

Enhancement of the Dissolution Rate of Ketoconazole Through a Novel Complexation with Fulvic Acid Extracted from Shilajit

SURAJ P. AGARWAL, SHAHID H. ANSARI and RITESH R. KARMARKAR*

Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard

Hamdard Nagar, New Delhi-110 062, India

E-mail: riteshkar@rediffmail.com

The objective of the current research work was to investigate the potential of fulvic acid, extracted from shilajit, as a bioavailability enhancer. Ketoconazole was selected as a model drug because of its insolubility in water except at low pH, leading to poor bioavailability. Complexes of fulvic acid with ketoconazole were prepared in a molar ratio of 1:0.5 and 1:1 by solvent evaporation, spray drying and physical mixing methods. The prepared complexes were characterized by differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). Phase solubility and intrinsic dissolution rate study of ketoconazole with fulvic acid were carried out in phosphate buffer of pH 5 and 6 to study the interaction between ketoconazole with fulvic acid. Phase solubility study indicates that solubility of ketoconazole was significantly increased at fulvic acid concentration greater than 0.3 % w/v due to micellization of fulvic acid. DSC and XRD studies showed differences between ketoconazole and ketoconazole-fulvic acid complexes prepared by various methods. Solvent evaporated and spray dried complexes showed significant improvement in dissolution rate as compared to ketoconazole or ketoconazole-fulvic acid physical mixture. Further, spray-dried complexes showed better dissolution rate than solvent evaporated complexes. The results indicate that the fulvic acid extracted from shilajit could be used to increase the dissolution rate and hence the bioavailability of poorly water-soluble drugs.

Key Words: Shilajit, Fulvic acid, Ketoconazole, Complexation, Dissolution enhancement.

INTRODUCTION

Humic substances are the most important source of organic carbon in the environment. They are product of the decomposition of dead and decaying plant and animal tissues. Their colloidal characteristics and the high surface functionality make them excellent adsorbents, possessing a superior capacity for the retention of ionic and molecular pollutants and

for facilitating the processes of mobilization/immobilization of these in the environment. They are mainly fractionated into fulvic acid, humic acid and humin, as a function of their solubility at different pH values. Fulvic acid is soluble at any pH; humic acid is soluble at alkali pH and humins are insoluble at any pH. The solubility of these fractions is closely related to molecular mass, structural branching complexity, molecular polarity and chemical composition. The structure of humic substances are at present ill defined despite many decades of scientific research. However the structural study so far carried out on fulvic acid showed that they have open flexible structure having voids in its structure. The porous structure of this acid indicates that they have inner hydrophobic and outer hydrophilic structure and thus have a capability to form a complex with non-polar solute and hydrophobic drug molecules¹. Humic substances occur ubiquitously in the environment such as in soil, peat, coal, shilajit, *etc.* Shilajit is one of the richest source of humic substances and has been used for thousands of years under the indigenous systems of medicine like Ayurveda, Siddha and Unani as a health tonic and rejuvenator². Chemical studies showed that it comprised of humus (60-80 %) along with other components such as benzoic acid, hippuric acid, fatty acid, ichthyol, ellagic acid, resin, triterpenes, sterol, aromatic carboxylic acid, 3,4-benzocoumarins, amino acids and phenolic lipids. The major physiological action of shilajit was found to be due to the presence of the bioactive dibenzo- α -pyrones along with humic and fulvic acid which acted as a carrier molecules for the active ingredients^{3,4}.

The toxicity study reported on fulvic acid extracted from shilajit showed that it is completely non-toxic compound⁵. The structural report on fulvic acid of shilajit showed that it has an open flexible structure having a void in its structure⁶. Fulvic acid of shilajit has an average molecular weight of 1200. In the present study we have extracted the fulvic acid from shilajit and was evaluated for its dissolution rate enhancement (bioavailability enhancement) capacity for the poorly water-soluble drug. To study the potential of fulvic acid as a bioavailability enhancer, ketoconazole was selected as a model drug. Ketoconazole (KET) is an imidazole antifungal agent used in the treatment of candidiasis, blastomycosis and other systemic fungal infections⁷. Ketoconazole is a weak base with pKa of 2.94 and 5.61. It is practically insoluble in water except at pH < 3. The major drawback associated with the therapeutic application of ketoconazole was poor bioavailability upon oral administration because of its very low aqueous solubility and hydrophobic structure⁸. The aim of the present study was to investigate the complexation behaviour of fulvic acid with KET and its effect on solubility and dissolution rate enhancement of KET in aqueous buffer of pH 5.0 and 6.0.

EXPERIMENTAL

Ketoconazole, (*cis*-1-Acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2(1H-imidazol-1-ylmethyl)-1,3 dioxolan-4-yl]methoxy]phenyl]piperazine) was supplied by Skylark, (New Delhi, India) and shilajit by Pioneer Enterprises (Mumbai, India). Tulsion ADS-400 and Indion 225 H was generously given by Thermax Ltd., India. All other solvent and materials used were of analytical grade.

Analysis of ketoconazole: KET was analyzed by HPLC method reported by Ene *et al.*⁹ with minor modification. Briefly, the samples were injected manually into Licrosorb RP-18 column (125 × 4.6 mm, particle size 5 μ) attached to the 2487 Waters HPLC model. The mobile phase used was water:acetonitrile:diethylamine (50:50:0.05 v/v/v) at a flow rate of 1.5 mL min⁻¹. The detection was carried out using UV detector at 260 nm.

Extraction of fulvic acid from shilajit: Finely powdered shilajit (200 g) was extracted with 500 mL each of hot chloroform, ethyl acetate and methanol to remove the bioactive components. 50 g of the extracted shilajit was then taken and dispersed in 500 mL of 0.1 N sodium hydroxide solution with intermittent shaking in presence of nitrogen at room temperature for 24 h. The suspension was filtered and the filtrate was acidified with dilute hydrochloric acid to a pH of less than 3. The solution was finally allowed to stand at room temperature for overnight leading to separation of humic acid as coagulate, which was filtered, dried and pulverized.

The filtrate obtained in the above step was shaken with 20 g of macroporous ion-exchange resin, Tulsion ADS-400. Adsorbed fulvic acid was then eluted with 100 mL of 0.1 N aqueous sodium hydroxide solution and the process was repeated several times (6-8 times) in order to complete the extraction of fulvic acid from shilajit. The fulvic acid solution thus obtained was shaken with 10 g of hydrogen saturated cation-exchange resin Indion 225 H for 5 min in order to exchange the sodium ion with hydrogen ion. The fulvic acid solution was finally freeze dried to obtain amorphous fulvic acid.

Phase solubility studies: Phase solubility study was carried out as reported by Higuchi and Connors¹⁰. Excess KET was added to vials containing various concentrations of fulvic acid and were shaken in water bath at 25 ± 2 °C until equilibrium was reached (7 d). The contents of each vial were filtered (0.22 μm pore size) and the concentration of KET was measured by a validated HPLC method.

Preparation and characterization of solid complexes and physical mixture: The solid complexes of KET-fulvic acid were prepared at a molar ratio of 1:0.5 and 1:1 by solvent evaporation, spray drying and physical mixing methods.

Solvent evaporation method: KET and fulvic acid were taken in an appropriate molar ratio and were dissolved in acetic acid and distilled water, respectively in a separate beaker. The KET and fulvic acid solutions were then mixed together in appropriate molar ratio before solvent evaporation. The solvent was removed using Rotaevaporator (Scientific Corporation, New Delhi) under reduced pressure. The solvent evaporated complex was collected from the flask, dried and pulverized. The yield was about 95 %.

Spray drying method: KET and fulvic acid were taken in an appropriate molar ratio and were dissolved in acetic acid and distilled water, respectively in a separate beaker. The KET and fulvic acid solutions were then mixed together in appropriate molar ratio before spray drying. The spray drying of the prepared solutions was carried out in a mini spray dryer (SMST Corp., Kolkota, India) using the following optimized condition-flow rate: 4 mL min⁻¹, outlet temperature: 115 °C, atomizing air pressure: 3 kg cm⁻². The yield of the spray drying process was 55 %.

Physical mixing method: Physical mixture of KET with fulvic acid in an appropriate molar ratio were prepared by pulverizing and mixing KET with fulvic acid in a mortar with pestle.

The complexes were characterized by powder X-ray diffractometry and differential scanning calorimetry.

Powder X-ray diffraction patterns were carried out with a Philips X-ray diffractometer (Panalytical 1830 BASED) using Cu-K_α radiation.

Thermal analysis was performed using a Perkin Elmer differential scanning calorimeter equipped with a computerized data station (scanning rate 10 °C min⁻¹).

Dissolution rate studies: Intrinsic dissolution rate study of KET and the prepared complexes was performed in 100 mL of 0.1 M phosphate buffer of pH 5 and 6 as a dissolution medium at 37 ± 0.5 °C for 3 h. The samples, corresponding to 30 mg of KET, was placed in the dissolution medium and magnetically stirred at 200 rpm. The concentration of the drug in solution at various time intervals was analyzed by HPLC at 260 nm. All dissolution studies were carried out in triplicate.

RESULTS AND DISCUSSION

Extraction of fulvic acid from shilajit: The extraction of shilajit with solvent of graded polarity *viz.*, chloroform, ethyl acetate and methanol removed the fatty acids, benzoic acid and hydroxy benzoic acid (chloroform soluble), benzo-pyrones, triterpenes and sterols (ethyl acetate soluble) and α-amino acids, ellagic acid, *etc.* (methanol soluble) components¹¹. Finally, the acidic treatment of extracted shilajit lead to separation of humic acid, which was collected, dried in oven at 100 °C for 2 h and pulverized. The

yield of humic acid was 8.0 %. The ion-exchange treatment with Tulsion ADS-400 and Indion 225 H extracted the fulvic acid from the acidic filtrate. The freeze-drying of the fulvic acid solution gave the amorphous fulvic acid. The yield of fulvic acid was found to be 14 %.

Interaction between KET and fulvic acid in aqueous media: Phase solubility profile of KET-fulvic acid is shown in Fig. 1. It was observed that solubility of KET at pH 5 and 6 was increased significantly by fulvic acid at concentration greater than 0.3 % w/v. Gaffaney *et al.*¹² reported that fulvic acid extracted from soil forms micelles in aqueous solution at 0.7-0.8 % w/v concentration, which further supports and suggest that the increase in solubility of KET in presence of fulvic acid is because of micelle formation.

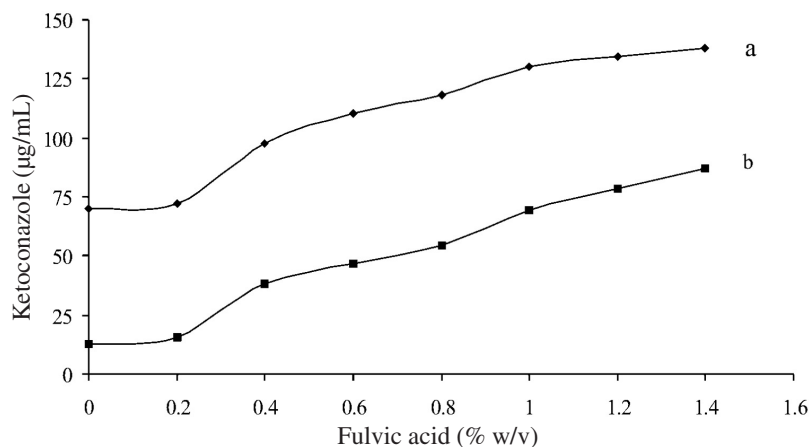


Fig. 1. Phase solubility diagram of ketoconazole and fulvic acid in buffer of pH 5(a) and pH 6(b) at 25 °C

Characterization of solid complexes: The complexes were characterized by powder X-ray diffractometry and differential scanning calorimetry.

The X-ray diffractogram of KET and prepared complexes are shown in Fig. 2. The diffraction pattern of 1:0.5 and 1:1 physical mixture corresponds to the superimposed diffractogram of KET and fulvic acid, while those of spray dried and solvent evaporated complex prepared in different molar ratio shows absence of peaks suggesting formation of complexation between KET and fulvic acid.

Fig. 3 shows the DSC thermograms of the KET and the prepared complexes. The DSC thermogram of 1:0.5 and 1:1 physical mixture were superimposed as that of fulvic acid and KET and showed one characteristics

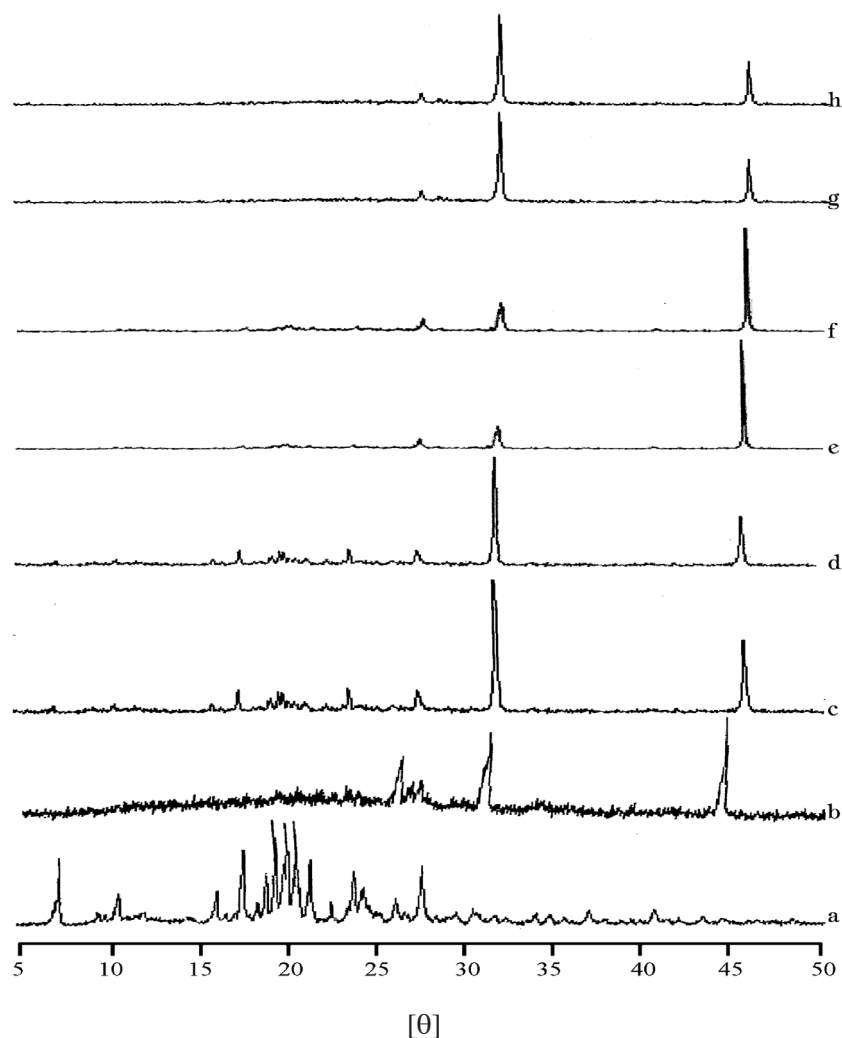


Fig. 2. Powder X-ray diffractogram pattern of different KET-fulvic acid system: (a) KET; (b) Fulvic acid; (c) KET-fulvic acid (1:0.5) physical mixture; (d) KET-fulvic acid (1:1) physical mixture; (e) KET-fulvic acid (1:0.5) solvent evaporated complex; (f) KET-fulvic acid (1:0.5) spray dried complex; (g) KET-fulvic acid (1:1) solvent evaporated complex and (h) KET-fulvic acid (1:1) spray dried complex

endothermic peak at 151°C (due to melting point of KET). However, this peak disappeared in the complexes prepared by solvent evaporation and spray drying methods. This suggests the formation of complex between KET with fulvic acid.

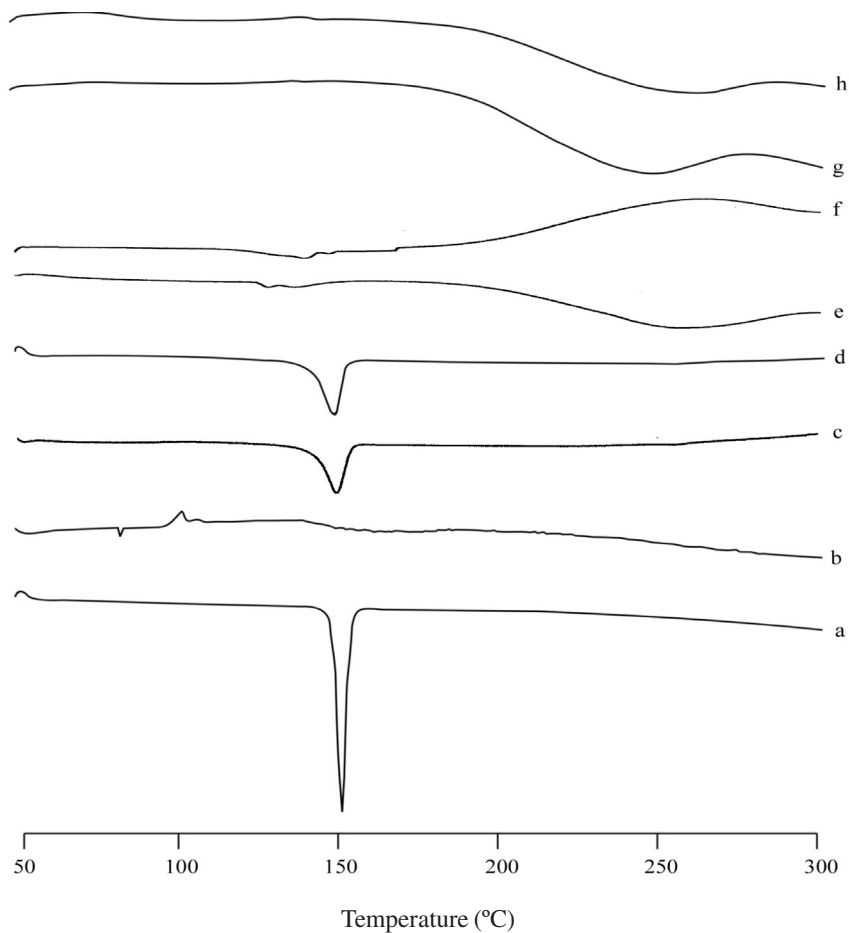


Fig. 3. Differential scanning calorimetry of different KET-fulvic acid system: (a) KET; (b) Fulvic acid; (c) KET-fulvic acid (1:0.5) physical mixture; (d) KET-fulvic acid (1:1) physical mixture; (e) KET-fulvic acid (1:0.5) solvent evaporated complex; (f) KET-fulvic acid (1:0.5) spray dried complex; (g) KET-fulvic acid (1:1) solvent evaporated complex and (h) KET-fulvic acid (1:1) spray dried complex

Effect of complexation on the dissolution of the drug: Figs. 4-7 illustrate the dissolution profile of KET and its complexes with fulvic acid prepared in different molar ratio by various methods in buffer solutions of pH 5 and pH 6.0. The Sheff's test (F-test) was used for comparing the dissolution efficiency of the prepared formulations at both pH 5 and 6. The Sheff's test grouped the formulation as

(A) KET; KET-fulvic acid (1:0.5) physical mixture; KET-fulvic acid (1:0.5) solvent evaporated complex; KET-fulvic acid (1:0.5) spray dried

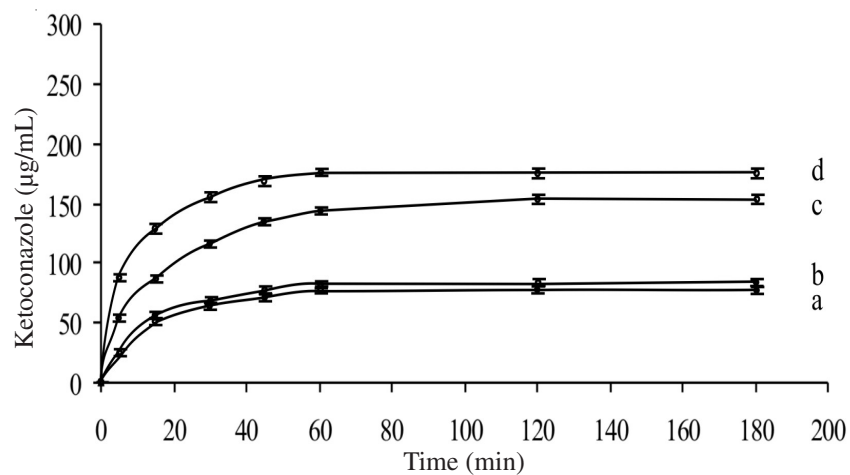


Fig. 4. Dissolution profile of ketoconazole and its 1:0.5 complexes at pH 5.0: (a) KET; (b) KET-fulvic acid (1:0.5) physical mixture; (c) KET-fulvic acid (1:0.5) solvent evaporated complex and (d) KET-fulvic acid (1:0.5) spray dried complex

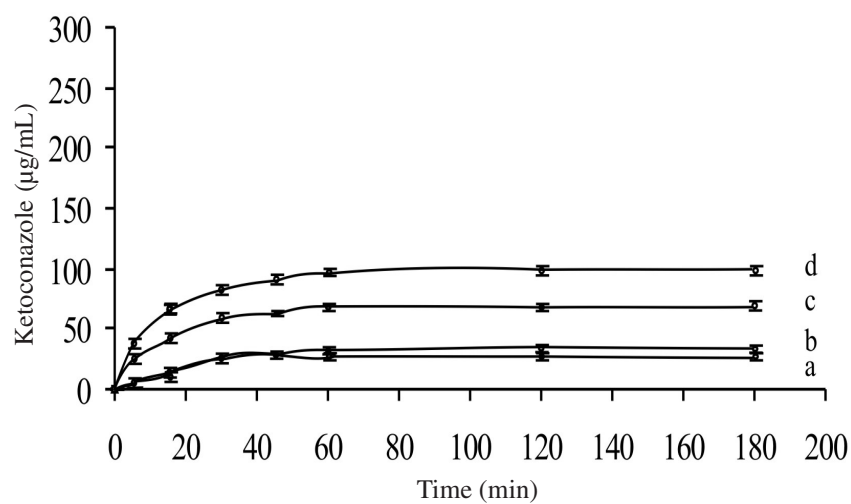


Fig. 5. Dissolution profile of ketoconazole and its 1:0.5 complexes at pH 6.0: (a) KET; (b) KET-fulvic acid (1:0.5) physical mixture; (c) KET-fulvic acid (1:0.5) solvent evaporated complex and (d) KET-fulvic acid (1:0.5) spray dried complex

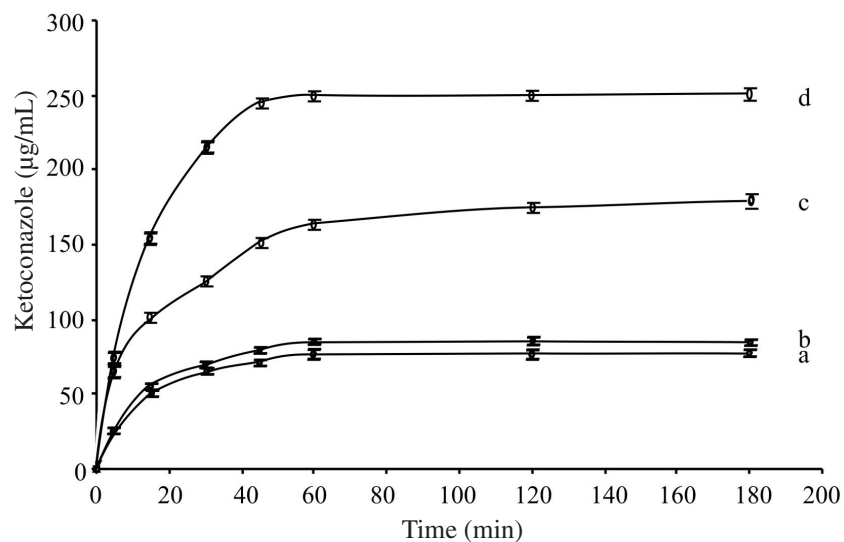


Fig. 6. Dissolution profile of ketoconazole and its 1:1 complexes at pH 5.0: (a) KET; (b) KET-fulvic acid (1:1) physical mixture; (c) KET-fulvic acid (1:1) solvent evaporated complex and (d) KET-fulvic acid (1:1) spray dried complex

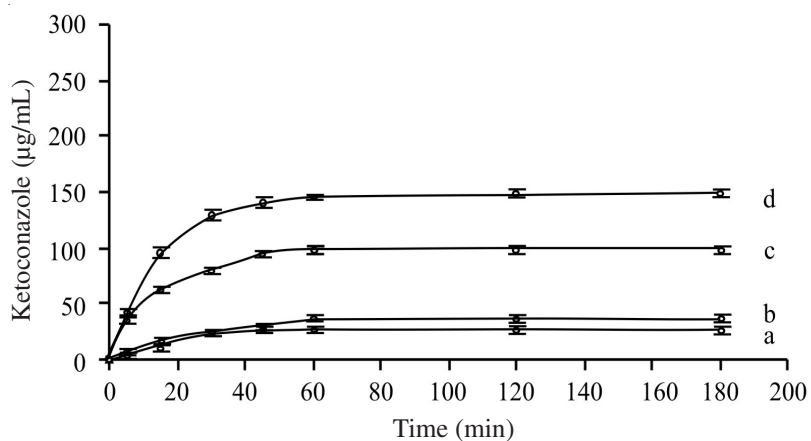


Fig. 7. Dissolution profile of ketoconazole and its 1:1 complexes at pH 6.0: (a) KET; (b) KET-fulvic acid (1:1) physical mixture; (c) KET-fulvic acid (1:1) solvent evaporated complex and (d) KET-fulvic acid (1:1) spray dried complex

complex; and (B) KET; KET-fulvic acid (1:1) physical mixture; KET-fulvic acid (1:1) solvent evaporated complex; KET-fulvic acid (1:1) spray dried complex.

One-way analysis of variance (Anova) in dissolution efficiency (0-180 min) reveals significant difference between the different formulations of KET. The dissolution efficiency for KET-fulvic acid (1:0.5) complexes were $F_{(3,24)} = 5.07$ and $F_{(3,24)} = 24.32$ for pH 5 and pH 6.0, respectively at a 5 % level of significance. Similarly for KET-fulvic acid (1:1) complexes the calculated values were $F_{(3,24)} = 17.69$ and $F_{(3,24)} = 35.69$ for pH 5 and pH 6.0, respectively at a 5 % level of significance. The F-test calculated values for the KET formulations at 5 % level of significance in both the dissolution media are much higher than tabulated value ($F_{(3,24)} = 3.008$), which suggest and supports that the dissolution efficiency of the prepared complexes is much higher as compare to KET alone.

Conclusion

Complex of KET-fulvic acid at a molar ratio of 1:0.5 and 1:1 can be obtained by spray drying and solvent evaporation method. The intrinsic dissolution rate studies showed that the dissolution of KET from KET-fulvic acid physical mixture prepared in a molar ratio of 1:0.5 and 1:1 was almost similar to KET alone but the complexes prepared by solvent evaporation and spray drying method exhibited a marked increase in the dissolution rate of KET. Further, the spray dried complexes showed better dissolution rate as compared to solvent evaporated complexes, which may be ascribed to the amorphous nature of the spray dried complex. The dissolution profiles in buffer solution of pH 5 and 6 are almost similar except that higher dissolution rate was observed in buffer solution of pH 5 which is most probably due to ionization of drug at lower pH. The study proved that fulvic acid has a potential to increase the dissolution rate of a poorly water-soluble drug(s) and hence could be used as a bioavailability enhancer.

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(Received: 4 November 2006; Accepted: 27 September 2007) AJC-5911