β-CD often gives rise to B type curves due to their poor water solubility, whereas the chemically modified CDs like HP-β-CD usually produce soluble complexes and thus give A type systems[24]. The phase-solubility diagram obtained for SMZ and β-CD is shown in Figure 1. According to Higuchi and Connors, the solubility curve can be classified as B_S type. The stoichiometric ratio of complex determined from the descending part of the diagram was found to be 1:1 (SMZ/β-CD).

Since, solubility of SMZ linearly increased with the increase of both β-CD-PEG 20000 and β-CD-PS20 concentrations, it is considered to be an Al type phase-solubility curve[22] (Figure 2). The apparent stability constants (K_c) calculated from the Equation 1 and the solubility values are shown in Table 1 and Table 2, respectively.

The solubility of active material was enhanced in the presence of β-CD, β-CD-PEG 20000 and β-CD-PS20 at either pH 4.5 or pH 7.0 (Table 2).

Characterization of inclusion formation

Evidence for an inclusion formation between drug and β-CD was provided by the analysis of the results obtained

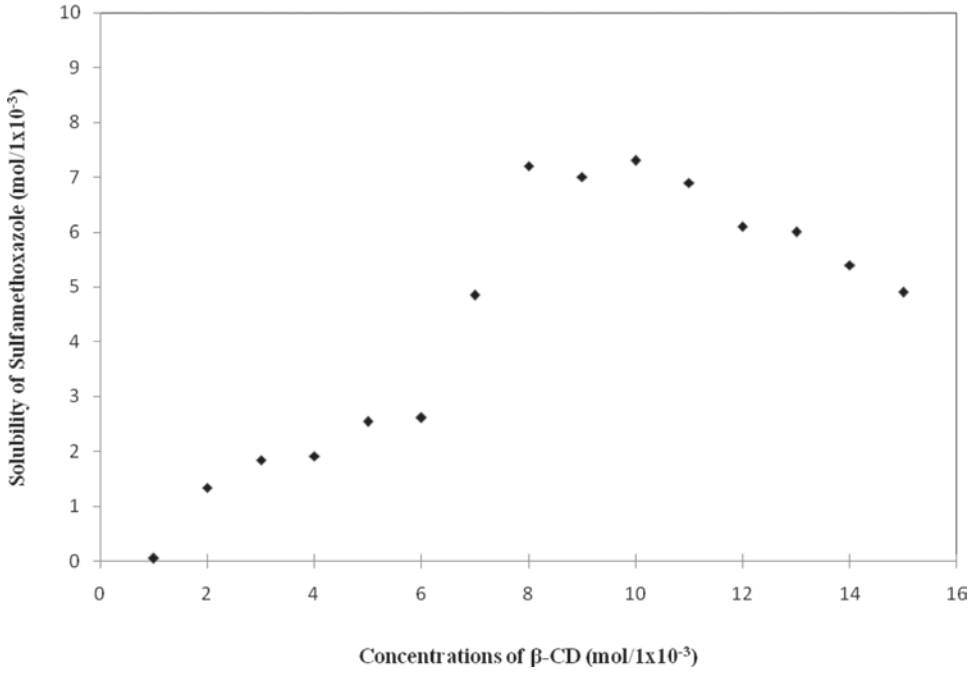

Figure 1. Phase-solubility diagram of SMZ/β-CD system in distilled water.

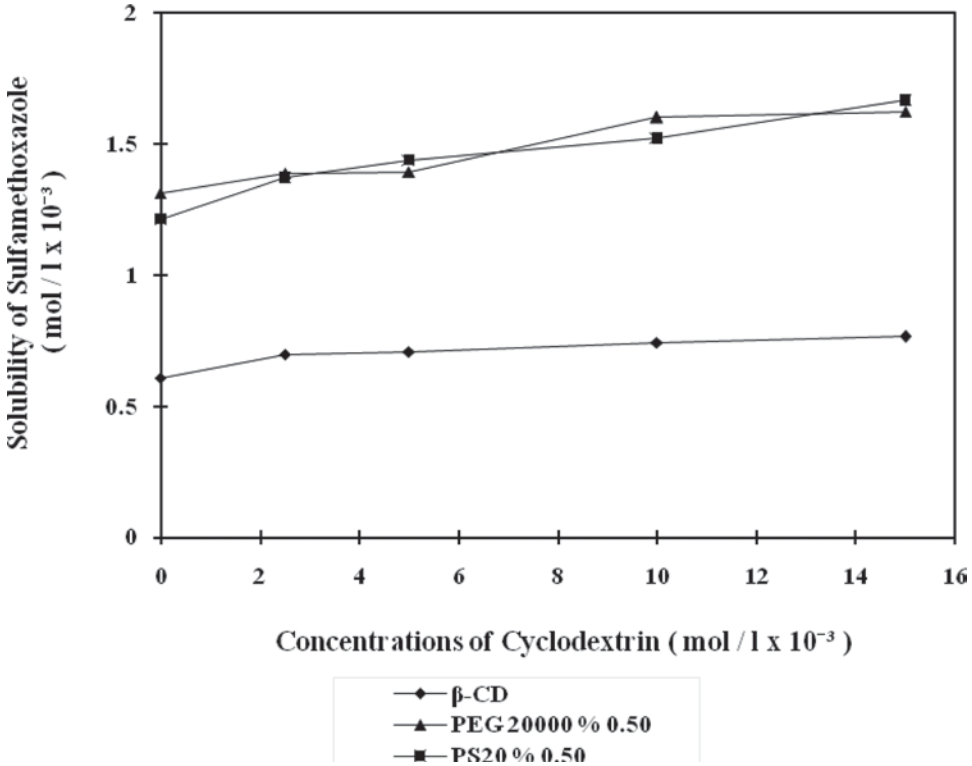

Figure 2. Effect of PEG 20000 (0.50% w/v), and PS 20 (0.50% w/v) on the solubility of SMZ at pH 4.5.

from infrared spectroscopy and differential scanning calorimetry.

The IR spectra of SMZ, β-CD, PEG 20000 and the inclusion complexes are shown in Figure 3. The characteristic bonds of SMZ at 1599 cm^{-1} (-NH2), 1306 cm^{-1} (-SO2) were modified significantly in the solid complexes as a result of complex formation. The observed decreases in the intensities of the characteristic bonds of SMZ may be due to its restriction within the β-CD cavity.

Figure 4 shows the thermogram of SMZ, β-CD and the inclusion complexes obtained from DSC measurements. The β-CD displayed no peaks in the temperature range between 50°C and 300°C while the SMZ exhibited its characteristic endothermic peak associated with the melting point of the drug around 171°C. However, inclusion complexes showed very broad peak, the peak of the SMZ disappeared especially in the presence of PEG 20000 and PS 20 without distinct phase transition around this temperature. The SMZ-β-CD coprepicipitated complex had a less intense peak. The absence of the peak in DSC curves may be considered as a strong indication for the formation of inclusion of the drug into the β-CD cavity.

Table 1. The apparent stability constants (K_c) of the complexes.

Formulations	K_c (M^{-1}) at pH 4.5	K_c (M^{-1}) at pH 7.0
Sulfamethoxazole: β-CD (1:1)	136.40	122.3
Sulfamethoxazole: β-CD (1:1)-PEG20000 (0.50% w/v)	243.80	553.9
Sulfamethoxazole: β-CD (1:1)-PS20 (0.50% w/v)	352.30	147.1

Table 2. The solubility values of sulfamethoxazole in presence of various concentrations of different polymers alone and with β-CD at 25°C.

	Solubility (mg/mL)	
Formulations	pH 4.5	pH 7.0
Sulfamethoxazole	0.086	0.124
Sulfamethoxazole: β-CD(1:1)	0.175	0.188
Sulfamethoxazole: β-CD(1:1) PEG 20000 (0.50% w/v)	0.377	0.406
Sulfamethoxazole: β-CD(1:1) PS20 (0.50% w/v)	0.349	0.386

Dissolution rate studies

The dissolution rate profiles of SMZ and the complexes are shown in Figure 5. The figure illustrates that the release of active material was strongly affected by the method of formulation. The coprecipitated products exhibited the best dissolution properties and were followed by the kneaded product and physical mixtures, respectively. The all complexes studied in this work exhibited better dissolution properties, then the pure drug alone. 37.4% of active material of kneaded product is dissolved within 180 min, whereas the values measured for the coprecipitated, physical mixture and pure drug were 28.4, 12.4

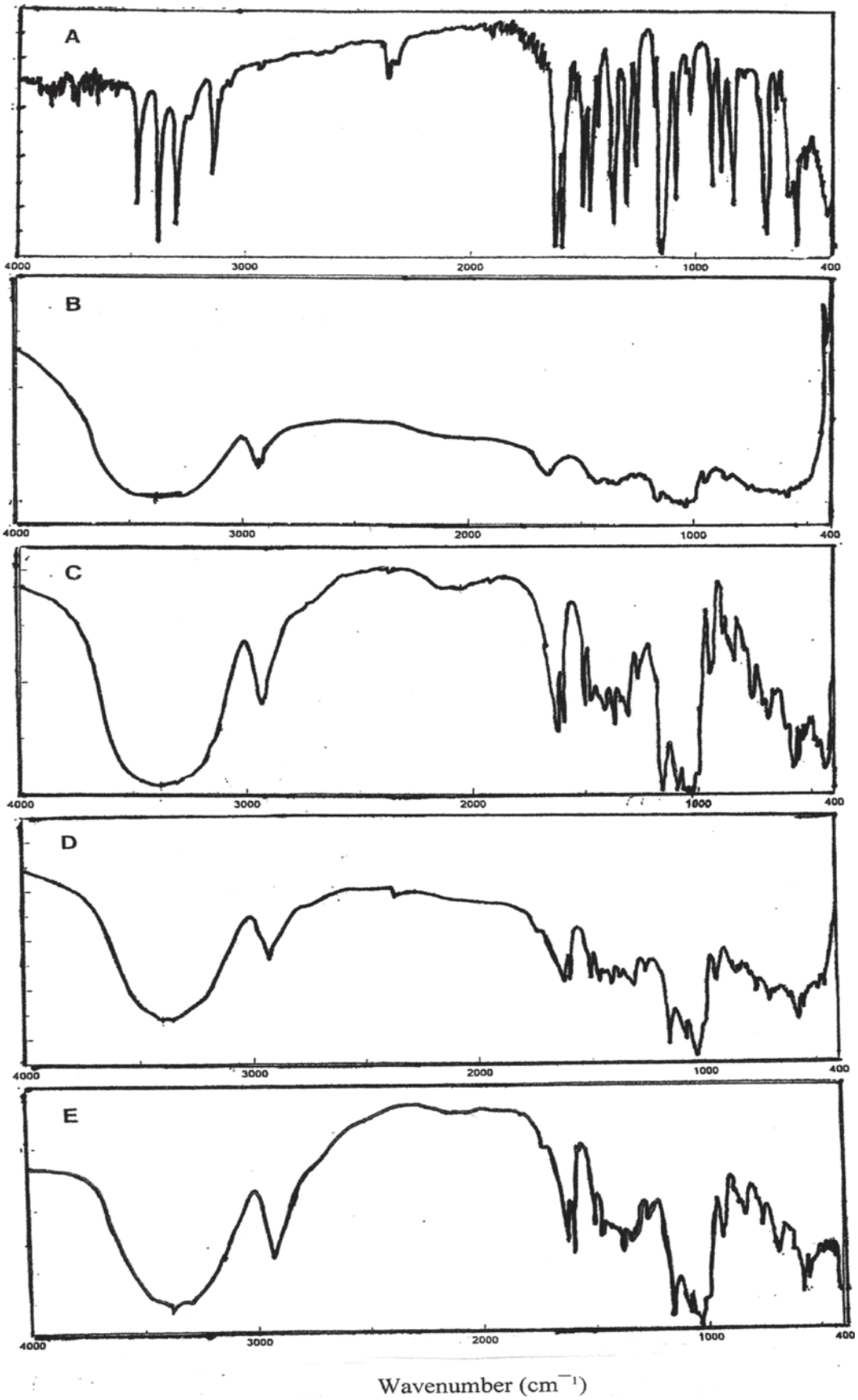

Figure 3. The IR spectra of (A) Sulfamethoxazole, (B) β-Cyclodextrin, (C) SMZ-β-CD coprecipitated complex, (D) SMZ-β-CD-PEG20000 coprecipitated complex, (E) SMZ-β-CD-PS20 coprecipitated complex.

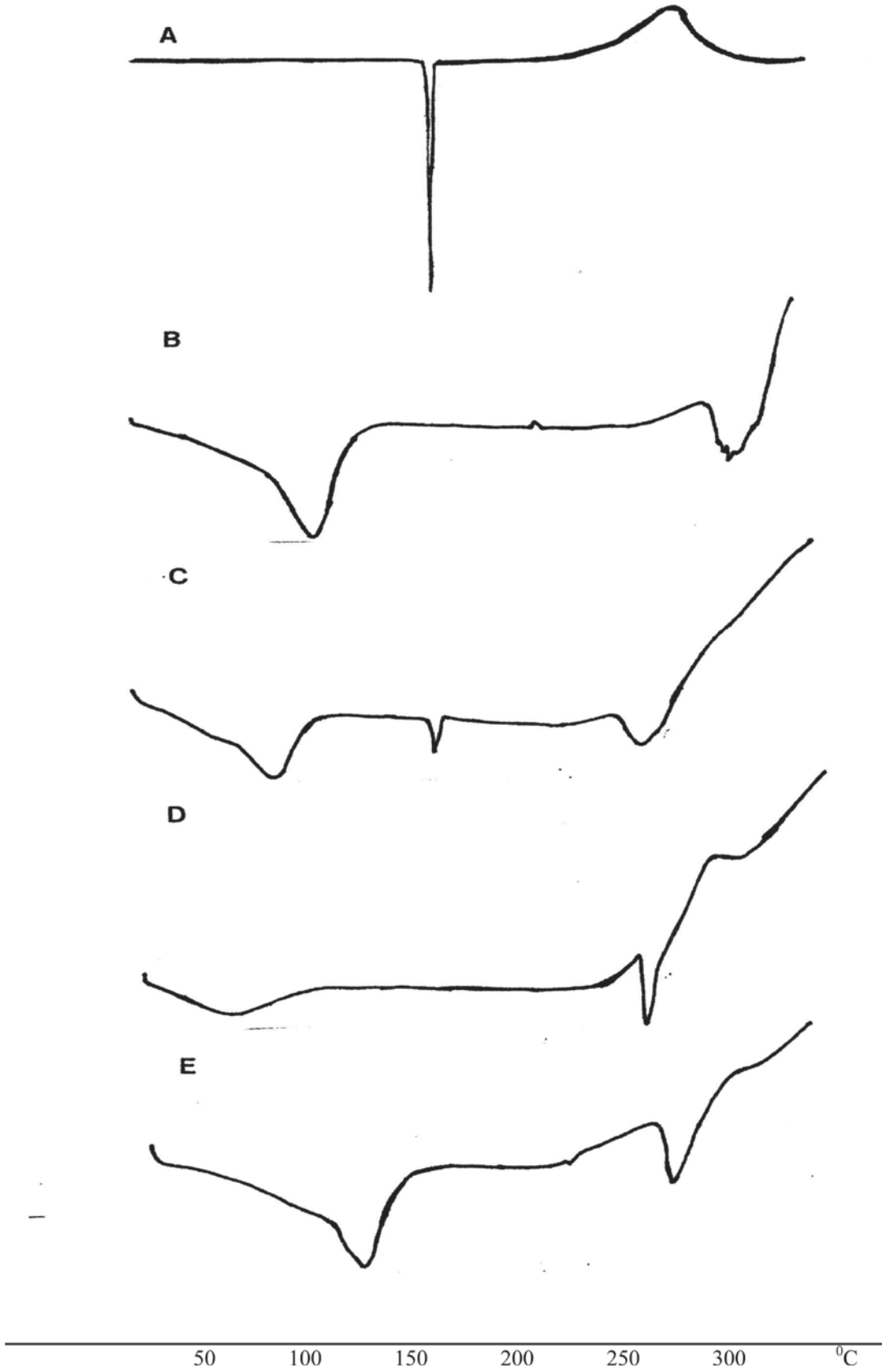

Figure 4. The DSC thermograms of (A) Sulfamethoxazole, (B) β-Cyclodextrin, (C) SMZ-β-CD coprecipitated complex, (D) SMZ-β-CD-PEG20000 coprecipitated complex, (E) SMZ-β-CD-PS20 coprecipitated complex.

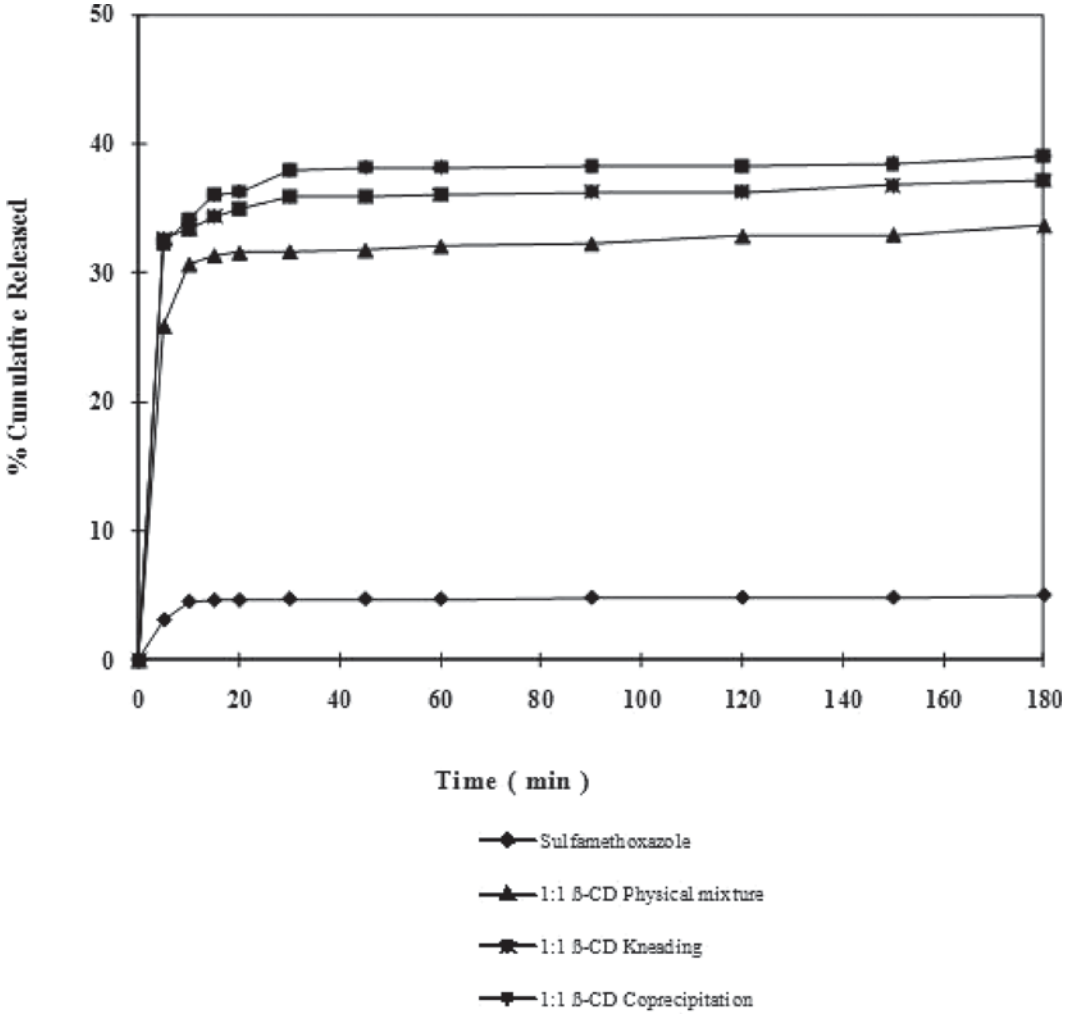

Figure 5. The dissolution profiles of SMZ and its β-CD complexes in pH 4.5 at 50 rpm and 37°C.

and 5%, respectively. The presence of PEG 20000 and PS-20 with the concentration of 0.50% (w/v) enhanced these values to the 42.6 and 44.1%, respectively (Figures 6 and 7).

In vivo *Studies*

In order to investigate the effects of inclusion complexes on the bioavailability of pure drug, β-CD:drug complex and β-CD:drug:PEG 20000 complexes prepared by coprecipitation method were given to healthy volunteers. As shown in Figure 8, the cumulative amount of active material excreted urinary was found to be highest in SMZ:β-CD:PEG 20000 complexes which was followed by SMZ:β-CD complex and powder form of active material, respectively. From Figure 9 active material area under the curves were 90.5, 90.9 and 97.7 mg/ml.hour for pure drug, β-CD:drug complex and β-CD:drug:PEG 20000 complex, respectively. This enhancement may be attributed to the high dissolution rate and enhanced bioavailability of active material by SMZ:β-CD:PEG 20000 complex.

Conclusion

As a result of this study, it may be concluded that the solubility, the dissolution rate and the bioavailability of SMZ was significantly enhanced by the complex formation. Preparation of inclusion complexes by using polymers and non-ionic surfactants increases the solubilizing effect of β-cyclodextrin. SMZ-β-CD-polymer complexes are more powerful solubilizers than the simple cyclodextrin complexes and can enhance the drug dissolution from solid cyclodextrin complexes. The bioavailability of active material can also be enhanced by the preparation of SMZ-β-CD-polymer complexes.

Acknowledgments

We would like to thank Mrs. Gülderen Göğüş for measurement of DSC thermograms. We gratefully acknowledge the supply of SMZ from Fako Pharmaceuticals Co. Ltd.

Declaration of interest

The authors report no conflicts of interest.

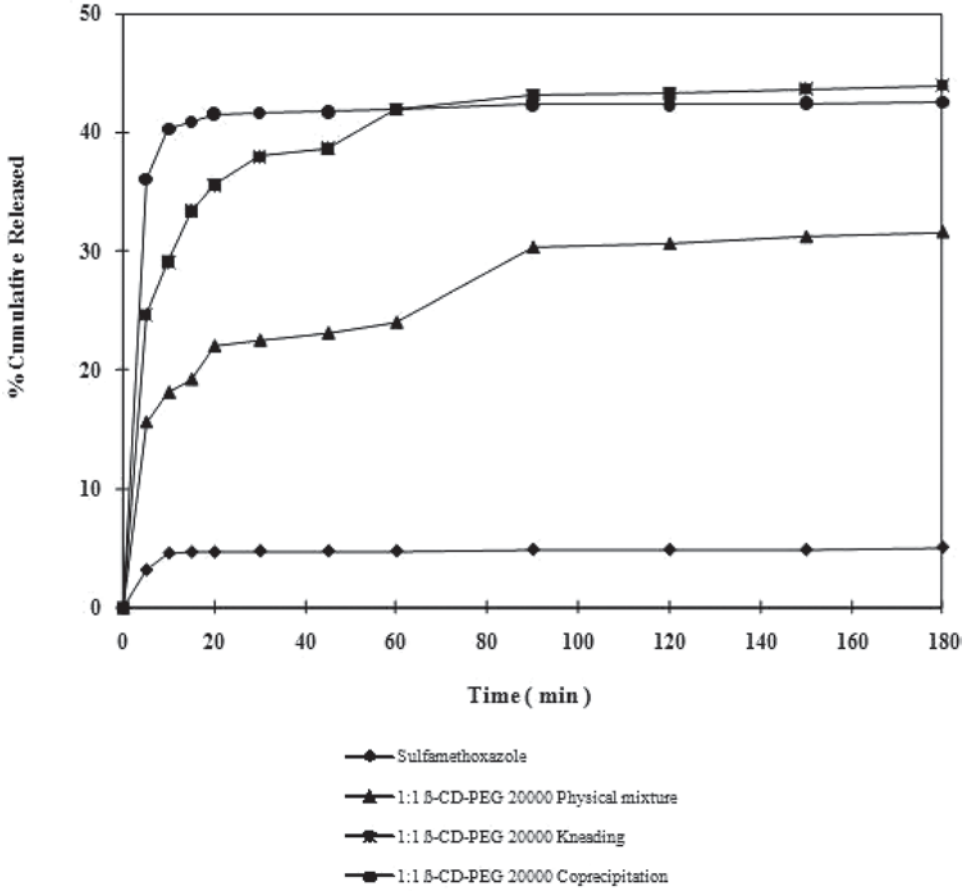

Figure 6. The dissolution profiles of SMZ and its β-CD-PEG20000 complexes in pH 4.5 at 50 rpm and 37°C.

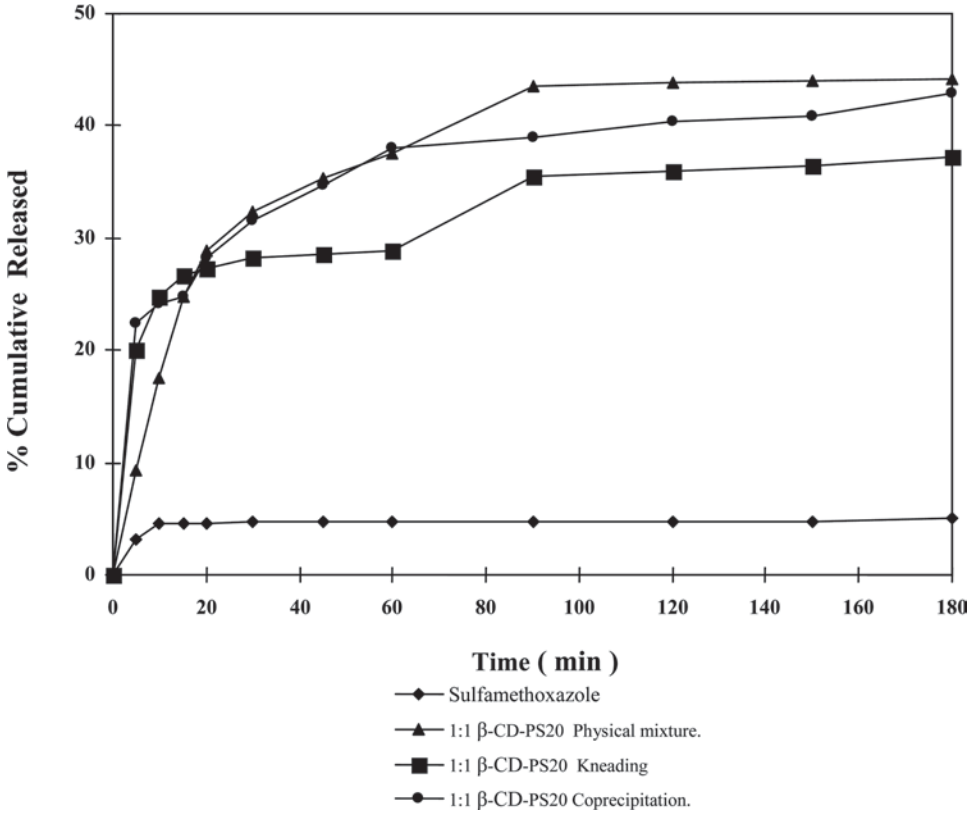

Figure 7. The dissolution profiles of SMZ and its β-CD-PS20 complexes in pH 4.5 at 50 rpm and 37°C.

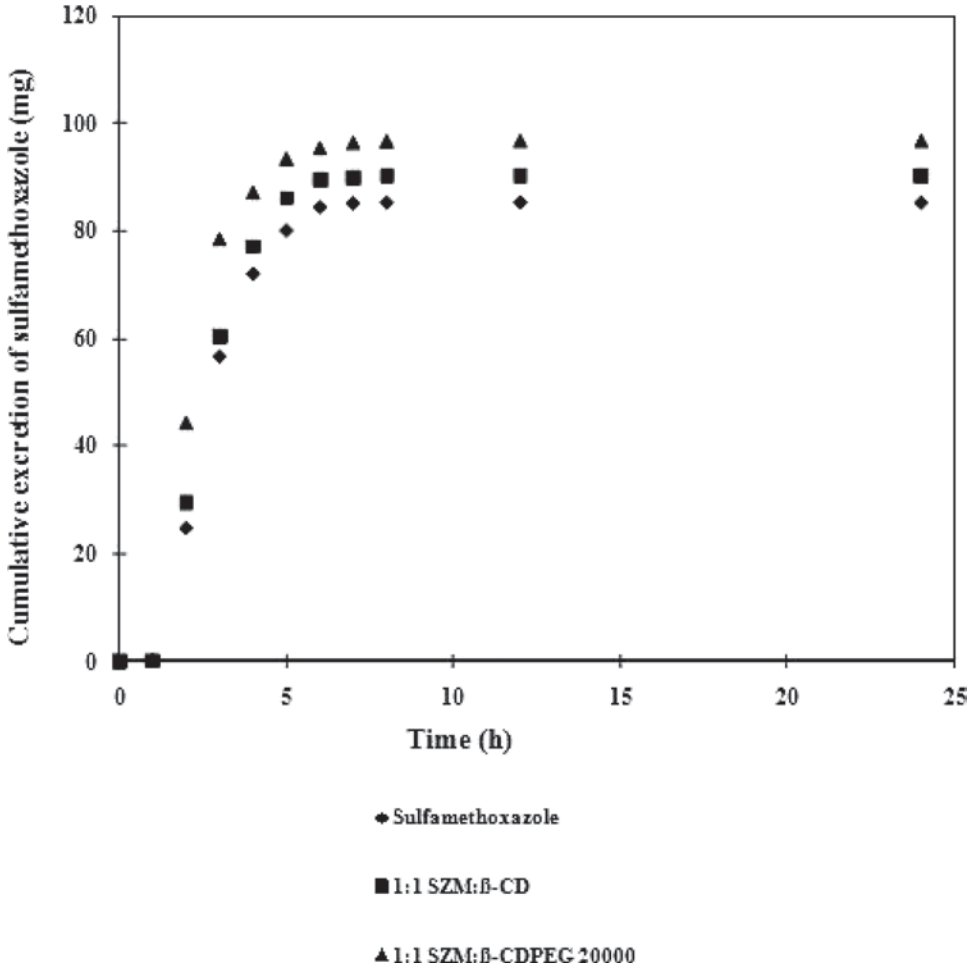

Figure 8. Cumulative urinary excretion profiles.

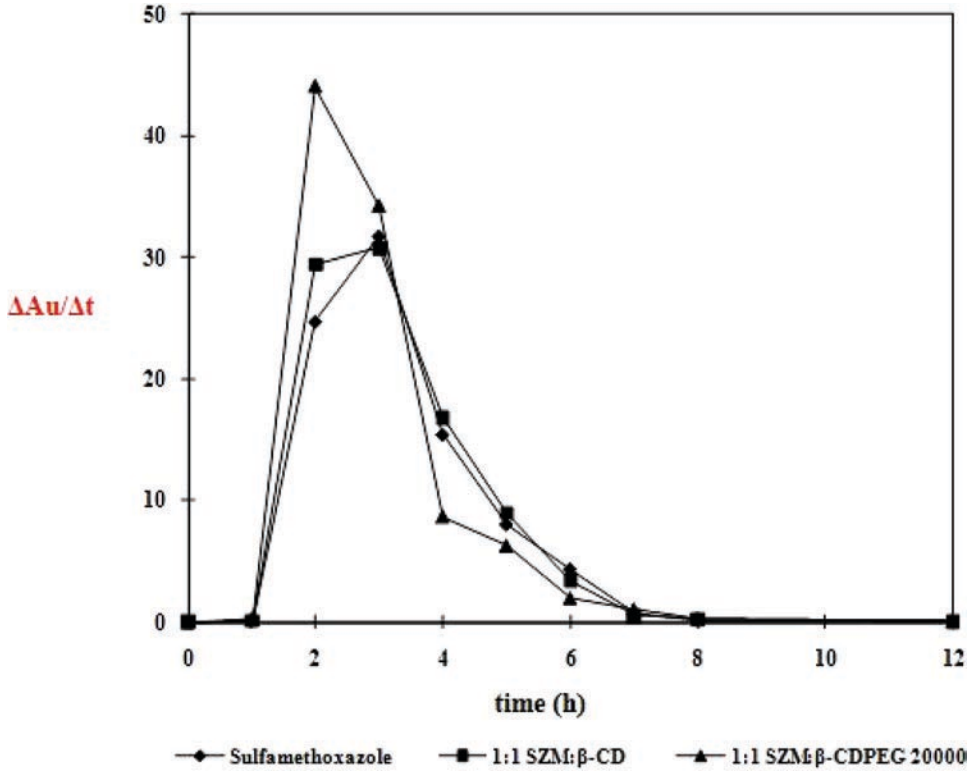

Figure 9. Urinary excretion rates of the subjects with the formulations.

References

1. Martindale, the extra pharmacopoeia.; (1993). 30th Edn. Reynold J.E.F. (ed.), Pharmaceutical Press London, England, p. 208.
2. Raja GV, Sekaran CB, Teja DW, Madhuri B, Jayasree B. (2009). Simple spectrophotometric methods for the determination of sulfamethoxazole in pharmaceuticals using folinciocalteau and orcinol as reagents. Journal of Chemistry, 6:357–360.
3. Bekers O, Uıjtendaal EV, Beıjnen JH, Bult A, Undrberg WJM. (1991). Cyclodextrins in the pharmaceutical field. Drug Dev Ind Pharm, 17:1503–1549.
4. Uekama K. (2004). Design and evaluation of cyclodextrin-based drug formulation. Chem Pharm Bull, 52:900–915.
5. Fromming VK, Szejtli J. (1994). Cyclodextrins in Pharmacy Dordrehcht, Kluwer Academic Publishers.
6. Szejtli J. (1988). Cyclodextrin Technology, Dordrecht: Kluwer Academic Publishers.
7. Sinha VR, Anitha R, Ghosh S, Amita, Kumria R, Bhinge JR et al. (2007). Physicochemical characterization and *in vitro* dissolution behaviour of celecoxib-beta-cyclodextrin inclusion complexes. Acta Pharm, 57:47–60.
8. Loftsson T, Friðrıksdottır H, Olafsdottır BJ. (1991). Solubilization and stabilization of drugs through cyclodextrin complexation. Acta Pharm Nord, 3:215–217.
9. Loftsson T, Friðrıksdottır H, Thorısdottir S, Stefansson E. (1994). The effect of hydroxypropyl methylcellulose on the release of dexamethasone from aqueous 2-hydroxypropyl-β-cyclodextrin formulations. Int J Pharm, 104:181–184.
10. Loftsson T, Guðmundsdottir TK, Friðrıksdottır H. (1996). The influence of water soluble polymers and pH on hydoxypropyl-β-cyclodextrin complexation of drugs. Drug Dev Ind Pharm, 22:401–405.
11. Hirlekar RS, Sonawane SN, Kadam VJ. (2009). Studies on the effect of water-soluble polymers on drug-cyclodextrin complex solubility. AAPS Pharm Sci Tech, 10:858–863.
12. Elgindy N, Elkhodainy K, Molokhia A, Elzoghby A. (2011). Lyophilized flutamide dispersions with polyols and amino acids: Preparation and *in vitro* evaluation. Drug Dev Ind Pharm, 37:446–455.
13. Polonisamy M, Khonam J. (2011). Solid dispersion of prednisolone: Solid state characterization and improvement of dissolution profile. Drug Dev Ind Pharm, 37:373–386.
14. Patel SG, Rajput SJ. (2009). Enhancement of oral bioavailability of cilostazol by forming its inclusion complexes. AAPS Pharm Sci Tech, 10:660–669.
15. Wang S, Ding Y, Yao Y. (2009). Inclusion complexes of fluorofenidone with beta- cyclodextrin and hydroxypropyl-beta-cyclodextrin. Drug Dev Ind Pharm, 35:808–813.
16. Özdemir N, Ordu S. (1998). Improvement of dissolution properties of furosemide by complexation with beta-cyclodextrin. Drug Dev Ind Pharm, 24:19–25.
17. Dhanaraju MD, Kumaran KS, Baskaran T, Moorthy MS. (1998). Enhancement of bioavailability of griseofulvin by its complexation with beta-cyclodextrin. Drug Dev Ind Pharm, 24:583–587.
18. Shargel L, Andrew BC. (1985). Applied Biopharmaceutics and Pharmacokinetics, Norwalk, Connecticut, USA Appleton-Century Crofts.
19. Bratton AC, Marshall EK. (1939). A new coupling component for sulphanilamide determination. J Biol Chem, 128:537.
20. Ağabeyoğlu İ, Kaynar-Özdemir N. (1989). Studies on sustained release XII: performance of sulfamethizole granules employing hydrogenated castor oil. 49th International Congress of Pharmaceutical Sciences of F.I.P. Munich, Germany. 127.
21. Loftsson T, Duchêne D. (2007). Cyclodextrins and their pharmaceutical applications. Int J Pharm, 329:1–11.
22. Higuchi T, Connors KA. (1965). Phase solubility techniques. Adv Anal Chem Instrum, 4:117–149.
23. Connors KA, Mollica JA Jr. (1966). Theoretical analysis of comparative studies of complex formation. J Pharm Sci, 55:772–780.
24. Challa R, Ahuja A, Ali J, Khar RK. (2005). Cyclodextrins in drug delivery: An updated review. AAPS Pharm Sci Tech, 6:329–350.
25. The United States Pharmacopeia. (USP XXII). (1990). United States Pharmacopeial Convention Inc. 1260, Twinbrook Parkway Rockwille, MD 20852.