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Full Length Research Paper

Physicochemical characterization and dissolution properties of binary systems of pyrimethamine and 2-hydroxypropyl-β-cyclodextrin

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Pyrimethamine (PYR), a drug effective against protozoan parasites, such as *Toxoplasma gondii* and *Plasmodium falciparum,* **is poorly water soluble and exhibits marked variation in oral bioavailability. This study was aimed at investigating the possibility and extent of enhancement of the dissolution properties of PYR via complexation with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) as well as characterization of the complex formation of the drug with the cyclodextrin. The interaction between PYR and HP-β-CD in solution was studied by phase solubility analysis while binary systems of the compounds at 1:1 molar ratios were prepared by using the physical mixture, kneading, co-evaporation and freeze-drying methods. The binary systems were characterized using differential scanning calorimetry (DSC), powder x-ray diffractometry (PXRD) and Fourier transform infrared (FT-IR) spectroscopy. Phase solubility studies revealed an A_L-type diagram indicating a 1:1 stoichiometric inclusion complex and a stability constant value of 914 M^{-1}. Solubility and dissolution rates of PYR and the binary systems were determined and found to be markedly enhanced by cyclodextrin complexation. The extent of enhancement of dissolution properties was dependent on the preparation method of the complex, and the product prepared by the freeze-drying method was shown to have the most superior dissolution efficiency than the other binary systems. The PXRD patterns and DSC curves especially for the co-evaporated and freeze-dried systems indicated strong drug amorphization and/or inclusion of PYR in the CD cavities. The results of this study suggest that the complexation of PYR with HP-β-CD could reduce variability in the drug absorption and improve therapeutic efficacy of the drug through increased drug dissolution efficiency.**

Key words: Pyrimethamine, 2-hydroxypropyl-β-cyclodextrin, complexation, enhanced dissolution, physicochemical characterization.

INTRODUCTION

Pyrimethamine (2,4-diamino-5-*p*-chlorophenyl-6-ethyl-pyrimidine) (PYR) (Figure 1) is a very sparingly water-soluble and weakly basic drug (pKa 7.34) that belongs to the group of antifolate drugs. The drug reversibly binds to and inhibits the enzyme dihydrofolate reductase in protozoa. It is generally administered in conjunction with a sulphonamide resulting in a synergistic effect due to the sequential inhibition of enzymatic steps in the folate synthesis provided by the combination (Chulay et al., 1984; Sirawaraporn and Yuthavong, 1986). Pyrimethamine in synergistic combination with either sulfadiazine or clindamycin is the treatment of choice for encephalitis caused by *Toxoplasma gondii* (toxoplasmic encephalitis [TE]) (Dannemann et al., 1992) and, pyrimethamine is also one of the important and powerful antifolates used with sulphadoxine in malaria chemotherapy. TE is the second most common opportunistic infection of the cen-

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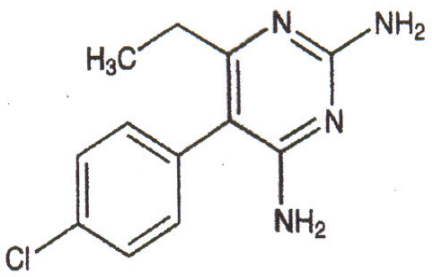

Figure 1. Chemical structure of pyrimethamine.

tral nervous system in patients with AIDS (Richards et al., 1995). The clinical response to pyrimethamine-sulfadiazine is highly variable (Luft et al., 1993) and marked variation has been observed in the plasma concentrations of PYR in AIDS patients treated for TE (Winstanley et al., 1995). This has been attributed to inter-individual differences in oral bioavailability of the drug (Almond et al., 2000). This observation is not surprising since drugs like PYR with poor aqueous solubility have the potential to lead to variable bioavailability. Hence, to overcome this limitation, increasing the aqueous solubility of PYR is an important objective. Solubility enhancement of poorly aqueous soluble drugs is a significant aspect of formulation development. There is plethora of reports of solubility improvement using different techniques. Buffers, co-solvency, surfactants and complexation are most commonly encountered pharmaceutical techniques for solubilizing drug(s) with low aqueous solubility (Yalkowsky, 1983). Studies have shown that reduction in variability in oral drug absorption can be achieved through improved aqueous drug solubility by cyclodextrin complexation of the drug (Arima et al., 2001; Challa et al., 2005).

Cyclodextrins (CDs) are cyclic oligosaccharides containing D-(+) glucopyranose units attached by α-(1, 4) glucosidic bonds, containing a relatively hydrophobic central cavity and hydrophilic outer surface. The commonly used forms of these ring-shaped molecules are α-, β-, and γ-CDs formed by 6, 7 and 8 glucose units, respectively (Szejtli, 1988). CDs have received significant interest in the pharmaceutical field because of their ability to favourably modify physical, chemical and biological properties of a wide variety of hydrophobic drug molecules through formation of inclusion complexes (Loftson and Brewster, 1996; Connors, 1997; Uekama et al., 1998; 1999). Applications have been made of CDs in oral drug delivery for different objectives including reduction of drug-induced gastrointestinal tract (GIT) irritation, taste masking, and improvement of drug bioavailability through enhancement of the solubility and dissolution rate, and/ or increased stability of the drug at the absorption site in the GIT (Challa et al., 2005). Among the above-mentioned CDs, β-CD is the most widely used because it is readily available and has cavity size suitable for the widest range of drugs. However, its low aqueous solubility poses a major drawback in its wider utilization but this has been overcome by chemical modification of the CD through substitution of some of the hydroxyl groups of the α-D-glucose, resulting in derivatives with enhanced solubility. One of such derivatives is HP-β-CD, a hydroxyalkylated β-cyclodextrin, which has been widely used in pharmaceutical applications because of its high water solubility and solubilizing power, low cost, and low toxicity (Challa et al., 2005).

In the course of executing this study, a report emerged in the literature (de Araujo et al., 2007), in which it was demonstrated with physicochemical techniques that PYR forms inclusion complexes with HP-β-CD. However, the report did not evaluate the dissolution properties of the complexes. Thus, in the present study, further information was provided on complex formation of PYR with HP-β-CD by determining the extent of improvement of the pharmaceutical properties (that is, aqueous solubility and dissolution rate) of the drug as well as identifying the method of complex preparation associated with most superior drug dissolution efficiency.

MATERIALS AND METHODS

Materials

Pyrimethamine base (mol wt. 248.7) was a gift from Swiss Pharma Nigerian Ltd (Lagos, Nigeria) and 2-hydroxypropyl-β-cyclodextrin with degree of substitution of approximately 0.6 (mol wt. 1380) was purchased from Fluka through Sigma-Aldrich Chemical Company (Japan). Other chemicals and reagents were of analytical grade

Phase-solubility studies

Phase-solubility studies were performed in accordance with the method of Higuchi and Connors (1965). Amounts of PYR that exceeded its aqueous solubility (30 mg) were introduced into glass tubes to which were added 10 mL of distilled water containing various concentrations of HP-β-CD (3 - 30 mM). These tubes were screw-capped and shaken for 36 h at 25°C on a rotary flask shaker. Thereafter, as shaking continued, 1.0 ml aliquots were withdrawn at intervals of 12 h and filtered immediately using a 0.45 µm nylon disc filter. The filtered samples were diluted suitably and assayed for PYR by UV spectrophotometer at 222 nm against blanks prepared in the same concentration of the cyclodextrin in water, so as to cancel any absorbance that may be exhibited by the HP-β-CD. The shaking continued until 3 consecutive determinations were the same (72 h). The pH values of the contents of the tubes were measured. The solubility experiments were conducted in triplicate. The apparent stability constant (K_c) according to the hypothesis of 1:1 stoichiometric ratio of complexes was calculated from the phase-solubility diagram using the following equation:

$$K_c = \text{slope} / [\text{Intercept } (1 - \text{slope})] \quad (1)$$

The slope was obtained from the straight-line of the plot of PYR concentration (in mM) against HP-β-CD concentration (in mM).

Preparation of solid binary systems

The binary systems of PYR and HP-β-CD were prepared at 1:1 molar ratios using the following methods.

Physical mixture

PYR and HP-β-CD were separately pulverized and sieved (80 µm). The calculated and weighed (1:1 molar) amounts of the powders were carefully and homogenously blended in a mortar, to prepare the physical mixture.

Kneading method

The physical mixtures of PYR and HP-β-CD in 1:1 molar ratios were prepared as earlier described. The binary mixtures were triturated in a mortar with a small volume of water-methanol (1:1 vol/vol) solution to obtain a homogeneous paste. The thick slurry was kneaded for 1 h and during this process, an appropriate quantity of water-methanol was added to maintain a suitable consistency. The paste was dried in oven at 40°C for 24 h. The dried complex was pulverized into a fine powder and sieved (80 µm).

Co-evaporation method

The weights of PYR and HP- β-CD described for the preparation of the physical mixtures in 1:1 molar ratios were used. HP-β-CD was dissolved in an adequate volume of water while PYR was dissolved in methanol. The two solutions were mixed, stirred for 1 h and evaporated under vacuum at a temperature of 45°C in a rotary evaporator. The solid residue was further dried completely at 40°C for 24 h. The dried product was pulverized into a fine powder and sieved (80 µm).

Freeze-drying method

The aqueous solution of HP-β-CD and alcoholic solution of PYR containing the required 1:1 stoichiometric quantities of the compounds were mixed and agitated with magnetic stirrer for 24 h. The resulting solution was kept in a freezer at -20°C and lyophilized in a freeze-dryer (Pump deluxe series, DD 150) for 24 h. The resultant product was pulverized into a fine powder and sieved (80 µm).

Solubility measurements

Aqueous solubility measurements of the pure drug powder and different binary systems with HP-β-CD were carried out by adding an excess amount of the drug or each product, corresponding to 50 mg of PYR, to 5 ml simulated gastric fluid (without enzyme) pH 1.2 [containing 2.0 g/L NaCl and 0.065 M HCl in water (USP 2003)] or simulated intestinal fluid (without enzyme) pH 6.8 [containing 6.805 g/L KH_2PO_4 and 0.896 g/L NaOH in water (USP 2003)] contained in glass tubes. The glass tubes were screw-capped, immersed for 48 h in thermostat-controlled water bath at 37 ± 0.5°C and shaken vigorously every 6 h to attain equilibrium. From phase-solubility studies, equilibrium was found to be reached in 48 h. Before analysis, solutions were brought to room temperature, aliquots of the supernatant liquid were withdrawn, filtered through 0.45 µm membrane filters, appropriately diluted and analyzed for PYR by UV spectrophotometry at 222 nm wavelength (Unicam UV, England). At least triplicate determinations were performed on each sample.

Dissolution rate studies

Dissolution rate studies were performed according to the United States Pharmacopoeia (USP) XXII rotating basket method. The pure drug (50 mg) and each of the binary systems corresponding to 50 mg of PYR were encapsulated in empty colorless soft gelatin capsules and placed into the rotating basket in the dissolution medium. The dissolution medium was 900 mL of simulated gastric fluid (SGF) pH 1.2 without pepsin or simulated intestinal fluid (SIT) pH 6.8 (without enzymes). The stirring speed was 100 rpm and the temperature was maintained at 37 ± 0.5°C. Five ml of the dissolution medium was withdrawn at various time intervals, filtered through 0.45 µ nylon disc filter, and replaced with the same volume of fresh dissolution medium. The filtered samples were analyzed for PYR spectrophotometrically at 222 nm. Triplicate dissolution rate determinations were made for each product and average percent of PYR dissolved was plotted versus time. Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time (Khan, 1975). Values for time taken for 50% drug dissolution ($T_{50\%}$) or 100 % dissolution ($T_{100\%}$) were recorded from the dissolution profiles.

Statistical analysis of data

Differences between the values of the aqueous solubility, drug dissolution efficiency (DE), and percentage of drug dissolved at a given time for the pure drug and the binary systems were evaluated using one-way analysis of variance (ANOVA). Significance of difference in the means was tested using Fishers LSD at 95% confidence.

Characterization of solid complexes

The complexes were characterized and evaluated by the following methods:

Differential scanning calorimetry (DSC)

DSC analyses of the different samples were carried out using DSC 204 F1 (Netzsch, Germany). Weighed samples (2 - 3 mg) were placed in covered aluminum pans, before heating under nitrogen flow (20 ml min^{-1}) at a scanning rate of 10°C min^{-1} over a temperature range of 30 – 500°C.

Fourier transform infrared spectrophotometry

An IR spectrophotometer (FT-IR Nicolet Avatar 330, Thermo Electron Corporation, England) was used for the analysis. The spectra for each of the binary systems prepared by different methods as well as for pure drug and HP-β-CD were generated using the KBr disk method in a frequency range between 4000 and 400 cm^{-1}.

Powder X-ray diffractometry

Powder X-ray diffraction (PXRD) patterns of the pure compounds (PYR and HP-β-CD) and the binary systems were recorded using an X-ray diffractometer 25 KV MD10 from Rasicon Ltd (Saint-Petersburg, Russia). Each sample was pulverized into fine powdery form and loaded into the sample holder and irradiated with monochromatized Cu Ka radiation and analyzed between 2θ angles of 3 and 70°. The sample exposure time was 1200 s.

RESULTS AND DISCUSSION

Phase solubility studies

The phase solubility method described by Higuchi and

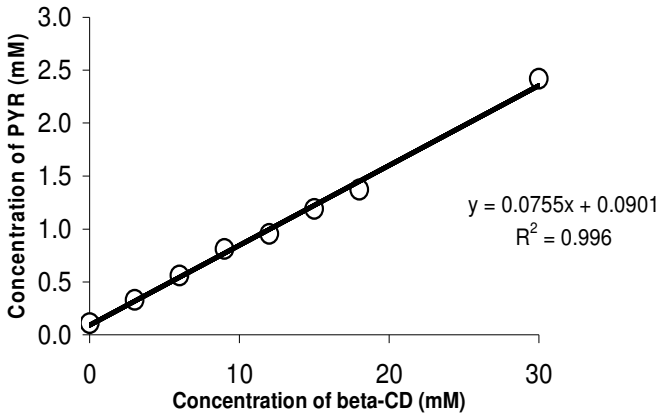

Figure 2. Phase solubility diagram of pyrimethamine in aqueous 2-hydroxypropyl-β-cyclodextrin solution. The experimental conditions were as described in the text.

Connors (1965) is the most widely used approach to study inclusion complexation in solution. The phase solubility diagram for the complex formation between PYR and HP-β-CD is shown in Figure 2. The aqueous solubility of PYR increased linearly (r^2 = 0.996) as a function of the concentration of cyclodextrin, and can be classified as type A_L according to Higuchi and Connors (1965). A-type curves indicate the formation of soluble inclusion complexes. Chemically modified β-CDs like HP-β-CD and Sulfobutylether-β-CD usually produce soluble complexes and thus give A-type systems (Challa et al., 2005). The solubility of PYR increased to about 22 times in the presence of 30 mM CD. pH played no role in increased solubility of PYR observed in aqueous HP-β-CD solutions since the pH values of various aqueous concentrations of the CD containing the drug were comparable to that of the drug suspension without CD (pH range 8.56 – 8.57). Since the plot (Figure 2) had a slope of <1, the increase in PYR solubility can be ascribed to formation of 1:1 molar complex with HP-β-CD. The apparent stability constant (Kc), calculated from the equation described in the methods section (Equation 1), was found to be 914 M^{-1}. This value of Kc indicated that the complex formed between PYR and HP-β-CD is stable as the value is within the range of 200 – 5000 M^{-1} considered adequate for the formation of an inclusion complex which may contribute to improving the bioavailability of poor water soluble drugs (Yamada et al., 2000). This shows the relevance of the phase-solubility studies since it enables determination of not only the solubilizing ability of the CD, but also provides the stability constant through the analysis of the curve. The Kc obtained in this study is less than the value (1900.6 M^{-1}) obtained by de Araujo (2007) for the same drug with HP-β-CD. This discrepancy in the values is most probably related to the difference in the degrees of molar substitution of the HP-β-CD that were employed in the two studies. The HP-β-CD used in this study had a degree of substitution of ~0.6 (mol wt. 1380) as against that with a degree of substitution of ~1.0 (mol wt. 1540) used by de Araujo (2007). Degree of substitution is known to play an important role in balancing β-CD water solubility and its complexing ability. Increasing the degree of substitution can increase the binding of guests to CDs by increasing the surface area of binding; however, steric hindrances can impair CD complexing efficiency after an optimum degree of substitution (Muller and Brauns, 1986).

Solubility and dissolution rate studies

The results of the aqueous solubility of PYR and its binary systems with HP-β-CD in simulated gastric fluid (SGF) (without enzyme), pH 1.2, and simulated intestinal fluid (SIF) (without enzyme), pH 6.8 are shown in Table 1. The aqueous solubility was expectedly comparatively higher in SGF than in SIF since the drug is weakly basic (pKa 7.34), hence, its ionization increases in acidic medium resulting in improved dissolution of the drug. All the binary systems yielded significantly higher ($p < 0.05$) solubility compared to the pure drug. Complexation of PYR with HP-β-CD did not have as much profound effect on the drug solubility compared to enhancement of solubilization of another weakly basic antimalarial, halofantrine, by HP-β-CD complex formation (Onyeji et al., 2007). Halofantrine is more hydrophobic (log P = 8.5) than PYR (log P = 2.69) (Cavallito et al., 1978) and this is an important physicochemical property that influences the incorporation of a drug into the hydrophobic cavity of CD. Halofantrine has a stronger association with HP-β-CD since its Kc value is as high as 2300 M^{-1} (Onyeji et al. 2007) compared to 914 M^{-1} obtained for PYR in the present study. The freeze-dried (FS) and co-evaporated systems (CS) were comparable ($p > 0.05$) in enhancement of drug solubility and both were significantly higher than those of kneaded system (KS) and physical mixture (PM) in both SGF and SIF. It is known that in most cases, method of preparation can affect drug/CD complexation and, effectiveness of a method depends on the nature of the drug and CD. In many studies, spray drying (Moyano et al., 1997; Mura et al., 1999) and freeze drying (Onyeji et al., 2007; Castillo et al., 1999; Pose-Vilarnovo et al., 2001) methods were found to be most effective for drug complexation and enhanced drug solubility, but kneaded systems have also been reported to exhibit superior improvement of dissolution properties (Chowdary and Nalluri, 2000).

On the other hand, method of preparation may not influence the dissolution performance of a drug-CD complex as shown for tolbutamide-β-CD complexes (Viega et al., 2001). The effect of complexation with cyclodextrin on the solubility of drugs can be explained in terms of the reduction in the crystallinity of the drug caused by preparation process and the inclusion of the drug into the hydrophobic cavity of the cyclodextrin.

Table 1. Solubility of pyrimethamine and its binary systems with 2-hydroxypropyl-β-cyclodextrin at 37°C in simulated gastric fluid (SGF) (pH 1.2) and simulated intestinal fluid (SIF) (pH 6.8).

Compound	Aqueous solubility (mg/ml)	
	SGF	SIF
Pure Pyrimethamine	2.25 ± 0.13	0.11 ± 0.005
Physical mixture	5.40± 0.24	0.26 ± 0.01
Kneaded system	5.71± 0.19	0.27± 0.01
Co-evaporated system	6.68 ± 0.27	0.36± 0.02
Freeze-dried system	6.78 ± 0.30	0.37± 0.02

Values are mean ± S.D; n = 3.

Table 2. Mean ± SD values of percent drug dissolved at 20 min (PD_{20}) and dissolution efficiency at 30 min (DE_{30}) of pyrimethamine and its binary systems with 2-hydroxypropyl-β-cyclodextrin at 37°C in simulated gastric fluid (SGF) (pH 1.2) and simulated intestinal fluid (SIF) (pH 6.8).

Sample	SGF		SIF	
	DP_{20} (%)	*DE_{30}	DP_{20} (%)	*DE_{30}
Pyrimethamine	34.73 ± 2.75	24.20 ± 1.61	8.82 ± 0.93	6.31 ±0.56
Physical mixture	44.81 ± 3.76	33.47 ± 3.66	24.31 ± 2.31	15.73 ± 1.24
Kneaded system	53.72 ± 2.02	43.67 ± 2.40	32.43 ± 2.50	22.45 ± 2.13
Co-evaporated system	80.70 ± 4.54	58.17 ± 3.42	49.53 ± 2.40	28.83 ± 1.92
Freeze-dried system	89.02 ± 6.03	65.33 ± 3.62	55.65 ± 3.71	30.83 ± 2.31

*DE_{30} was calculated as described in the text from the area under the dissolution curve at 30 min expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

The dissolution properties of the pure drug and binary systems were assessed using percent of active ingredient dissolved at 20 min [$DP_{(20)}$] in the dissolution media and dissolution efficiency determined at 30 min [DE_{30}]. The dissolution efficiency can have a range of values depending on the time interval chosen but, a constant time interval should be chosen for comparison. The use of SGF and SIF as dissolution media was to provide information on comparative dissolution profiles of the products at the locations of the gastrointestinal tract where the drug is expected to dissolve. The mean dissolution curves and derived dissolution data are presented in Figure 3 and Table 2, respectively. In line with the solubility data, the dissolution rates of the products were corresponding higher in SGF than in SIF, due to greater extent of drug ionization and solubilization at the lower pH. In both dissolution media, all the binary systems significantly improved ($p < 0.05$) the drug dissolution rates compared to the pure drug and there was also a clear evidence of the effect of method of preparation on the drug dissolution. The dissolution profiles of any pair of the treated samples were significantly different ($p < 0.05$), except between the freeze-dried and co-evaporated systems. Overall, the rank order of dissolution rates of the products was: FS ≥ CS > KS > PM > Pure Drug (Table 2). To further differentiate the dissolution profiles of the pure drug and binary systems in SGF, time for 100% drug dissolution ($T_{100\%}$) was determined from the curve (Figure 3A). The values were 30, 40, 50, 80, and >180 min. for the FS, CS, KS, PM and pure drug, respectively. The DE_{30} values for the binary systems in SGF have a good correlation with $T_{100\%}$ ($r = -0.95$) and also with DP_{20} values ($r = 0.99$). These correlations indicate that either of these parameters can be used to adequately evaluate the dissolution profiles. Similarly, the time for 50% drug dissolution obtained for the pure drug and binary systems in SIF were 25, 30, 40, 100 and >180 min. for FS, CS, KS, PM and Pure drug, respectively, and these significantly correlate ($r < -0.97$) with the DE_{30} and DP_{20} values. The 100% dissolution of PYR from the freeze-dried system within 30 min compared to incomplete dissolution of the pure drug over 180 min in the simulated conditions of the stomach is of profound significance since this suggests that the product can reduce the high variability in GIT absorption of pyrimethamine. Marked variation in oral bioavailability of PYR (Almond et al., 2000) results in unpredictability of clinical response to the drug in the treatment of toxoplasmic encephalitis in AIDS patients (Luft et al., 1993).

The DP_{20} and DE_{30} values of the Freeze-dried system were higher than those of the pure drug by up to 5 – 6 times in the simulated intestinal fluid. The degree of im-

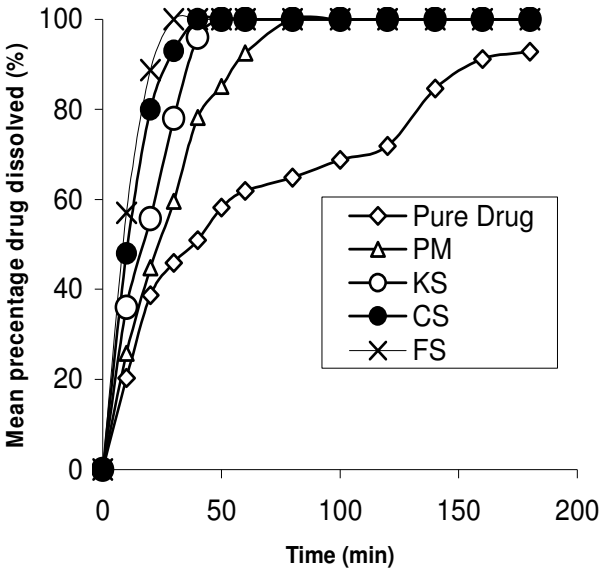

Figure 3. Dissolution profiles of pyrimethamine alone and from its equimolar binary systems with 2-hydroxypropyl- β-cyclodextrin in simulated gastric fluid, pH 1.2 (A) and simulated intestinal fluid pH 6.8 (B) at 37 ± 0.5°C.

provement in dissolution rate of a drug may not correspond with that of solubility enhancement since dissolution rates from drug–CD binary systems are also dependent on other factors, such as diffusion and dissociation of the complex in the dissolution medium in addition to reduction of drug crystallinity and enhanced wettability of the drugs by the inclusion complexation (Uekama and Hirayama, 1996; Nallury et al., 2003). It is pertinent to note that some degree of improvement in drug dissolution rate can be obtained even when there is no interaction between a drug and CD in the solid state. This is because, since CD dissolves more rapidly in aqueous medium than the pure drug, it is possible that in the early stages of the dissolution process, the CD molecules will operate locally on the hydrodynamic layer surrounding the particles of the drug, and this action results in an *in situ* inclusion process, which produces an increase of the amount of the dissolved drug (Corringan and Stanley, 1982). Therefore, characterization of drug-CD binary systems is essential as it provides insight into the basis for improvement of drug dissolution characteristics by cyclodextrin complexation.

Differential scanning calorimetry (DSC)

DSC was used to characterize the pyrimethamine-HP-β-CD solid binary systems prepared by different methods. The DSC thermograms of the various products, with the endothermic events directed upwards, are presented in Figure 4. The DSC curve of PYR (Figure 4A) showed a single sharp endothermic peak at 240°C, corresponding to its melting point, and indicating the crystalline anhydrous state of the drug. A broad endothermic effect associated with loss of water was recorded over a range of 40 – 100°C in the thermogram for HP-β-CD along with a second broad endothermic event at 290 - 340°C, related to the thermal decomposition of the compound. The thermal curves of the binary systems exhibited broadening and marked reduction in intensity of the endothermic peak of PYR to varying degrees in all the products (Figure 4C – F). Also, the PYR peaks shifted to lower temperatures. For the freeze-dried system, the PYR peak was very small, and this can be explained on the basis of major interaction between the drug and cyclodextrin. Reduction in PYR endothermic peak height in the binary products was of the same order as observed in the enhancement of dissolution profiles of the drug by the different methods of the complex preparation. Thus, the PYR peak intensity was of the order: freeze-dried system < co-evaporated system < kneaded system < physical mixture. Furthermore, the endothermic peak associated with thermal decomposition of HP-β-CD showed broadening and varying degrees of diminution of its intensity in the binary products. This phenomenon is also indicative of strong interaction between the drug and cyclodextrin in the solid state. The marked reduction in intensity and/or broadening and shift to a lower temperature of the PYR endotherm indicates a partial inclusion of PYR in the CD cavities. The freeze-drying method produced the strongest interaction between the two compounds.

Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra of the binary systems were compared to those of the pure compounds (pyrimethamine and HP-β-CD). Changes in the characteristic bands of the pure substances indicate the existence of a complex as a new compound with different spectroscopic bands. The FT-IR spectrum of PYR showed bands with peaks at 3467 and 3149 cm^{-1} (Figure 5 a) and these are indicative of stretching vibrations of N-H and aromatic ring C-H, respectively. The presence of the N-H band provides a strong diagnostic point for detection of interactions between the drug and other molecules due to possibility of intermolecular H-bonding. The spectrum also displayed absorption bands between 1400 and 1649 cm^{-1}, which correspond to the stretching vibrations of C=C and C=N from the aromatic rings in PYR. The peaks at 1394 and 1280 cm^{-1} of the spectrum represent C-H bending vibration from CH3 and C-N stretching vibration, respectively. These bands are not of high value in evaluating intermolecular hydrogen bonding and hence not diagnostic of interaction between PYR and other molecules. The FT-IR spectrum of HP-β-CD (Figure 5b) showed a broad absorption band at 3423 cm^{-1} for O-H stretching vibrations. Other prominent peaks were at 2931 cm^{-1} (for C-H stretching vibrations), and bands in the range of 1384–

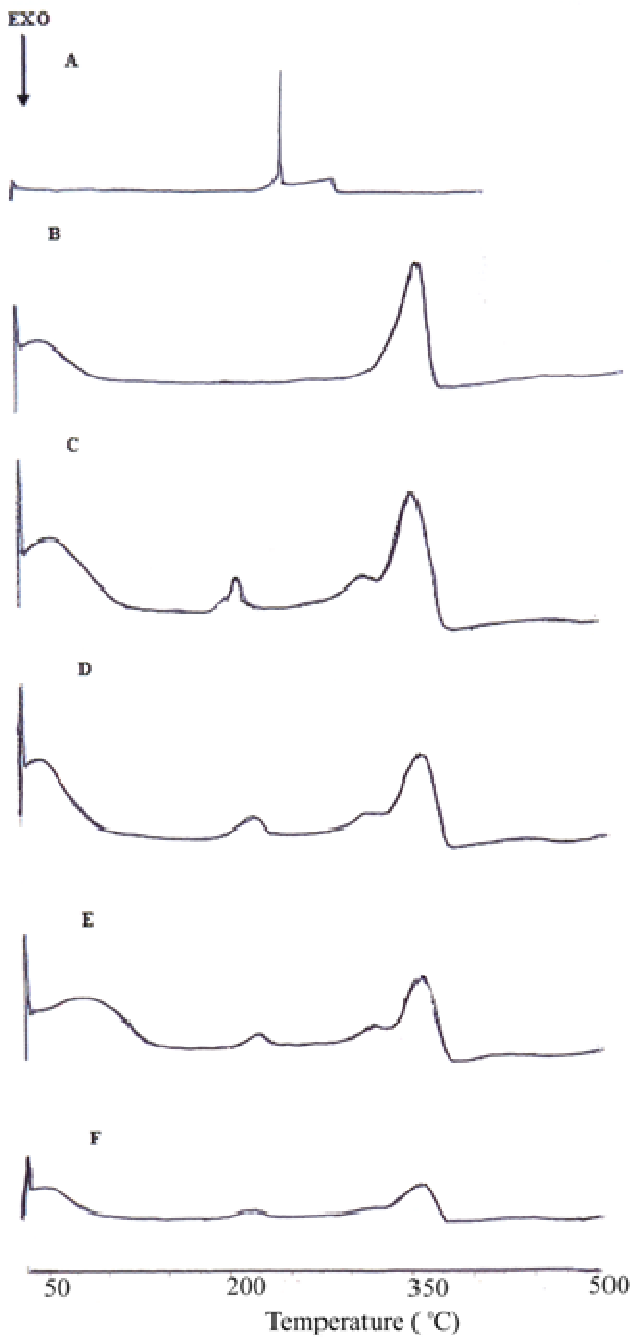

Figure 4. Differential scanning calorimetry (DSC) curves of pyrimethamine (A), 2-hydroxypropyl- β-cyclodextrin (B), and their equimolar binary systems: physical mixture (C), kneaded system (D), co-evaporated system (E) and freeze-dried system (F).

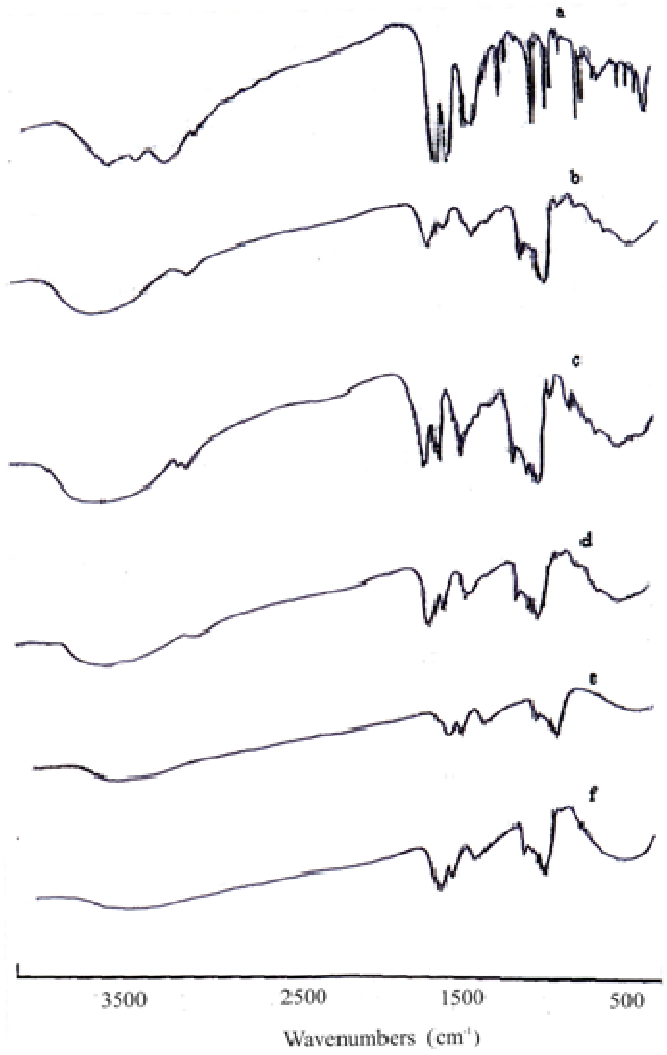

Figure 5. FT-IR Spectra of pyrimethamine (a), 2-hydroxypropyl-β-cyclodextrin (b), and their equimolar binary systems: physical mixture (c), kneaded system (d), co-evaporated system (e) and freeze-dried system (f).

1460 cm^{-1} representing CH_2 and CH_3 bending vibrations. The IR spectra of the binary systems compared to those of the pure drug and cyclodextrin showed different degrees of changes. There was an overlap of the O-H stretching vibrations of cyclodextrin and that of N-H of PYR in the IR spectra of the binary systems and, the new band decreased in intensity to varying degrees in the products (Figure 5c-f). Also, the intensities of the bands between 1400 and 1649 cm^{-1}, corresponding to the stretching vibrations of C=C and C=N from aromatic ring were similarly affected by complex formation with the cyclodextrin. Decrease in band intensity is indicative of increased level of interaction between molecules of the two compounds. These interactions result from weak H-bonding between the N-H of PYR molecules and the O-H groups of the cyclodextrin in addition to non-H-bonding (hydrophobic) interactions occurring in varying degrees in the binary systems, with the highest degree of interaction produced by the freeze-drying method. Furthermore, there were qualitative differences in the finger print regions of the IR spectra of the pure compounds (PYR and cyclodextrin) compared to those of the various binary

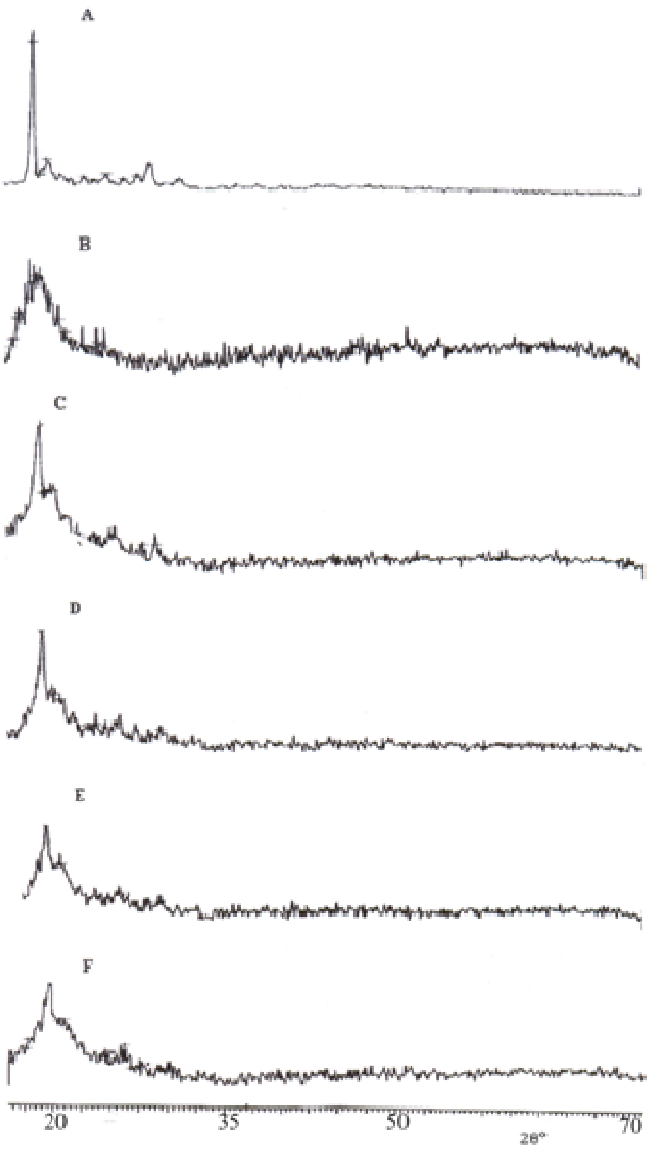

Figure 6. Powder X-ray diffractograms of pyrimethamine (A), 2-hydroxypropyl- β-cyclodextrin (B), and their equimolar binary systems: physical mixture (C), kneaded system (D), co-evaporated system (E) and freeze-dried system (F).

systems. The finger print regions of the binary systems were not superimposable on the spectra of the individual pure compounds. Interactions between the molecules of the compounds reflected in reduction to varying extents along with slight shifts in the peaks relative to those of the pure compounds. These provide further evidence of interaction between the two compounds.

X-ray diffractometry studies

The Powder X-ray diffractometry (PXRD) patterns of PYR, HP-β-CD, and prepared binary systems are presented in Figure 6A – F. PXRD is a useful method for the detection of cyclodextrin complexation in powder or microcrystalline states. A diffraction pattern that is clearly distinct and not a superposition of each of the components of the binary system are indicative of formation of a true inclusion complex. The extent of complex formation can be assessed quantitatively by calculation of relative degree of crystallinity (RDC). RDC = I_{sam}/I_{ref} where I_{sam} is peak height of the sample under investigation and I_{ref} is the peak height of the same angle for the reference with the highest intensity (Ryan, 1986). The crystalline nature of PYR was demonstrated in its diffraction pattern which showed peaks that were intense and sharp (Figure 6A). On the other hand, the PXRD pattern of HP-β-CD had a single broad peak and many undefined, diffused peaks with low intensities and, this is a reflection of the amorphous nature of cyclodextrins (Baboota et al., 2005; Onyeji et al., 2007). The diffraction patterns of PM and KS contained some of the major peaks of PYR but the intensities were lower while the diffractogram of CS had fewer sharp peaks with considerable diminution of the intensities. In contrast, the FS exhibited a diffraction pattern with many undefined and diffused peaks, similar to that of pure HP-β-CD, but with a reduction in peak intensities, suggestive of amorphization of the drug. Employing RDC to quantitatively measure the extent of complex formation in the binary systems, and using PYR peak at 18.85° 2θ to calculate the RDC, the calculated values were 0.168, 0.188, 0.228 and 0.235 for the FS, CS, KS and PM, respectively. The marked reduction in the RDC relative to unity (that is, that of PYR) in all the binary systems provides an explanation for the significant increase in the solubility and dissolution rates of PYR by cyclodextrin complexation. The order of decrease in crystallinity as indicated by the RDC values is in accordance with the observed trend in the increase in dissolution characteristics of the products (Tables 1 and 2). Thus, the freeze-drying method produced the most efficient amorphization and/or inclusion complexation of the drug while the physical mixture method was the least effective.

Conclusion

The study demonstrated the possibility of significantly improving the aqueous solubility and dissolution performance of pyrimethamine by inclusion complex formation with 2-hydropropyl-β-cyclodextrin. Phase-solubility studies indicated that pyrimethamine forms a complex with the cyclodextrin at a 1:1 molar ratio and the apparent stability constant is adequate for the formation of an inclusion complex which may contribute to improving the bioavailability of a poorly water soluble drug like PYR. Results obtained by different physicochemical characterization techniques indicate that the freeze-drying method produces the most efficient complexation of pyrimethamine with 2-hydropropyl-β-cyclodextrin. Improved disso-

lution characteristics of pyrimethamine may reduce the inter-individual variability in the drug absorption, with a resultant predictability of clinical response to the drug.

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