Multicomponent Systems as Potential Economic Approach for Enhancing the Therapeutic Characteristics of an Antimycotic Drug

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Abstract:

***Purpose*:** The poor aqueous solubility of miconazole nitrate (MN), a broad spectrum antimycotic drug of interest, limited its dissolution, therapeutic efficiency and pharmaceutical applications. The current work aimed to widening pharmaceutical applications of MN and potentiating its therapeutic characteristics through the concept of multicomponent systems. Further, the work aimed to enhance the solubilizing and complexing power of β-cyclodextrin (β-CD) to reduce its amount required for MN solubilization.

***Methods*:** Multicomponent systems of MN with β-CD in association with polyvinyl pyrrlidone (PVP-k90), (MN/β-CD/PVP-k90), prepared by physical mixing, kneading and grinding method were characterized for their physicochemical, dissolution and antimycotic characteristics and comparatively evaluated by their corresponding binary systems (MN/β-CD). Phase solubility studies of different MN systems were also carried out.

***Results*:** The marked enhancement in MN solubilization and stability constants values of MN/β-CD/PVP-k90 systems clearly proved the benefit of PVP-k90 addition to increase the solubilization and complexation efficiency of β-CD toward MN. The results also displayed a progress reduction in MN crystallinity and a highly degree of amorphousity offered by MN/β-CD/PVP-k90 and MN/β-CD systems prepared by grinding method rather than kneading one. The extent of enhancement in the dissolution and antimycotic characteristics of MN displayed by its MN/β-CD/PVP-k90 systems was found to be higher than that of its corresponding MN/β-CD systems and increased by increasing PVP-k90 concentration to 5% w/w.

***Conclusion*:** The use of multicomponent systems of β-CD in association with PVP-k90 for solubilization of MN not only offer synergistic solubilization effects, but also it reduced the required amount of β-CD, which is good from the production economic strategy. The current work reflected the potential efficiency of multicomponent systems as an effectively economic approach for enhancing the therapeutic characteristics of MN.

Key words: Miconazole nitrate, β-CD, polyvinyl pyrrlidone**,** multicomponent systems and therapeutic characteristics.

1. Introduction:

Cyclodextrins (CDs) play an important role in the formulation development. CDs are able to form inclusion complexes with many of poorly water-soluble drugs and the physicochemical and biopharmaceutical characteristics of these drugs were largely improved [1-3]. For a series of reasons, (price, availability, cavity dimensions, etc…), the parent β-CD is the most widely used among the natural CDs [4]. β-CD forms complexes with many drugs by inclusion a whole drug molecule or only some nonpolar part of it inside their cavity and hence, the characteristics of guest molecules can be changed in this manner [4-6]. Unfortunately, the low aqueous solubility of β-CD, restricted its solubilizing and complexing power toward a drug and hence more amounts of β-CD are needed to solubilize small amounts of a poorly water-soluble drug which is more demand on the formulation bulk making the solubilization of a drug by β-CD is impractical and more cost from the production economic strategy [7-9].

The enhancement of the solubilization and complexation efficiency of β-CD is of a pharmaceutical importance in the recent literatures [2, 8]. Among the approaches have been applied to enhance the solubilization and complexation efficiency of β-CD, the approach based on multicomponent systems (blending of β-CD with small amounts of water-soluble polymers) has been proved to be the most promising one [8,9]. The use of multicomponent systems of β-CD in association with water-soluble polymers for solubilization of a drug, not only potentiate the solubilization and complexation power of β-CD toward a drug but also it offers synergistic

enhancement effects resulting in further improvement in the aqueous solubility and biopharmaceutical characteristics of the drug embedded into the multicomponent systems. These could be related to the formation of co-complexes between drug, cyclodextrin and polymer [8, 9].

MN is a broad spectrum antimycotic drug having powerful antimycotic activities against many of mycoses accompying some dangerous infections of the intersest in the recent years [10]. MN, a pooly water-soluble drug belonging to BCS Class Π, displays limited dissolution characteristics leading to irreproducible clinical response or some therapeutic defects [11]. Furthermore, the poor aqueous solubility of MN restricted its pharmaceutical application to the topical use [11].

This study investigated the potential of multicomponent system (MN/β-CD/PVP K-90) as an approach for enhancing the aqueous solubility and dissolution of MN. The impact of these effects on the antimycotic characteristics of MN prepared systems was also investigated. The results obtained were comparatively evaluated by that obtained by MN/β-CD binary systems.

2. Materials and methods:

2.1 Materials:

Miconazole nitrate (MN) was kindly supplied by Medical Union Pharmaceuticals company, Ismailia, Egypt. β-Cyclodextrin (β-CD) and Polyvinyl pyrrlidone (PVP-k90) obtained from Sigma Chemical Company, U.S.A. Methanol, ethanol and tween 80, all obtained from El-Nasr Company, Abu-Zabal, and Cairo, Egypt. Strains of Candida albicans (C. albicans) and a modified Sabouraud agar were gifted by Microbiology Department, Faculty of Pharmacy, Minia University, Minia, Egypt.

2.2 Solubility studies:

Phase solubility studies were carried according to Higuchi and Connors [12] method in distilled water at room temperature (25°C). Excess amounts of MN were added to stoppered flasks containing aqueous solutions of increasing amounts of βCD (0–15 mM) with or without PVP K-90 at 1% w/v. The flasks were mechanically shaken using thermostatic controlled water bath (Polyscience, USA) until equilibrium was achieved (48 h). Aliquots were drawn, filtered, and spectrophotometrically (UV/VIS spectrophotometer, Spectronic Genesys 2PC, USA) analyzed at 273 nm for their MN contents. The presence of β-CD and PVP K-90 did not interfere with the spectrophotometric assay of the drug. The apparent stability constant (Kc) and complexation efficiency (CE) were calculated from the slope of the linear plot of the phase solubility diagram according to the following equations:

$$Kc = slope/S_0 (1- slope) \quad \text{equation (1)}$$

$$CE = slope/ (1- slope) \quad \text{equation (2}$$

2.3 Preparation of MN solid systems:

Physical mixtures of MN/β-CD in equimolar ratio with or without PVP-k90, at concentration 2 and 5 % w/w, were prepared by simple mixing the appropriate amounts in a mortar for 5 min using spatula. Kneading systems were prepared by wetting the prepared physical mixtures using ethanol/water mixture (1:1) untill obtain dough homogeneous mass. The mass was dried in a vacuum oven at 40°C until complete drying. Ground systems were prepared by triturating the prepared physical mixtures in a glass mortar for 20 min. All the prepared systems were appropriately sieved and stored in a desiccator till used.

However, physical mixtures, kneading systems and ground systems were donated as Bin-PM, Bin-KS and Bin-GS for the binary system, as Trn-PM_1, Trn-KS_1 and Trn-GS_1 for the multicomponent system containing 2% w/w of PVP-k90, and as Trn-PM_2, Trn-KS_2 and TrnGS_2 for the multicomponent system containing 5% w/w of PVP-k90, respectively.

2.4 Drug contents:

A certain amount of MN solid systems was dissolved in 30 ml methanol, and then completed with distilled water to 100 ml. The contents were filtrated and assayed for their MN content spectrophotometrically at $\lambda_{max.}$ 273 nm. The average of triplicates was reported.

2.5 Differential Scanning Calorimetry (DSC):

DSC studies were carried out using DSC-shimadzu apparatus (DSC-50, Shimadzu Co., Japan). DSC technique was adjusted for purging of nitrogen at rate of 40 ml/min and heating rate at 10°C/min. The temperature scale was ranged from 30-300ºC, and the instrument was calibrated with indium as a standard.

2.6 X-ray diffractometry (XRD):

XRD studies were determined by Philips diffractometer (Philips 1710, Holland) using Cu α k radiation. A copper target tube operated at a voltage of 40 kV and a current of 40 mA. The scanning speed was 0.6/min and a wide angle diffraction of $4^o < 2\theta < 60^o$ were employed.

2.7 Dissolution studies:

The dissolution of MN and its different prepared systems were carried out using USP XXI-dissolution apparatus II (Hanson Research Corporation, California, USA). Samples of 100 mg of MN or its equivalent of its different prepared systems were introduced into 500 ml distilled water containing 0.02% w/v of Tween 80. The whole contents were stirred at rate of 100 rpm and maintained at 37 ± 0.5^oC. At predetermined time intervals, 5-ml sample of the dissolution medium was withdrawn and replaced by fresh 5-ml of the same dissolution medium under the investigation kept at 37 ± 0.5^oC. The dissolute (MN) was determined spectrophotometrically at $\lambda_{max.}$ 273 nm. The average of three determinations was taken.

2.8 Microbiological assay:

The microbiological assay (cup diffusion method) was carried out to evaluate the effect of the synergistic enhancement in the solubilizing and complexing power of β-CD toward MN in their MN/β-CD/PVP-k90 systems on the antimycotic activity of MN in comparing by that of the corresponding MN/β-CD systems. The aqueous solutions of MN/β-CD/PVP-k90 and MN/β-CD systems employed in solubility studies were used in this microbiological assay. The assay was carried out in a similar manner described by Pederson et al. [13]. A modified Sabouraud agar medium was freshly prepared and sterilized by autoclaving at 120°C for 1 hr. The indicator strains of C. albicans was grown for approximately for 48 hrs at 31°C, in a dish containing the Sabouraud agar medium. The strain of C. albicans was seeded to a concentration of 10^5 yeast cells per ml in the agar medium at 40-50°C. The seeded agar medium was poured in lots of 35ml into 14 cm-Petri dishes and six wells in each dish were cut, each 6 mm in diameter. 50 ul sample of each of the investigated solubility diagram solutions were placed in each wells according to randomization scheme. The dished were incubated at 32°C for 18 hrs before the diameters of inhibition zones were measured. The results reported are inhibition zone diameter minus well diameter (6mm). Each of the used solutions was applied twice and the average of the results of two applications was taken.

3. Results and Discussion:

3.1 Solubility studies:

As shown in Fig. (1), the Phase solubility diagram of either MN/β-CD or MN/β-CD/PVP-k90 systems, was found to be of Higuchi's A_L type: that means a linear increase in MN concentration was observed as a function of β-CD concentrations**.** The slopes of solubility diagrams of both binary and ternary systems were less than unity, thus confirming the formation of 1:1 complexes [14]. The values of Kc and CE of MN systems in absence and presence of 1% w/v PVP-k90 were listed in table (1). It is clearly that the presence of PVP-k90 resulted in a significant increase in Kc and CE values. This increase in Kc and CE of MN systems could be attributed to the increase in the complexing ability of β-CD towards MN via the formation of interactions through hydrophobic bonds, Van der Waals dispersion forces, hydrogen bonds and/or promoting the release of high-energy water molecules present in the cyclodextrin cavity [15]. The results reflected the superior performance of the multicomponent (ternary complex) system than binary system in promoting MN and β-CD interaction in solution state.

Table (1): The apparent stability constant (Kc) and complexation efficiency (CE) of MN/β-CD and MN/β-CD/PVP-k90 systems.

System	**Stability constant (Kc) (M^{-1})**	**complexation efficiency (CE)**
MN/β-CD	637.21	0.198
MN/β-CD/PVP-k90	1055.31	0.482

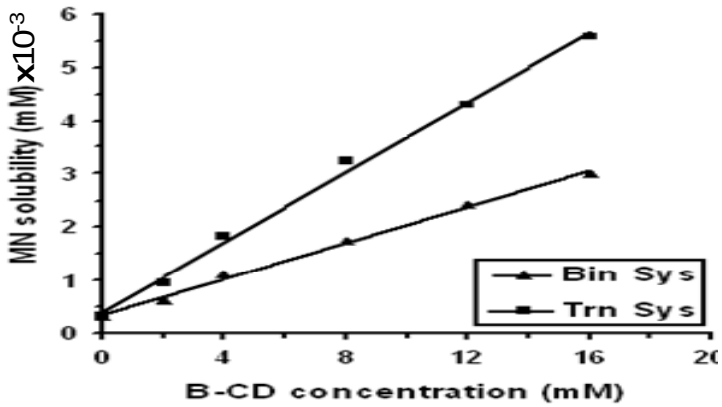

Fig.(1): Phase solubility diagram of different MN systems in distilled water at 37 ºC.

3.2 Drug contents:

The values of drug content of different MN solid systems were found between and 28.1 and 32.4% ± 4.55%.

3.3 DSC studies:

Figure (2) demonstrated DSC thermograms of MN intact, β-CD and their different prepared systems. DSC thermogram of MN intact displayed a single sharp endothermic peak at 183.7Cº corresponds to its melting point. DSC thermogram of β-CD showed a broad endotherm in the region of 70–120°C, which was attributed to the release of water molecule from the cavity. As evident in this figure, DSC thermogram of MN/β-CD or MN/β-CD/PVP-k90 physical mixture reflected a combination of DSC thermograms of their plain components, with a slightly reduction in the intensity of MN melting peak attributable to the dilution effect of the carrier investigated.

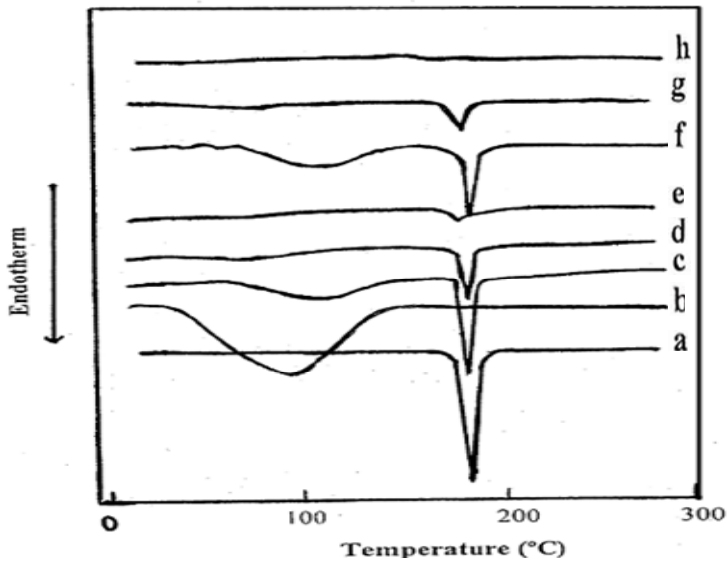

Fig.(2): DSC thermograms of different MN solid systems. Key: a) MN intact, b) β-CD, c) Bin-PM, d) Bin-KS, e) Bin-GS, f) Trn-PM1, g) Trn-KS1, h) Trn-GS1.

On the other side, DSC thermogram of MN/β-CD or MN/β-CD/PVP-k90 kneading systems displayed small narrow endothermic peaks at 175.3 and 171.1Cº, respectively, indicating to partially dispersion of MN through the carrier systems investigated. DSC thermogram of MN/β-CD ground systems showed undistinguchable shallow peak which may be attributed to the fusion of some MN free crystals. While, MN/β-CD/PVP-k90 ground systems displayed DSC thermogram devoid of any peaks: that is, MN melting peak was completely disappeared owing to the formation of amorphous dispersion complex system. Similar results of the presence of a very shallow peak in case of binary system with its complete disappearance in ternary systems were explained by Diaz et al. [16].

The obtained DSC data demonstrated that the values of fusion heats (ΔH) of the different MN systems could be arranged in the following order: MN intact >> Bin-PM > Trn-PM_1 = Trn-PM_2 > Bin-KS > Trn-KS_1 > Trn-KS_2 > Bin-GS > Trn-GS_1 > Trn-GS_2. It is worth to state that the lower ΔH values, the greater loss of drug

crystallinity and the more amount of drug found in an amorphous state upon dispersion into the investigated polymeric matrices [17].

3.4 XRD studies:

XR diffractograms of MN intact, β-CD and their different prepared systems are demonstrated in Fig. (3). XR diffractogram of MN intact or β-CD exhibited numerous sharp intense diffraction peaks reflecting their crystaline nature. XR diffractogram of MN/β-CD or MN/β-CD/PVP-k90 physical mixture was corresponded to the superposition of simply diffractograms of their plain components with a slight reduction in the diffraction peaks intensity, conforming the absence of any molecular interaction between the components. While, XR diffractogram of MN/β-CD or MN/β-CD/PVP-k90 kneading systems displayed more significant reduction in the intensity of diffraction peaks with absence of other peaks, indicated to partial interaction between the components may be found [17]. These events are greatly pronounced by XR diffractograms of MN/β-CD ground systems. As for, MN/β-CD/ PVP-k90 ground systems, a diffuse XR diffractogram with a few undistinguishable diffraction peaks was obtained indicating to probability of the formation of an amorphous complex system. Similar results were obtained by Ammar et al. [18].

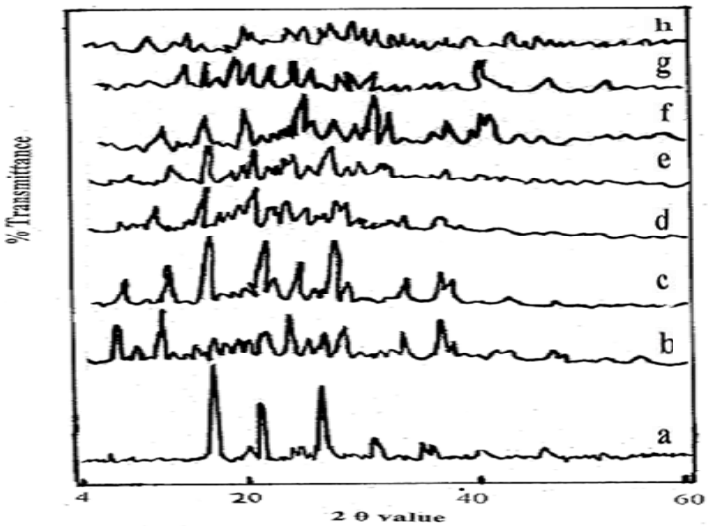

Fig.(3): XR diffractograms of different MN prepared systems. Key:a) MN intact, b) β-CD, c) Bin-PM, d) Bin-KS, e) Bin-GS, f) Trn-PM1, g)Trn-KS1, h) Trn-GS1.

The results of XRD studies were consistent with DSC results and confirmed the superior performance of the multicomponent (ternary complex) system than the binary system in establishing effective solid-state interactions with the drug.

3.5 Dissolution studies:

The dissolution profiles of MN intact and its different prepared systems are demonstrated in Figs. (4, 5). As evident, the dissolution profile of MN intact reflected poor dissolution characteristics: that is about 26% dissolved in the first 30 minutes and only 33% of the drug dissolved at the end of the dissolution period (2 h). A slight to moderate increase in the dissolution profile of MN was displayed by Bin-PM, Trn-PM_1, and Trn-PM_2 which may be due to the local solubilization action of the carrier systems operating in the microenvironment or to the state of better dissociation and less aggregation found in these systems [19].

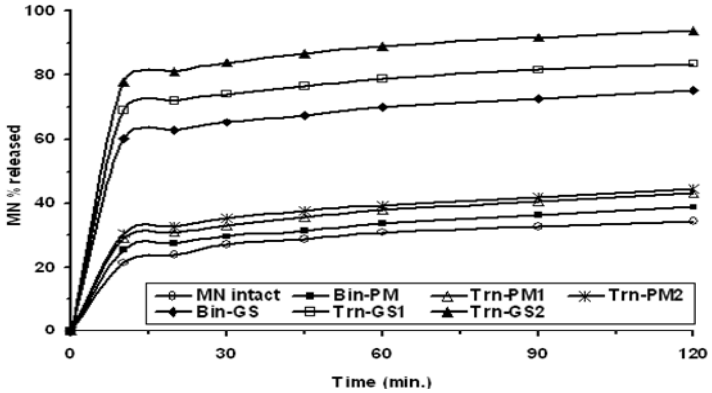

Fig.(5): Dissolution profiles of MN from its different solid systems in distilled water containing 0.02% w/v of tween 80.

On the other side, the binary and multicomponent MN systems that prepared by kneading or grinding method showed marked enhancement in their MN dissolution profile as compared to MN intact. In accordance with the results of DSC and XRD studies, the MN systems prepared by grinding method showed more MN dissolution enhancement than their corresponding systems prepared by kneading method. The extent of enhancement in the dissolution profile of MN from MN/β-CD/PVP-k90 systems was higher than that of the corresponding MN/β-CD systems and progressely increased by increasing PVP-k90 concentration to 5% w/w. Generally, MN prepared systems are arranged according to the extent of enhancement in the dissolution profile of MN in the following order: Trn-GS_2 > Trn-GS_1 > Trn-KS_2 > Trn-KS_1 > Bin-GS > Bin-KS >> Trn-PM_2 > Trn-PM_1 > Bin-PM > MN intact.

The above results proved the effectiveness of PVP-k90, as a water-soluble polymer, on improving the dissolution characteristics of MN/β-CD systems [20]. This effect could be attributed to increasing the drug particle wettability, reduction in the drug crystallinity and to the presence of the drug in highly energetic amorphous state [21, 22] as evident by PXRD and DSC studies.

3.6 Microbiological studies:

The microbiologic studies displayed that the plot of the determined inhibition zone measurements against the various concentrations of β-CD, is consistent with the solubility diagrams, as shown in Fig. (6). Where it was found that as the concentrations of β-CD increase, inhibition zone measurements increase and the highest inhibition zone measurement was obtained by the aqueous solutions of MN/β-CD/PVP-k90 systems which showed the higher solubilization power as proved by solubility studies. This is attributed to the increase of the amount of MN solubilized and to the good wettability effects of these systems, resulting in a good improvement in the diffusion of MN into the agar medium. However, it is worth to note that PVP-k90 and β-CD up to a concentration 18 mg /ml have no effect on the growth of test organisms in their own rights.

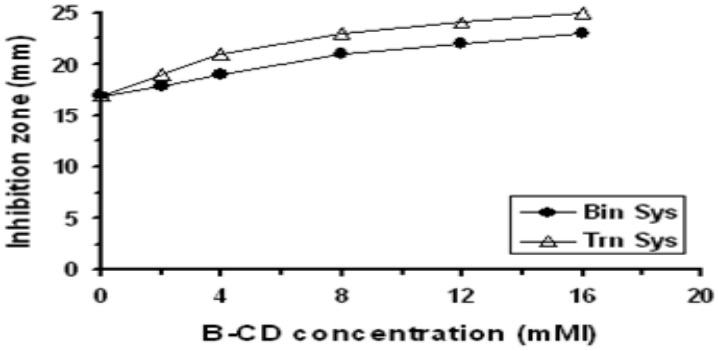

Fig. (6): Inhibition zone measurements of MN ternary systems (Trn Sys) compared by that of MN binary systems (Bin Sys) using C. albicans as a test organism.

4. Conclusion:

The results obtained by this work proved that the use of β-CD in association with PVP-k90 (a water-soluble polymer) offer synergistic enhancement in the solubilization and complexation efficiency of β-CD toward MN as evident by the greater Kc and CE values of the multicomponent (ternary) systems in comparison with the corresponding binary systems. Also, the results demonstrated that incorporation of PVP-k90 through MN/β-CD systems by grinding method rather than kneading one, further aid in amorphization and particle size reduction

of this MN systems, resulting in further improvement in the dissolution and antimycotic characteristics of this system (MN/β-CD/PVP-k90) as compared by that of MN/β-CD systems. Moreover, the extent of enhancement in both MN dissolution and antimycotic characteristics displayed by MN/β-CD/PVP-k90 systems was progressely increased by increasing PVP-k90 concentration to 5% w/w. The current work reflected the potential efficiency of multicomponent systems (ternary complexes) as an effectively economical approach for enhancing the a poorly water-soluble drug.

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References:

[1] Carrier RL, Miller LA, Ahmed I. The utility of cyclodextrins for enhancing oral bioavailability. J Control Release. 2007; 123: 78–99.
[2] Loftsson T, Duchene D. Cyclodextrins and their pharmaceutical applications. Int J Pharm. 2007; 329: 1–11.
[3] Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilisers. Adv Drug Deliv Rev. 2007; 59: 645–666.
[4] Laura SSR, Domingos CF, Francisco JBV. Physicochemical investigation of the effects of water-soluble polymers on vinpocetine complexation with β-cyclodextrin and its sulfobutyl ether derivative in solution and solid state. Eur J Pharm Sci. 2003; 20 (3): 253-266.
[5] Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. Drug solubilization and stabilization. J Pharm Sci. 1996; 85:1017–1025.
[6] Ukama K, Otagiri M, Cyclodextrins in drug carrier systems. Chem Rev. 1987; 98: 2045-2076.
[7] Szejtli J, Medicinal applications of cyclodextrins. Med Res Rev. 1994; 14: 353-386.
[8] Fareen S, Betty Ph, Kamla P. Effect of auxiliary substances on complexation efficiency and intrinsic dissolution rate of Gemfibrozil–β-CD complexes. AAPS PharmSciTech. 2010; 11 (1):27-35.
[9] Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech. 2005; 06: E329–E357.
[10] Isham N, and Ghannoum MA, Antifungal activity of miconazole against recent candida strains, Mycoses, 2010; 53 (5): 434-437.
[11] Amidon GL, Lennernas H, Shah VP, Crison JR, A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res. 1995; 12: 413-420.
[12] Higuchi T, Connors KA. Phase solubility diagram. Adv Anal Chem Instr. 1965; 4: 117–212.
[13] Pedersen M, Rassing MR, Miconazole chewing gum as a drug delivery system application of solid dispersion technique and lecithin. Drug Dev. Ind. Pharm. 1990; 16 (13): 2015-2030.
[14] Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solublizers. Adv Drug Deliv Rev. 2007; 59: 645–666.
[15] Rekharshy MY, Inoue Y. Detection of paramagnetic pH dependent inclusion complexes. Chem Rev. 1998; 98: 1875–1896.
[16] Diaz MTE, Mfndez MG, Pfrez-Marcos MB, Vila-Jato JL, Torres-Labandeira JJ. Characterization and in-vitro dissolution behavior of ketoconazole/β-and 2-hydroxypropyl-β-cyclodextrin inclusion compounds. Int J Pharm. 1996; 143: 203–210.
[17] Carpenter TO, Gerloczy A, Pith J. Safety of parenteral hydroxy propyl β-CD. J Pharm Sci. 1995; 84 (2): 222-225.
[18] Figueiras A, Ribeiro L, Veira MT. Preparation and characterization of omeprazole:methyl–beta-cyclodextrininclusion complex in solid state. J Incl Phenom Macroc Chem. 2007; 57: 173–177.
[19] Ammar HO, Salama HA, Ghorab M, Mahmoud AA. Formulation and biological evaluation of glimepiride–cyclodextrin–polymer systems. Int J Pharm. 2006; 309: 129–138.
[20] Fromming K, Szejtli J. Cyclodextrin in pharmacy. Kluwer Acad Publ Dordrecht. 1994; 110
[21] Ammar HO, Salama HA, Mahmoud AA. Implication of inclusion complexation of glimepiride in cyclodextrin–polymer systems on its dissolution, stability and therapeutic efficacy. Int J Pharm. 2006; 320: 53–57.
[22] Naidu NB, Chowdary KP, Murthy KV, Satyanarayana V, Hayman AR, Bechet G. Physicochemical characterization of meloxicam-cyclodextrin binary systems. J Pharm Biomed Anal. 2004; 35: 75–86.
[23] Mura P, Faucci MT, Maestrelli F, Furlanetto S, Pinzauti S. Characterization of physicochemical properties of naproxen systems with amorphous beta-cyclodextrin-epichlorohydrin polymers. J Pharm Biomed Anal. 2002; 29: 1015–1024.