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COMPARATIVE STUDY ON INCLUSION COMPLEXATION OF HEPTAKIS (2,6-di-O-METHYL)-β-CYCLODEXTRIN, β-CYCLODEXTRIN AND γ-CYCLODEXTRIN WITH KETOCONAZOLE IN AQUEOUS SOLUTION AND SOLID STATE.

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ABSTRACT

The interaction of Ketoconazole with three kinds of cyclodextrins, β-, γ- and dimethyl β-cyclodextrin in aqueous solution and solid state was investigated to improve solubility and dissolution rate of the drug. For β- and dimethyl-β-cyclodextrin, drug solubility increased linearly as a function of cyclodextrin concentration and the solubility curve could be classified as Higuchi A_L type which indicated the formation of soluble complexes. Complexation with γ-cyclodextrin, however, yielded a B_S type solubility curve. The solid complexes in all cases were obtained in a molar ratio of 1:1. The inclusion complexes of the drug with the three Cyclodextrins were investigated using DSC and powder X-ray diffractometry and their dissolution behaviour were examined. The dissolution rate of Ketoconazole-dimethyl-β-cyclodextrin complex was significantly faster than other complexes due to its high aqueous solubility and amorphous structure. It was concluded that cyclodextrins may be used to enhance the solubility and dissolution rate of Ketoconazole in water.

INTRODUCTION

There has been increasing interest in optimizing the efficacy of drug activity through the use of rationally designed drug carrier materials. Cyclodextrins (CyDs) are considered novel candidates for such purpose [1-3]. CyDs are cyclic oligosaccharides consisting of 6-8 glucopyranose units. The α -1, 4- glycosidic linkage of the glucose units results in the formation of torus-like molecules with a polar outer surface and a non polar interior cavity [4]. As a consequence of this non polar cavity, the CyDs are able to form inclusion complexes with a variety of guest molecules[5-7]. CyDs offer several advantages in drug delivery including improved drug solubility and protection against physico-chemical and enzymatic degradation, as well as the potential for enhanced absorption afforded by direct interaction of the CyDs with membrane component such as cholesterol, which might induce changes in fluidity and permeability [8].

Recently, many kinds of CyD derivatives have been prepared to extend the physicochemical and inclusion properties of host molecules[9,10]. For example, more water soluble derivatives of CyDs such as glucosyl CyD, di-o-methylβ-CyD, trimethylβ-CyD, hydroxyethylβ-CyD and hydroxypropylβ-CyD were recently prepared and investigated[11-16]. Different methods for the preparation of solid inclusion complexes including crystallization, co-precipitation, evaporation under vacuum, freeze drying, kneading, grinding and spray drying were applied in the preceeding literature.

Ketoconazole (*cis*-1- acetyl-4-[4-[[2- (2,4-dichlorophenyl) -2 (1H- imidazole = 1ylmethyl)-1,3-dioxolan-4-yl]phenyl]piperazine, is one of the antimycotic imidazole derivatives suitable for the treatment of candidiasis and other systemic fungal infections. However, this drug is a weak base with pKa values of 2.94 and 6.51, with very low aqueous solubility because of its hydrophobic structure and it can be solubilized only under extremely acidic conditions[17]. Accordingly, it is less absorbed in patients with achlorhydria.

The aim of the present study is to compare the inclusion behaviour of a chemically modified β-cyclodextrin (dimethyl-β-CyD) with natural β- and γ-CyD in solution and solid state with Ketoconazole. The dissolution characteristics of the resulting inclusion complexes were also investigated.

MATERIALS AND METHODS

Materials:

Ketoconazole (Janssen, Germany), β- , γ- and 2,6-dimethyl-β-
cyclodextrin (Nihon Shokuhin Kako Co. Ltd, Tokyo, Japan), were used as received and their particle size was 150-180 um. All other materials and solvents were of analytical grade., and deionized double distilled water was used throughout the study.

Solubility studies:

Phase-solubility studies were performed according to the method reported by Higuchi&Connors[18]. Excess amounts of Ketoconazole were weighed into 20 ml vials, to which 10 ml of deionized double distilled water containing various concentrations of cyclodextrins were added. With β-cyclodextrin, the study was carried out up to its maximum solubility (1.8% W/V), meanwhile with other CyDs it was carried out in the concentrations of (0.0-10% W/V). The securely capped vials were placed in a water bath and shaken continuously at a constant temperature (25 ±0.5 °C) until equilibrium was reached (approx.7 days). Each sample was centrifuged at 3000 r.p.m. for 10 minutes. Aliquots were withdrawn and filtered through a 0.2 um membrane filter (SM-66, Sartorius, Gottingen, Germany). The filtered solutions were analyzed for the total amount of ketoconazole dissolved.

Preparation of solid complexes:

Three methods were used for the preparation of the solid complexes. They were: co-grinding, evaporation under vacuum and kneading.

Co-grinding:

Mixtures of Ketoconazole and CyDs in equimolar ratios were ground for 40 minutes using an automatic laboratory vibratory ball mill which was made of tungsten carbide.

Coprecipitation with Evaporation:

The clear filtered solutions of equimolar mixtures of Ketoconazole with CyDs in water were evaporated under vacuum to dryness at 50 °C using a rotary evaporator (Buchi Rotavapor, Switzerland). The product was dried under vacuum over phosphorous-pentoxide (P_2O_5).

Kneading:

The drug was keanded with CyDs in equimolar ratios with a small amount of water for 1 hour. The paste thus obtained was dried under vacuum at room temperature for 4 days.

IR spectroscopy:

IR spectra were obtained using a Unicam SP 1000 (Pye Unicam, GB-Cambrige) with potassium bromide pellets. All determinations were performed with freshly prepared complexes directly after drying.

Differential Scanning Calorimetry:

A Perkin Elmer DSC-7 Differential Scanning Calorimeter was used. It was equipped with a Perkin Elmer 7700 professional computer, TAC 7 Instrument Controller; Perkin Elmer multitasking software and a Hewlett-Packard x-y plotter.

Indium was used as the standard and thermograms were obtained by heating at a constant rate of 10 °C/min and nitrogen was used as the purging gas for all determinations. Samples (5-8 mg) were weighed directly into an aluminum sample pans. An aluminum cover was placed on the sample and thermetically sealed. The individual substances and the prepared systems were heated over a temperature range of 30 to 230 °C.

X-ray powder diffraction analysis:

X-ray powder diffraction patterns were recorded by Stoe Powder Diffractometer, over 2 θ angle from 0 to 45 degrees.. The conditions were; Ni-filtered Co-radiation, voltage 42 kv, current 220 mA, scanning speed 2 degrees/minute.

Dissolution studies:

A USP (XXIII) paddle apparatus was used at 100 r.p.m. Dissolution medium was 500 ml of distilled water at 37 ± 0.5 °C. 100 mg of KET or an equivalent amount (containing 100 mg of KET) of the mixture or the complex under investigation were introduced into each of the dissolution flasks. Samples, 2 ml, were withdrawn at appropriate time intervals. Samples were suitably diluted and assayed spectrophotometrically for their contents of KET at 225 nm. Each experiment was performed at least three times and the mean was calculated in each case.

RESULTS AND DISCUSSION

Phase solubility studies:

The phase solubility diagrams of KET with CyDs obtained at 25 °C in water are shown in Fig. 1.

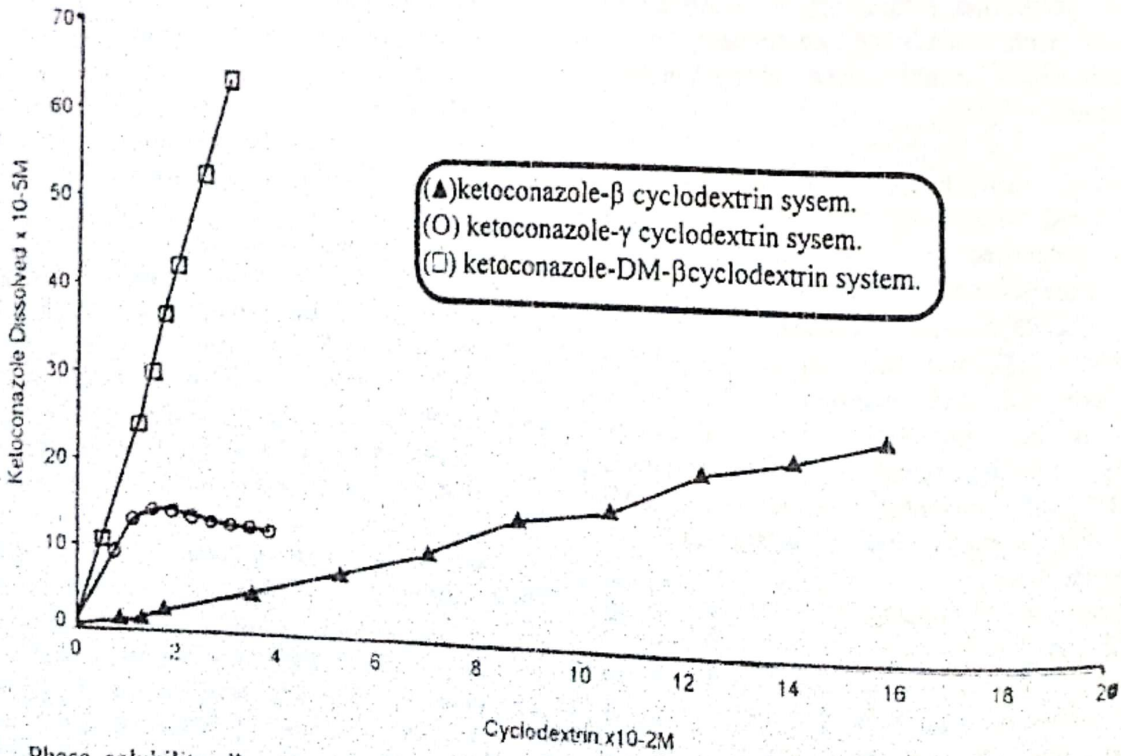

Fig (1): Phase solubility diagrams of ketoconazole-cyclodextrin systems in water at 25 °C. Keloconazole-β-cyclodextrin system.

The solubility of KET was increased with increasing concentrations of β-CyD and DM β-CyD showing the features of an A_L type solubility diagram, with no indication of limiting solubility of the complex; however, due to the limited solubility of β-CyD in water (18 g/l at 25 ?C) concentrations beyond 1.8 % can not be studied. However, the solubility curve in the case of γ-CyD, was a B_S type with the precipitation of microcrystalline KET-CD complex at high γ-CD concentrations. This precipitation started at a concentration of approximately 1.5 % γ-CyD. The apparent 1:1 stability constants ($K_{1:1}$) for KET with β-, γ- and DM-β-CyD were calculated as 439, 158 and 317 M^{-1}, respectively. This solubility enhancement is considered to be mainly due to the formation of inclusion complexes[18].

The solubility and stability constant obtained with DM-β-CyD were much greater than those obtained with the parent β-CyD and γ-CyD. This may be attributed to the different physicochemical properties of the derivative DM-β-CyD, such as its highest aqueous solubility and adequate cavity size as compared with natural CyDs[10]. The formation of 1:1 complex was assumed to take place and the mixtures of KET with CyDs were prepared hereafter according to their equimolar ratio.

Complexation in the solid state:

To examine the interaction of KET with cyclodextrins in the solid state, IR spectroscopy, X-ray diffractometry and DSC were employed to compare the corresponding physical mixtures in a 1:1 molar ratio. Table 1 shows the characteristic IR spectroscopic absorption bands of physical mixtures of KET and CyDs in their equimolar ratio as well as those of solid complexes prepared by different methods. The band corresponding to the carbonyl group appeared at 1660 cm^{-1} (C=O stretching of ketone) was shifted to lower value 1580 cm^{-1} in case of solid complex prepared by coprecipitation under vacuum and kneading. This could be attributed to the hydrogen bonding between KET and CyDs.

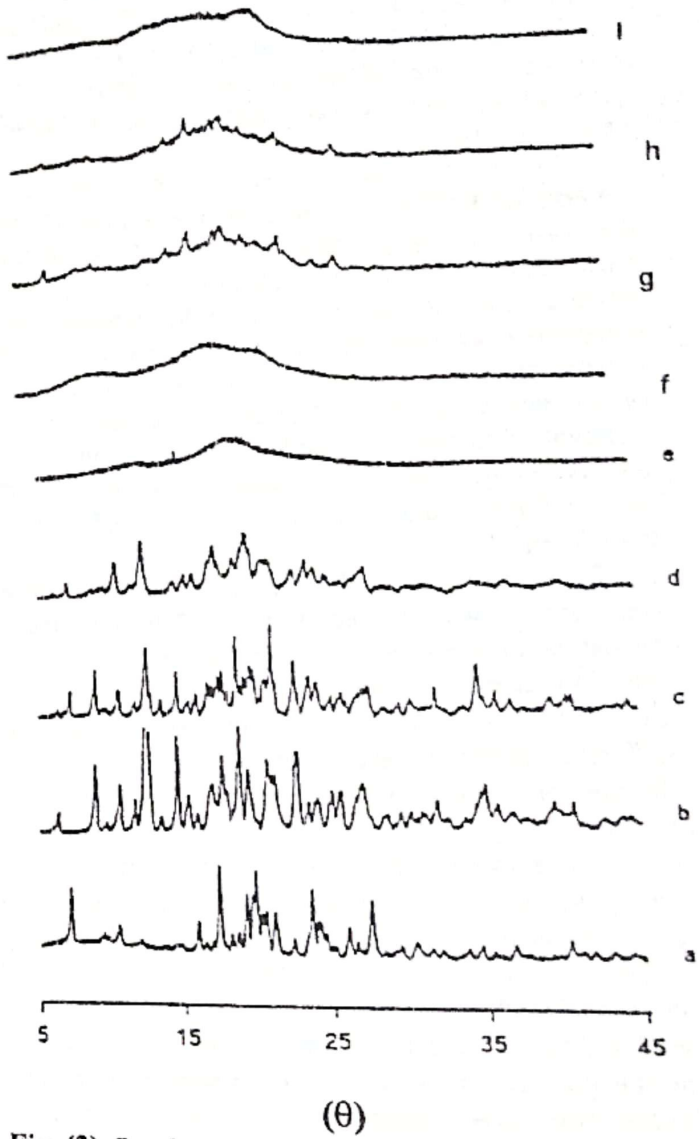

Fig. (2): Powder X-ray diffraction patterns of Ketoconazole-CD physical mixtures an dinclusion complexes. a: KET; b: βCD; c:" KET-βCD physical mixture; d: kneaded KET-βCD complex; e: evaporated KET-βCD com,plex; f: DM-βCD; g: KET-DM-βCD physical mixture; h: kneaded KET-DM-βCD complex; I: evaporated KET-DM-βCD complex.

Table 1: The IR absorption (nm) of the characteristic bands of Ketoconazole-CDs physical mixtures and solid inclusion complexes.

	KET-β CD PM	KET-DM β-CD PM	KET-β CD GM	KET-β CD EVAP	KET-γ CD PM	KET-γ CD GM	KET-DM CD EVAP	KET-γ CD EVAP
C=O Ketone	1660	1650	1650	1580	1650	1650	1580	1580

PM: Physical Mixture , GM: Ground Mixture , EVAP: Evaporated under vacuum

On the other hand, co-grinding of KET with CyDs showed very little shift which may indicate that the effect of hydrogen bonding between the drug and CyDs was compensated by the dissociation of intermolecular bonds between KET molecules[15]. This may also indicate that the inclusion of KET in CyDs was not complete in case of co-grinding [20, 21]. In contrast, the physical mixtures showed no appreciable spectral change in all cases.

Fig. 2 shows the powder X-ray diffraction patterns of KET-CyDs complexes in comparison with the corresponding physical mixtures at the same molar ratio. The diffractometry patterns of the physical mixtures are the superposition of the patterns of each component whereas, the amorphous character of the complexes were obvious and indicated the formation of a new solid phase. The complexes prepared by different methods exhibited hollow patterns in which it was no longer possible to distinguish the characteristic peaks of KET suggesting the formation of inclusion complexes[19].

The thermal behavior of CyDs inclusion complexes was studied using DSC to confirm the formation of the solid complexes. The thermograms of the physical mixtures of the KET and β-CyDs as well as those of the solid binary complexes prepared by different methods are shown in Fig.3 . The thermograms show two endothermic peaks at 100°C (corresponding to the loss of the water content of β-CyDs) and 149°C (due to melting of the drug) and for the physical mixtures and the kneaded systems this peak shows a slight change. The fact that the peak of these systems changed re ative to that of the pure drug showed that there was a weak interaction[22]. Besides, the KET fusion appeared to be more definite in the case of the physical mixture than for the kneaded complex. These two peaks disappeared in case of complex prepared by evaporation under vacuum which indicates the formation of an amorphous complex between β-CyDs and KET. These results indicate that the complexes obtained by the kneading method did not seem to be a true inclusion. However tie encapsulation of the drug within the β-CyDs can be achieved by evaporation under vacuum.

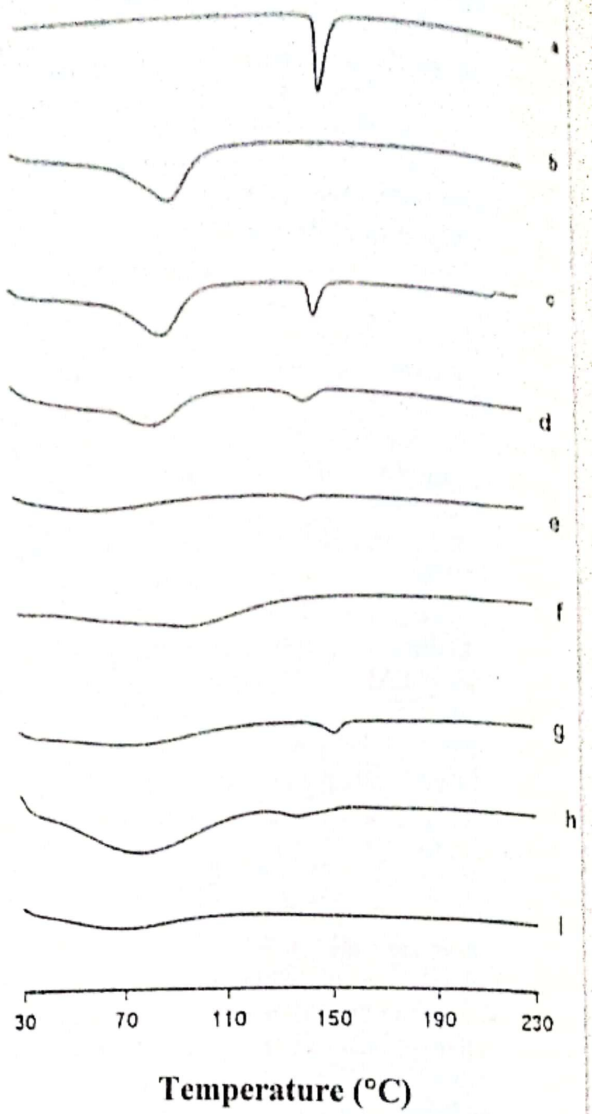

Fig. (3): DSC thermograms of Ketoconazole - CD physical mixtures and inclusion complexes. a: KET: b: βCD; c: KET=βCD physical mixture; d: kneaded KET-βCD complex; e: evaporated KET-βCD comple; f: DM-βCD; g: KET - DM-βCD physical mixture;h: kneaded KET -DM-βCD complex; I: evaporated KET-DM-βCD complex.

Dissolution studies:

Figures (4-6) show the dissolution profiles of KET, KET-CyDs physical mixtures, ground mixtures and KET-CyDs solid complexes. The dissolution results obtained in water show that the presence of CyDs leads to an improvement in the solubility of KET. A very rapid dissolution of KET-CyDs complexes occurred. For the KET-DM-β-CyD systems, at 20 minutes, more than 74 % of the drug is dissolved from the complex prepared by evaporation under vaccum compared with 47 % from the kneaded compounds or 30 % from the ground mixture. For the KET-β-CD systems, 64.6 % of KET is solubilized from the evaporated complex, from the kneaded system 31 % and from the ground mixture 29 % at the same time. For the KET-γ CD systems, only 37 % of KET is dissoluted from the evaporated complex, and from the kneaded system 27% and ground mixtures 32 % This enhanced dissolution could be attributed to the reduction in particle size of the drug, the amorphous state, the increased wettability and the inclusion complexation[22]. It is important to mention that simple size reduction of drugs leads to a limited improvement in the dissolution characteristics and sometimes it leads to impaired dissolution characteristics due to the induced aggregation tendency (ss, ss). On the other hand, physical mixtures gave rise to somewhat better dissolution profiles than in case of simple KET. This is due to the better dissociation and less aggregation in that case.

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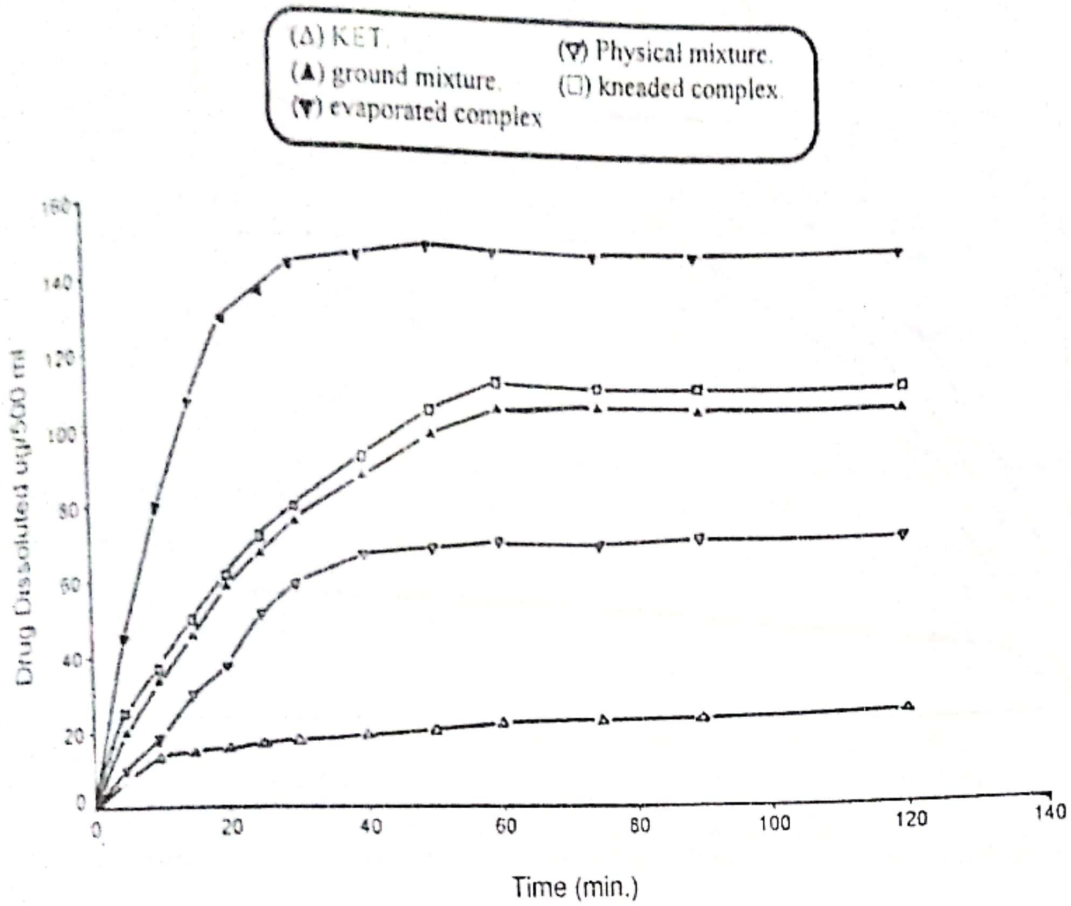

Fig. (4): Dissolution profiles of KET and its inclusion complexes with β-cyclodextrin

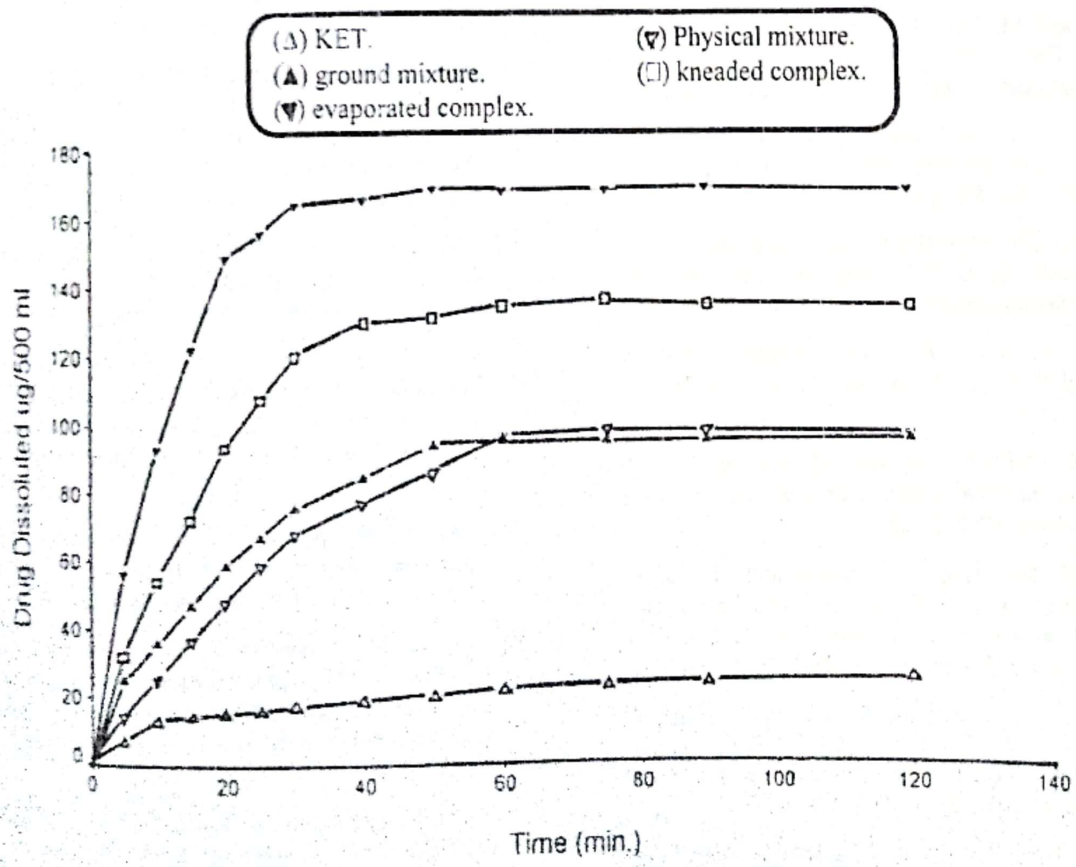

Fig. (5): Dissolution profiles of KET and its inclusion complexes with DM-β-cyclodextrin

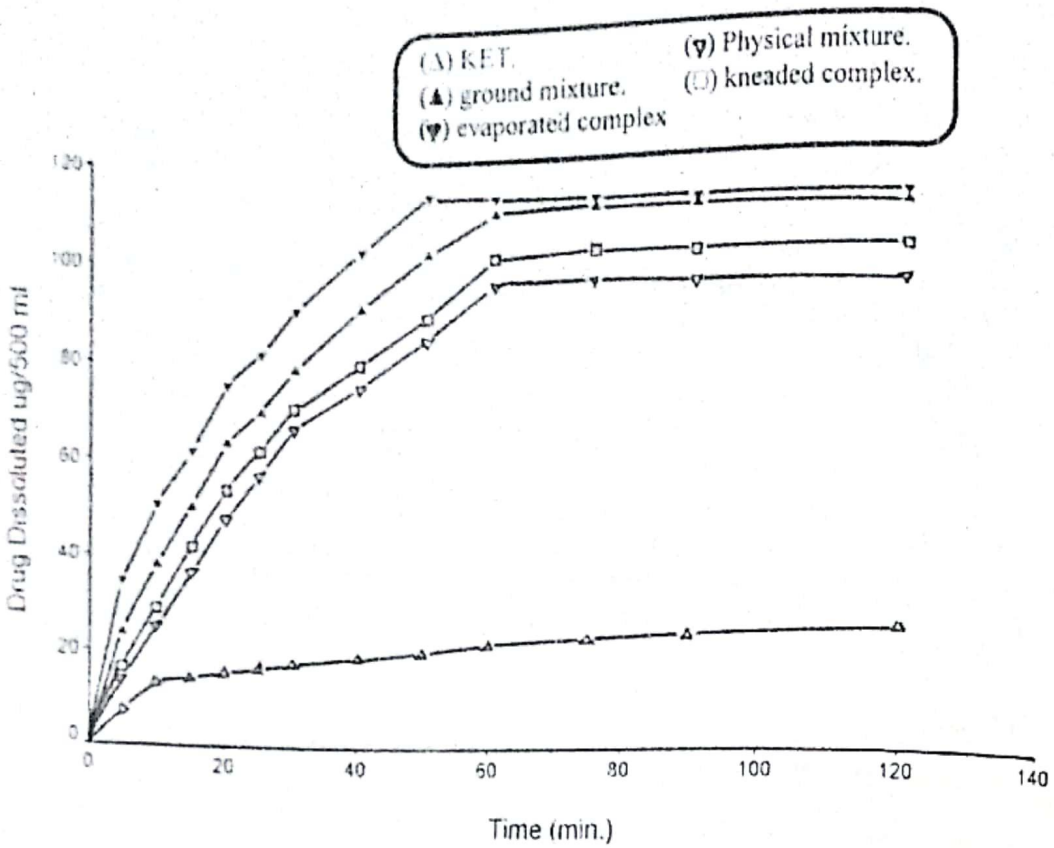

Fig. (6): Dissolution profiles of KET and its inclusion complexes with γ-cyclodextrin

REFERENCES

1. Szejtli, "Cyclodextrin Technology", Kluwer Academic Publishers, Dordrecht, The Netherlands, (1988).
2. Uekama and M. Otagiri, Cyclodextrins in drug carrier systems, CRC Critical Reviews in "Therapeutic Drug Carrier Systems", CRC Press Inc., Boca Raton, 1, 1-40 (1987).
3. Duchene, Cyclodextrins and Their Industrial Uses, Editions de Sante, Paris, (1991).
4. Szejtli, "Chemistry, characterization and properties of cyclodextrins". In D. D. Breimer, and P. Speiser (Eds.), Topics in Pharmaceutical Sciences, p. 151 (1987).
5. Saenger, "Cyclodextrin inclusion compounds in research and industry". Angew. Chem. Int. Ed. Engl., 19, 344-462 (1980).
6. Szejtli, E. Bolla-Pusztei and M. Kajatar, The β-cyclodextrin inclusion complex of menadione (vitamin K3). **Pharmazie.** 37, 725-728 (1982).
7. Uekama, S. Narisawa, F. Hirayama and M. Otagiri, Improvement of dissolution and absorption characteristics of benzodiazepines by cyclodextrin copmlexation. **Int. J. Pharm.**, 16, 327-338 (1983).
8. Irie, M. Otagiri, M. Sunada, K. Uekama, Y. Ohtani, Y. Yamada and Y. Sugiyama, Cyclodextrin induced hemolysis and shape changes of human erythrocytes in vitro. **J. Pharm. Dyn.**, 5, 741-744 (1982).
9. Duchene, "New Trends in Cylodextrins and Their Derivatives", Editions de Sante, Paris, 1991.
10. Uekama, F. Hirayama and T. Irie, "Pharmaceutical uses of cyclodextrin derivatives", in : M. Szycher (Ed.), High Performance Biomaterials, A Comperhensive Guide to Medical and Pharmaceutical Applications, Technomic Publishing Co. Inc., PA, , pp. 789-806, (1991).
11. A. El-Gendy, K. Terada, K. Yamamoto and Y. Nakai, Molecular behaviour, dissolution characteristics and chemical stability of aspirin in the ground mixture and the inclusion complex with di-O-methylβ-cyclodextrin. **Int. J. Pharm.** 31, 25-31 (1986).
12. Koizumi, Y. Okada, Y.Kubata and T. Utamura. Inclusion complexes of poorly water-soluble drugs with glucosyl-cyclodextrins. **Chem. Pharm. Bull.**, 35, 3413-3418 (1987).
13. Yamamoto, A. Yoshida, F. Hirayma, and K.Uekama. Some physicochemical properties of branched β-cyclodextrin and their inclusion characteristics. **Int. J. Pharm.**, 49, 163-171 (1989).
14. Yoshida, H. Arima, K. Uekama and J. Pitha. Pharmaceutical evaluation of hydroxyalkyl ethers of β-cyclodextrins. **Int. J. Pharm.**, 46, 217-222 (1988).
15. Yoshida, M. Yamamoto, T. Irie, F. Hirayama and K. Uekama. Some pharmaceutical properties of 3-hydroxypropyl and 2,3-hydroxypropylβ-cyclodextrins and their solubilizing and stabilizing abilities. **Chem. Pharm. Bull.**, 37, 1059-1063 (1989).
16. Nakai, K. Yamamoto, K. Terada, T. Oguchi, H. Saito and D. Watanabe. New methods for preparing cyclodextrin inclusion compounds: II. Effects of heating temperature, water content and drug properties on the inclusion formation. **Chem. Pharm. Bull.**, 37, 1055-1058 (1989).

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Vol. 7, No. 1, pp. 87-93

17. W. M. Van der Meer, J. J. Keunning, H. W. Scheijgrond, J. Heykants, J. Van Custem and J. Brugmans. The influence of gastric acidon the bioavailability of ketoconazole. **J. Antimicrob. Chemother.**, 6, 552-554 (1980).

18. Higuchi and K. A. Connors. Phase solubility techniques. **Adv. Anal. Chem. Instrum.**, 4, 117-212 (1965).

19. Uekama, T. Fujinaga, F. Hirayama, M. Otagiri and M. Yamasaki. Inclusion complexation of steroid hormones with cyclodextrins in water and in solid phase. **Int. J. Pharm.**, 10, 1-15 (1982).

20. Nakai. Molecular behaviour of medicinals in ground mixtures with microcrystalline cellulose and cyclodextrins. **Drug Dev. Ind. Pharm.**, 12, 1017-1039 (1986).

21. Oguchi, K. Terada, K. Yamamoto and Y. Nakai, Molecular state of methyl p-hydroxybenzoate in the solid dispersion prepared by grinding with α-cyclodextrin. **Chem. Pharm. Bull.**, 37, 1886-1889 (1989).

22. Vormans, A. C. Eissens and C. F. Lerk, Mechanism of dissolution of drug-cyclodextrin complexes; A pragmatic approach. **Acta Pharm. Technol.**, 35, 250-255 (1989).

23. I. Saleh, S. M. Ahmed and J. M. Aiache. Improvement of dissolution behaviour of temazepam. **Bull. Pharm Sci.Assiut Unversity**, 14, 95-102. (1991).

24. S. I. Saleh, S. M. Ahmed and A. E. Aboutaleb, Dissolution behaviour of cinnarizine from its ground mixture with microcrystalline cellulose. **S.T.P. Pharma**, 5, 745-749 (1989).

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المتراكب الأحتوائى لعقار الكيتوكونازول مع البيتاسيكلوديكسترين والجاماسيكلوديكسترين والدايمثيل ـبيتاسيكلوديكسترين فى المحاليل المائيه وفىالحاله الصلبه

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تمت دراسة المتراكب الاحتوائي لعقار الكيتوكونازول مع البيتاسيكلوديكسترين والجاماسيكلوديكسترين والدايمثيل –بيتاسيكلوديكسترين في المحاليل المائية وفىالحالة الصلبة

بواسطة رسوم أطوار الذوبان البيانية ، ومطياف الأشعة تحت الحمراء وأنماط الحيود الضوئي وكذال بواسطة التحليل باستخدام الأشعة السينية وقد تم تحضير مركبات الكيتوكونازول – سيكلوديكسترين المشتملة بطرق عديدة منها التبخير تحت ضغط مخلخل والطحن المشترك وقد تحسنت ذوبانية الكيتوكونازول المتراكب بشكل كبير مقارنا الكيتوكونازول أو المزيج منه مع البيتاسيكلوديكسترين والجاماسيكلوديكسترين والدايمثيل –بيتاسيكلوديكسترين بنفس النسبة.