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Comparative Studies on Dissolution and Bioavailability of Tamoxifen Citrate Loaded Binary and Ternary Solid Dispersions

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DSS and MKG designed the study and wrote the protocol. Author DSS wrote the first draft of the manuscript. Authors DSS and MKG managed the analyses of the study. Author MST managed the literature searches. Author MST run experiments and performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study focused on comparing binary and ternary solid dispersions (SD's) of water-insoluble Tamoxifen citrate (TMC) regarding drug dissolution and oral bioavailability. Basically, the enhanced dissolution of this drug by water-soluble polymer has not yet been reported in literature.
Study Design: In vitro and In-vivo characterization of Tamoxifen citrate loaded binary and ternary solid dispersion systems.
Place and Duration of Study: Department of Pharmaceutics and Industrial Pharmacy, Helwan University, Cairo, Egypt between June 2012 and June 2013.
Methodology: Amorphous SD's of TMC with two hydrophilic polymers, polyethylene glycol 6000 (PEG 6000) and Methyl cellulose (MC), were prepared by melting method. Binary SD's of TMC with PEG of different weight ratios were prepared. MC was used as third component to prepare ternary SD's. Physicochemical properties of SD's were characterized by FTIR, DSC, and *In-vitro* drug release in comparison with physical mixtures and the drug alone. Oral bioavailability of the optimized SD formula was compared with that of free TMC in rats.
Results: Infrared spectroscopic studies suggested no interaction between TMC and

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polymers rather than H-bond formation. A remarkably improved dissolution of drug from the ternary solid dispersion systems when compared to the binary solid dispersion systems was detected. On the basis of % dissolution efficiency (% DE), the SD composed of PEG: TMC: MC in a ratio 4:1:2 w/w/w was selected as the optimized SD. The *in-vivo* studies showed extremely significant higher values of C_{max} (P<.05), AUC_{0-24} (P<.05) and significantly (P<.05) lower values of T_{max} exhibited by SD compared with free TMC.
Conclusion: Highly enhanced TMC dissolution and bioavailability exhibited by PEG: TMC: MC ternary solid dispersion in a weight ratio 4:1:2 were promising to improve the therapeutic potential of TMC.

Keywords: Tamoxifen citrate; polyethylene glycol 6000; methyl cellulose; ternary solid dispersion.

1. INTRODUCTION

Drugs belonging to the biopharmaceutical classification system II (BCS- II) often exhibit low bioavailability due to their poor aqueous solubility. Therefore, it is important to improve the solubility of these drugs, which subsequently enhance their dissolution rate [1]. Studies with various approaches to improve aqueous solubility of poorly-water soluble drugs have been reported. These included ultrarapid freezing (URF) [2], solid dispersion [3], precipitation technique [4], media milling [5] and complexation [6].

Among all methods, solid dispersion (SD) is an effective one for enhancing drug dissolution rate [7]. Solid dispersions are prepared mainly by fusion (melting) method [8] or solvent evaporation method [9]. The solvent method has been discouraged by the pharmaceutical industry due to the safety regulations and environmental precautions imposed on the process for use of organic solvents, such as chloroform or dichloromethane [10]. A coground method has been reported [11] , in which a poorly water soluble drug was co-ground with water soluble polymers in the presence of small amount of water at controlled temperature. Although the composition of the coground mixture is similar to that of SD's, the drug crystallinity is maintained to some extent [12].From literature, physically unstable properties have been also reported in coground mixtures compared with melting method [13].

In this study, Tamoxifen citrate (TMC) as a class II drug according to the biopharmaceutical classification system [1] was loaded in solid dispersions. This drug is poorly insoluble in water (pKa 8, equilibrium solubility in water at 37°C is 0.5 mg/mL)[14] so that the dissolution from its dosage forms is too low and is a limiting step in the absorption process. It was reported that the dissolution of TMC could be improved to some extent by preparing microemulsions [15] and solid lipid nanoparticles [16]. The enhanced dissolution of this drug by water-soluble polymer has not yet been reported in literature. Thus, we focused in our present study on enhancing dissolution power of drug by SD technique. PEG 6000 was used in binary DS system and methyl cellulose (MC) was used as the third component in the ternary system.

2. MATERIALS AND METHODS

2.1 Materials

Tamoxifen citrate (98.9% purity), was kindly supplied by Amriya Company for Pharmaceuticals Industries, Cairo, Egypt. Polyethylene glycol 600 (PEG 6000) was purchased from El-Nasr for Chemicals Industry, Cairo, Egypt. Methylcellulose (MC) (viscosity 4200 mpas), was purchased from Fluka (Chemika), Steinheim, Switzerland, whereas HPLC grade ethanol and acetonitrile were obtained from Sigma-Aldrich, Inc., Munich, Germany. Disodium hydrogen phosphate, sodium dihydrogen phosphate and sodium chloride were purchased from EL-Gomhoria Company, Cairo, Egypt. Water for HPLC was obtained from ADWIC, Cairo, Egypt.

2.2 Phase Solubility Diagram of Tamoxifen Citrate in PEG 6000 Solution

A solubility study was performed according to the well-known method described by Higuchi and Connors in 1965. Briefly, a known excess weight of TMC (30 mg) was added to a series of screw capped vials containing 10 ml aqueous solutions of different molar concentrations of PEG 6000 from 0 to 16.67 mM with or without 1% w/v MC. Vials were mechanically shaken in a thermostatically controlled water bath shaker at 37 °C. Aliquots were withdrawn from the vials every 24 hours – till equilibrium was reached – and filtered through hydrophilic cellulose acetate syringe filters (pore size 0.45 µm, diameter 25mm). The filtrates were then analyzed spectrophotometrically for TMC content at 237 nm.

2.3 Preparation of Binary and Ternary Solid Dispersions of Tamoxifen Citrate

A binary solid dispersion of TMC was prepared by the melting method where poorly water-soluble drug was dispersed in a liquid melt of PEG 6000 at 70°C. Methylcellulose was added to the mixture at 70°C to prepare ternary SD's. After cooling to room temperature, the solidified mass was ground for 30 minutes using glass mortar and pestle. According to the experimental purposes 2-3 ml of deionized water was added to the mixture as the water was remarkably effective to improve apparent solubility of TMC, which was further ground for 10 min. After grinding, the deionized water was removed by drying at 40°C for 6 h. The resultant mass was lightly triturated and passed through sieve No. 8 [17].

2.4 Characterization and Evaluation of Solid Dispersions

2.4.1 Fourier Transform Infrared Spectroscopy (FT-IR)

To detect the presence of any interaction between TMC and the used polymers (PEG 6000 and MC), the pure drug, pure polymer, SD's and their physical mixtures were mixed separately with IR grade KBr in the ratio of 9:1 (KBr: TMC) and corresponding discs were prepared by applying 5.5 metric ton pressure in a hydraulic press. Infrared (IR) spectra for the prepared TMC-SD systems were obtained at room temperature using FT-IR spectrophotometer (Shimadzu, Kyoto, Japan). The scanning range was 400 to 4000 cm^{-1}. The spectra were collected with a resolution of 4 cm^{-1} using a mercury cadmium telluride (MCT) detector.The samples were run in triplicate.

2.4.2 Differential scanning calorimetry (DSC)

The thermal analysis of the prepared TMC-SD systems was conducted using Shimadzu differential scanning calorimeter (DSC-50). The measurement was carried out using a differential scanning calorimeter (TA-501; Shimadzu Corporation, Japan) with DSC-50 detector. Accurately weighed samples (~ 1.78 to 2 mg) were collected in an aluminum close-pan system and subjected to DSC run over the temperature range of 22°C ± 2°C to 200°C with heating rate of 10°C/min. The analysis was performed under a flow of nitrogen of 30 ml/min to avoid sample oxidation. An empty pan was used as a reference. The experiment was performed in triplicate.

2.5 Dissolution Study

The dissolution behavior of TMC from binary and ternary solid dispersions was investigated at 37 ± 0.5°C by using the USP paddle method at a rotating speed of 75 rpm. Freshly sieved fraction of the dispersion (through sieve No. 8, diameter 228 µm) equivalent to 10 mg of TMC was added to 900 ml of distilled water in the dissolution cups (Hanson SRII 6 regular cups, California, USA). At fixed time intervals 3 ml samples were withdrawn and filtered through a filter-syringe (micro-filter pore size 0.45 µm). Samples were spectrophotometrically assayed for drug content at 236.5 nm, and replaced with equal volume of fresh dissolution medium. The test was performed in triplicate.

A comparison of TMC dissolution profiles of different SD's was done using dissolution efficiency (DE) parameter. DE is defined as the area under the dissolution curve (Y) between time points' t_1 and t_2 expressed as a percentage of the curve at maximum dissolution, Y_{100}, over the same time period, and calculated using the following equation [18]:

$$DE = \left(\frac{\int_{t_2}^{t_1} Y.dt}{Y_{100}\left(t_2 - t_1\right)} \right) x100 \qquad (1)$$

2.6 *In vivo* Study

Female Sprague Dawley rats weighing 250 ± 20 gm and of 4 - 5 weeks old, were supplied by the Holding Company for Biological Products and Vaccines [VACSERA], Egypt. All rats were maintained in a light controlled room at temperature of 22°C ± 2°C and relative humidity of 55% RH ± 5% RH (Animal House of Pharmacology Department, Faculty of Pharmacy, Helwan University). The rats were divided into 2 groups (*n*=*6*) after fasting overnight (12 h) with free access to water before the experiments. First group of animals received oral TMC suspension while the second group of animals received oral TMC optimized solid dispersion (4:1:2, PEG 6000: TMC: MC). The two groups received TMC at a dose of 10 mg/ Kg body weight.

One hafter administration, the rats were mildly anesthetized using diethyl ether and blood samples (0.2 ml) were collected directly through retro-orbital puncture. Blood samples were taken regularly from each rat at 1, 2, 3, 4, 5, 6, 8, 12 and 24 h- intervals post dosing. The withdrawn samples were placed into previously heparinized eppendorff tubes and

centrifuged at 2000 rpm for 5 minutes at 4°C to separate plasma from red blood corpuscles' (RBC's) [19]. Plasma samples were stored at -80°C till quantitatively analyzed [20].

Plasma levels of TMC were analyzed using HPLC method where a mobile phase of acetonitrile: methanol (85: 15 % v/v) containing 0.02% triethylamine was used at a flow rate of 1.5 ml/ min [21]. Shimadzu 10A VP HPLC was used in the study,(degasser DGU 12A, Pump Model LC- 10A DVP, Shimadzu, Japan). Drug extraction from plasma was performed by adding acetonitrile to each sample (1:4 volume ratio), followed by vortexing for 30 seconds and centrifugation at 4000 rpm for 15 minutes. The upper layer of the centrifuged sample was withdrawn, filtered through 0.45 µm Millipore filter. Then, 10 µl of the filtrate was injected into the HPLC column (Teknokroma, C18, 5um, 25x 0.46, System controller Model SCL 10 VP). Blank plasma was spiked with 0.1 ml of TMC standard solution and treated in the same way as the test samples to develop the standard curve. Measurement was done with UV detector (Model SPD- 10A VP, Shimadzu, Japan) at 237 nm.

2.7 Statistical Analysis

The pharmacokinetic data were analyzed by unpaired student t-test for C_{max} and AUC_{0-24}, and by Wilcoxon test for T_{max}, using InStat3 program (version 3, Graph Pad software, Inc., La Jolla, CA 92037, USA). A statistically significant difference was considered at *P* value <.05.

3. RESULTS AND DISCUSSION

3.1 Construction of TMC-PEG 6000 Phase Solubility Diagrams in Presence and Absence of Methyl Cellulose

Phase solubility diagram of TMC in different concentrations of PEG 6000 aqueous solutions with and without 1% MC, was presented in Fig. 1. The diagram revealed enhancement in drug solubility according to carrier concentration. In presence of PEG only, curve linearity was observed till certain concentration of PEG 6000 (12.5 mM) beyond this concentration a plateau was formed indicating saturation. The aqueous solubility of TMC elevated from 0.92 mM in the absence of PEG 6000 to1.61 mM in the presence of 12.5 mM PEG 6000 ~ a one and half fold increase in drug solubility. Enhancement of TMC solubility in case of PEG was mainly attributed to increased drug wettability due to the higher level of hydrophilicity of this polymeric carrier [22,23].

On the other hand, in the presence of 1% MC, the phase diagram showed linearity but with more powerful increase in TMC solubility, which reached 1.92 mM in 12.5 mM PEG solution ~ two fold increase in drug solubility. The solubility enhancement could be explained on the basis that MC and its derivatives increase the solubility of sparingly soluble drugs by acting as precipitation inhibitors. In other words, a significant increase in free drug concentration above equilibrium solubility results in super saturation, which can lead to drug precipitation or crystallization. This can be prevented by use of inert polymers such MC, HPMC, PVP, PVA [23].

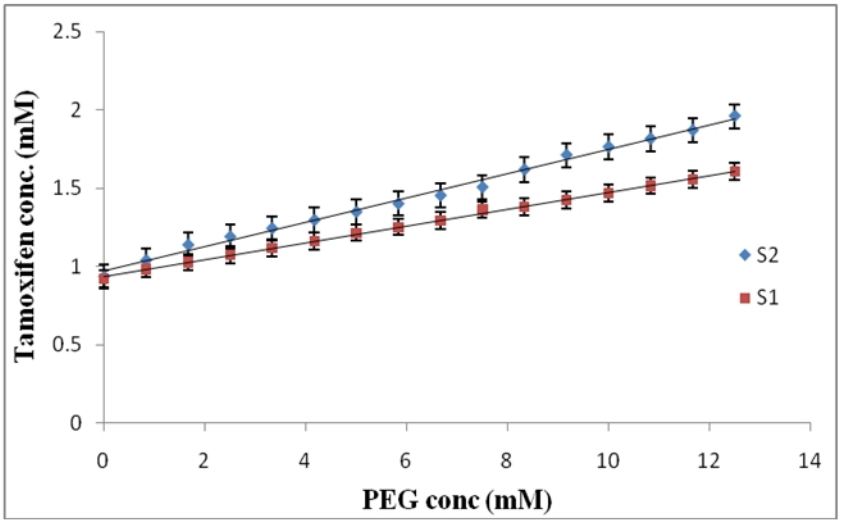

Fig. 1. Phase solubility diagram of TMC in aqueous solutions of different concentrations of PEG 6000 (S1) and in PEG 6000 / 1% MC (S2)

3.2 Characterization and Evaluation of Solid Dispersion

Solid dispersions of TMC in PEG 6000 in absence and presence of MC were prepared. The composition of binary and ternary SD's was presented in Table 1. Physical mixtures of similar ratios to ternary SD's were also prepared in order to evaluate the effect of MC on TMC dissolution.

Table 1. Composition of physical mixtures, binary and ternary solid dispersions of TMC with PEG and MC

Binary solid dispersion			Ternary solid dispersion			
Formula Code	Formula composition		Formula code	Formula composition		
	PEG	TMC		PEG	TMC	MC
B1	-	1	T1	-	1	-
B2 (SD)	2	1	T2 (SD)	2	1	2
B3 (SD)	3	1	T3 (Ph. Mix.)	2	1	2
B4 (SD)	4	1	T4 (SD)	3	1	2
			T5 (Ph. Mix.)	3	1	2
			T6 (SD)	4	1	2
			T7 (Ph. Mix.)	4	1	2
			T8 (SD)	2	1	1
			T9 (Ph. Mix.)	2	1	1

PEG: polyethylene glycol 6000; TMC: Tamoxifen citrate: MC: methyl cellulose; SD: solid dispersion; Ph. Mix: physical mixture.

3.2.1 Fourier Transform Infrared Spectroscopy

In the present study, the interaction between TMC and polymers was assessed by FT-IR spectral analysis, and the bands were assigned according to previously published data [24].

Fig. 2 showed the IR spectra for TMC, PEG 6000, MC, physical mixtures and solid dispersions of the drug in polymers. The spectrum of TMC was characterized by a peak at 3425 cm^{-1} which represents the stretching band of carboxylic and alcoholic hydroxyl group, 3028 cm^{-1} (=C-H stretching), and a highly intense narrow peak at 1732 cm^{-1} characteristic of the C=O group of citrate (Fig.2A). The region from 1377 cm^{-1} till 597 cm^{-1} represented a characteristic finger print for the compound.

Fig. 2B and 2C represented the spectra of PEG 6000 and MC, respectively. Stretched peaks at 1103 cm^{-1} and 1118 cm^{-1} represent C-O group in both polymers while the peaks at 3429 cm^{-1} and 2889 cm^{-1} are due to hydroxyl group OH of ethylene glycol chain and aliphatic C-H in PEG spectrum (Fig.2B). The spectrum of TMC-PEG 6000 binary SD (B4) (Fig.2E) exhibited different changes compared to binary physical mixture (Fig. 2D). In solid dispersion, the two bands representing TMC aromatic C-H at 3028 cm^{-1} and PEG hydroxyl group at 2889 cm^{-1} combined to form a sharp peak at 2889 cm^{-1} while the same bands appeared clearly in the spectrum of PEG/TMC physical mixtures by ratio 4:1. In other words, the IR spectrum of the binary physical mixture displayed a superimposition pattern of TMC and PEG. This change in SD spectrum is a strong indication of H-bonding occurring between TMC and PEG. Different proportions of the binary solid dispersions presented the same FTIR spectra as B4 (data not presented).

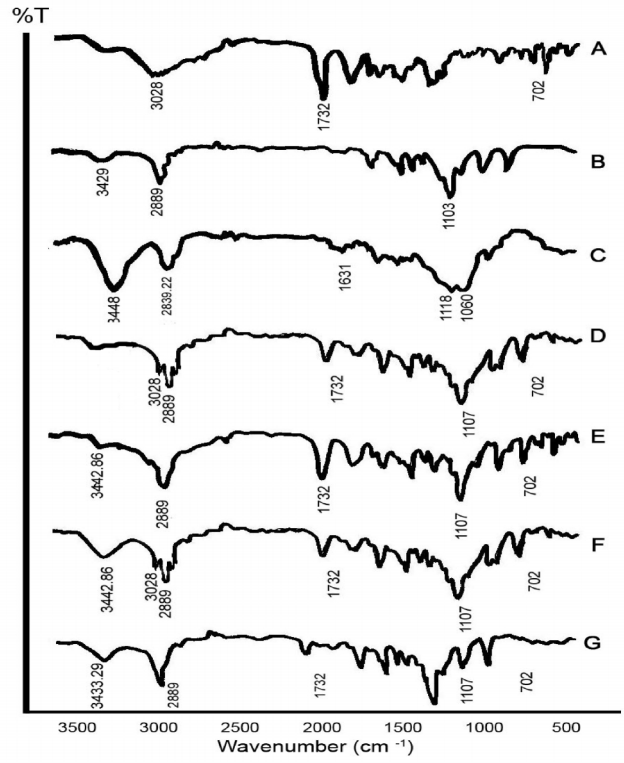

Fig. 2. FTIR spectra of (A)TMC , (B) PEG 6000 , (C) MC, (D) PEG/TMC physical mixture, (E) Solid dispersion TMC/PEG B4, (F) PEG/TMC/MC physical mixture T7 (G) PEG/TMC/MC solid dispersion T6

The ternary SD T6 (4:1:2) exhibited the same behavior as B4 (Fig.2G). In T6, the bands characterizing TMC and PEG were combined to form a sharp intense peak at 2889 cm^{-1}. The same bands appeared clearly in the spectrum of PEG/TMC/MC physical mixture by ratio 4:1:2 (T7) (Fig.2F). The resulted peak may appear mainly due to formation of H-bond that caused hydrogen de-shielding and bond shifting. On the other hand, the strong bands such as the carbonyl peak at 1732 cm^{-1} was clear at all points. Moreover, no additional peak was observed in any binary or ternary system, indicating absence of any chemical interaction between TMC and polymers. In conclusion, the spectrum suggested no interaction between drug and carrier rather than the weak H-bond. Different proportions of ternary solid dispersions presented the same FTIR spectra as T6 (data not presented).

3.2.2 Differential scanning calorimetry (DSC)

The results of the DSC study of TMC solid dispersions with PEG 6000 and MC were shown in Fig. 3. The thermograms of intact TMC and carriers were also given for comparison purposes. The DSC curve of intact TMC exhibited a sharp endothermic melting onset/peak/endset peak at 140.8/144.68/149.13 °C [25] with enthalpy of fusion H = 13.56 J/g, indicating its crystalline state, while that of intact PEG was observed at 58.55/ 61.98/ 65.45°C, 55.88 J/g enthalpy value (Fig. 3; I,II). On the other hand, MC exhibited a broad endothermic onset/peak/endset peak at 38.22/ 85.23/ 80.81°C and 5.19 J/g enthalpy value representing moisture loss from the polymer (Fig. 3; III).

In binary solid dispersions, The DSC thermogram of B4 showed that the characteristic melting peak of TMC had disappeared and sharp endothermic melting onset/peak/endset peak at 53.04/ 59.14/ 62.35°C, 22.13 J/g enthalpy (Fig. 3; IV). The disappearance of characteristic peak could be attributed to either the possible change of the drug to an amorphous form or due to molecular dispersion of the drug in the polymeric matrix. The thermal behavior of TMC in ternary systems (T6) was similar to that of binary systems (Fig. 3; V). These results indicated that MC did not play any role in the thermal behavior of TMC.

The systems in which the drug was in low proportion to carrier displayed DSC with only one peak that corresponded to PEG fusion. Therefore, the DSC result suggested that microcrystalline TMC in the TMC-PEG solid dispersions were dissolved in the melting PEG during the DSC scans. The DSC thermogram of physical mixture T7 (Fig. 3; VI) showed sharp endothermic melting onset/peak/endset peak at 53.60/ 59.81/ 66.60°C and 109.12 J/g enthalpy. The lower enthalpy change value ΔH of the SD (8.57J/g) compared to ΔH of physical mixture (95.56 J/g) indicated that TMC was dispersed in polymer in an amorphous form.As reported in literature, the heat of fusion of drug, its physical mixture and solid dispersions calculated from DSC thermograms in terms of area under endothermic peak, allowed quantitative estimates of their crystallinity [26]. Accordingly, the DSC thermograms together with FTIR spectra suggested the existence of TMC in amorphous state [24]. From literature, TMC exhibited two polymorphs, forms A and B, [27]. Form A was chemically unstable, whereas form B was stable against light irradiation. The maximum peak of UV/vis solid-state absorption spectra of form A was observed at 337 nm within the UVA range and was in longer wavelength regions than form B, which exhibited the strong UV absorption mainly in UVB and UVC region [28].From reported data and our FTIR results, polymorph B is more likely dispersed in PEG [27].

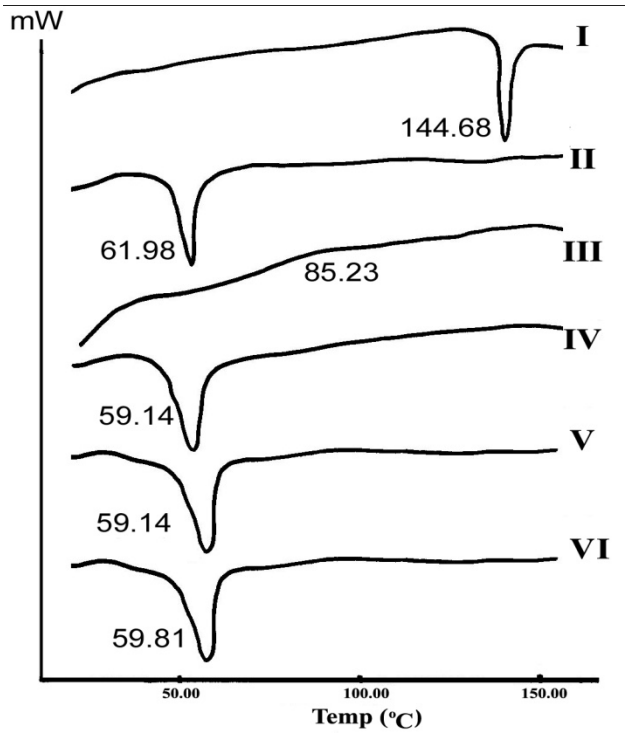

Fig. 3. DSC thermograms of (I) TMC, (II) PEG 6000, (III) MC, (IV) PEG/TMC solid dispersion B4, (V) PEG/TMC/MC solid dispersion T6, and (VI) PEG/TMC/MC physical mixture T7

3.3 Dissolution Study

The drug dissolution profiles of pure TMC, the physical mixture of TMC and polymers, and TMC-SD formulations were examined in distilled water up to 180 min (Fig. 4-6). Poor dissolution behavior was seen in pure TMC at dissolution efficiency after 30 min (DE_{30}) of 27.83 % (Table 2). The physical mixture of TMC also exhibited the limited dissolution behavior in the same manner as pure TMC (Fig. 5, 6), DE_{30} % ~ 48 % (Table 2). According to the limited dissolution properties of the physical mixtures, the use of PEG 6000 improved the dissolution of TMC, but better results was expected with dispersions.

Improved dissolution behavior was seen in most binary and ternary SD formulations of TMC prepared; however, SD's showed increase in extent of TMC solubility and enhancement of release rate with increment of PEG amounts. By comparing all binary SD's (Fig. 4) it is clearly that formula B4 (4:1 PEG/TMC) exhibited the best release profile and highest DE_{30} % compared with other binary formulae with lower PEG content. Moreover, among all prepared ternary SD's, Formula T6 of higher PEG content (weight ratio 4:1:2 PEG/TMC/MC) exhibited the best release profile (Fig. 5). These observations were consistent with previous reports, showing that the improved wettability resulted from the presence of hydrophilic groups like hydroxyl groups in hydrophilic polymers has a beneficial effect, possibly by a molecular interaction of drug and polymers or the ability to form hydrogen bonds with water and increase drug water solubility [22,23].

Table 2. Dissolution efficiency values of free TMC, physical mixtures and SD's

Binary SD	Y_{0-30} (ng.h/ml)	$\%DE_{30}$	Ternary SD	Y_{0-30} (ng.h/ml)	$\%DE_{30}$
B1	834.9 ± 0.54	27.83%	T1	834.9 ± 0.09	27.83%
B2	1230.8 ± 0.73	41.03%	T2	1795.13 ± 0.22	59.84%
B3	1653.68 ± 0.42	55.13%	T3*	1540.1 ± 0.18	51.34%
B4	1972.13 ± 0.23	62.74%	T4	1814.03 ± 0.065	60.47%
			T5 *	1009.2 ± 0.25	53.47%
			T6	2108.03 ± 0.076	70.27%**
			T7*	1210.28 ± 0.066	40.34%
			T8	1753.28 ± 0.33	58.44%
			T9 *	1498.1 ± 0.92	49.94%

SD is solid dispersion; Y_{0-30} is the area under the dissolution curve between time points' 0 and 30 min;DE_{30} was calculated from Y_{0-30} using equation 1; Physical mixture; **Significantly different.*

Comparing the dissolution profiles of binary (B4) and ternary (T6) SD's, the ternary SD exhibited the highest DE_{30} % (~ 70%) (Table 2). The bioavailability of class II drugs like TMC is rate-limited by its dissolution and generally a small increase in the dissolution rate results in a large increase in the bioavailability [1,29]. Therefore, the findings of improved dissolution properties of ternary SD formulations of TMC (T6) encouraged us to clarify the pharmacokinetic behavior and enhancement of the bioavailability in rats.

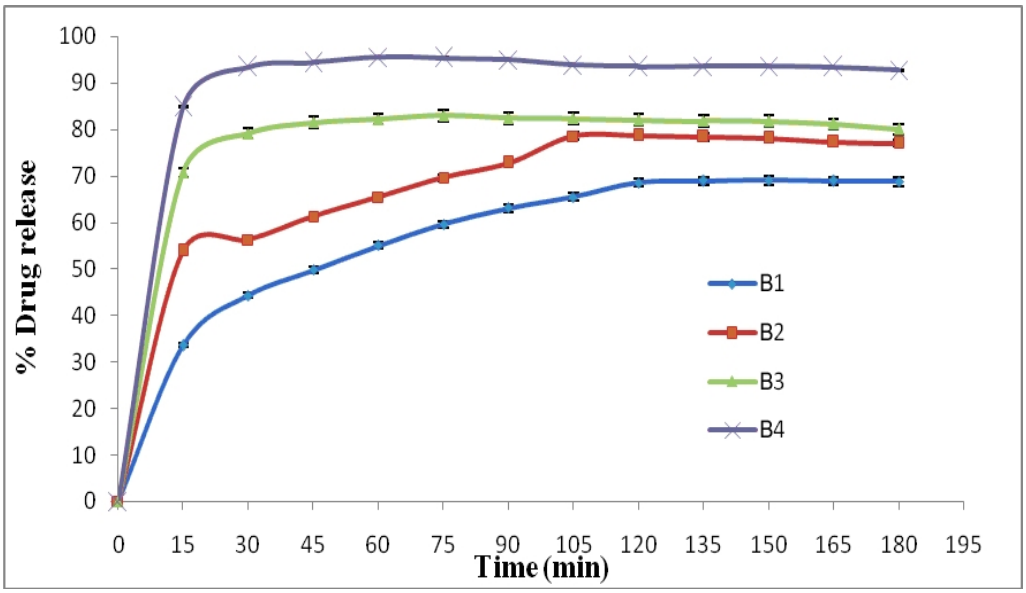

Fig. 4. Dissolution profiles of TMC (B1) and binary solid dispersions of PEG 6000/TMC in the ratios of 2:1 (B2), 3:1 (B3) and 4:1 (B4)

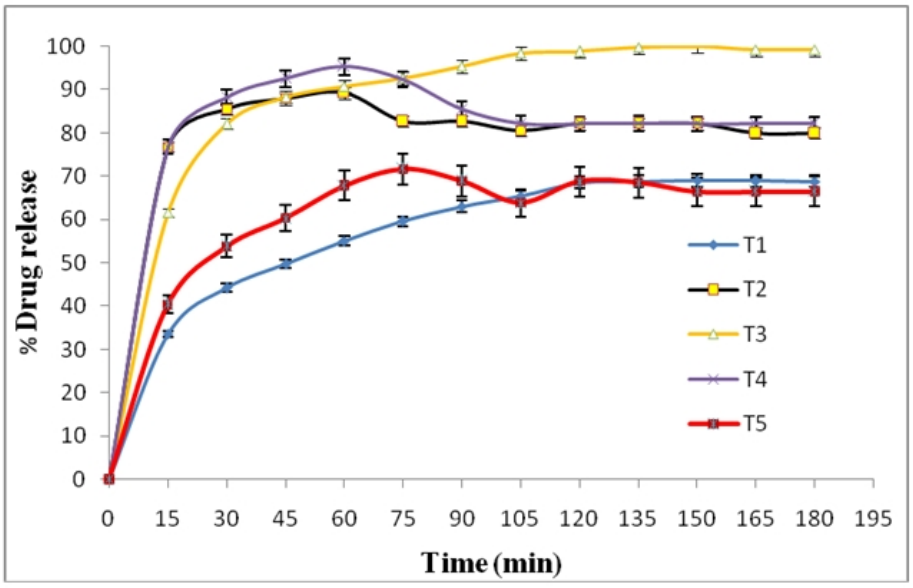

Fig. 5. Dissolution profiles of TMC, ternary SD's and physical mixtures. (T1) TMC, (T2) solid dispersion 2:1:2, (T3) physical mixture of 2:1:2, (T4) solid dispersion 3:1:2, (T5) physical mixture of 3:1:2

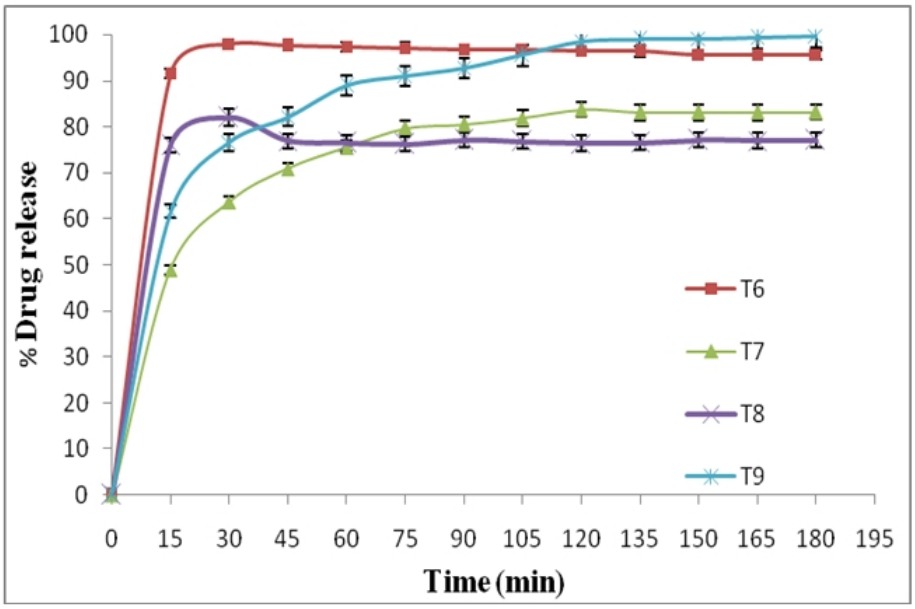

Fig. 6. Dissolution profile of TMC, ternary SD's and physical mixtures. (T6) solid dispersion 4:1:2, (T7) physical mixture of 4:1:2, (T8) solid dispersion 2:1:1 and (T9) physical mixture of 2:1:1

3.4 *In-vivo*Study and Pharmacokinetic Profiling of TMC SD

Fig. 7 shows the blood concentration–time profiles of TMC in rats after oral administrations of a dose of 10 mg/ Kg body weight, also the pharmacokinetic parameters including C_{max}, T_{max}, and AUC_{0-24} were listed in Table 3. The oral administration of the optimized SD of TMC resulted in a gradual elevation of blood TMC concentration up to C_{max} 476.8 ng/ml reached in 3.33 hours. In contrast, the free TMC showed about 3 h delay in the drug absorption and C_{max} 307.79 ng/ml that was achieved 5.8 hours post dosing (Fig. 7). This last observation was in agreement with the reported T_{max} of 4 to 7 h for free TMC [30]. From literature, TMC elimination is biphasic, with an initial half-life of around 7 h and a terminal half-life of 7-11 days [31]. TMC has two values for elimination rate constant K of 0.099 h^{-1} (initial half-life) and 0.004 h^{-1} (terminal half-life). The faster absorption, higher C_{max} and shorter T_{max} for SD might be attributed to higher *in-vitro* solubility and dissolution efficiency that reflected on the solubility and absorption of drug *in-vivo.*

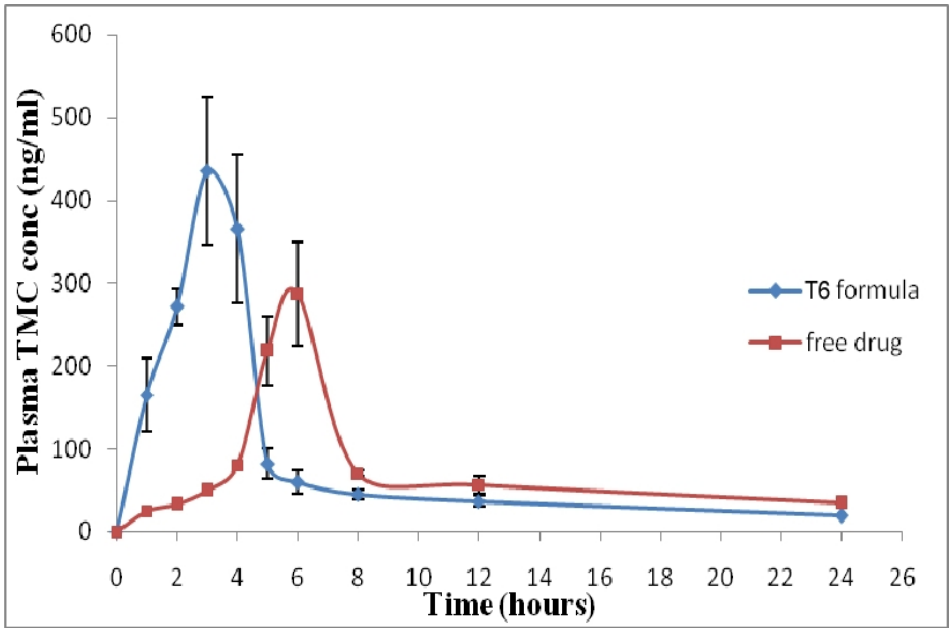

Fig. 7. Mean plasma concentration-time curves for TMC following oral administration of free TMC and the selected TMC solid dispersion formula (T6;SD of PEG/TMC/MC by weight ratio 4:1:2)

Statistical analysis of data showed high significant difference between SD and free drug regarding the values of C_{max} ($p < .05$), T_{max} ($p < .05$), and AUC_{0-24} ($p < .05$). Thus, there were 1.5-fold enhancement in C_{max} and AUC of TMC using the ternary solid dispersion technology. On the basis of AUC value of orally administered TMC, relative bioavailability of ternary SD to free drug was calculated to be ~115%, respectively. These findings were consistent with the results of dissolution study, demonstrating that ternary SD of TMC provided a marked increase in dissolution rate compared with that of binary SD and free TMC (70% DE compared to 48 and 27%).

Table 3. Pharmacokinetic parameters of TMC in rats after oral administration of a single dose (10 mg/kg) of ternary solid dispersion or free TMC

Parameter	TMC ternary SD	Free TMC
T_{max} (h)	3.33 ± 0.516	5.833 ± 0.41
C_{max} (ng/ml)	476.8 ±37.12	304.797± 20.16
AUC_{0-24}(ng.h/ml)	1954.6 ± 107.26	1704.76 ±115.11

T_{max}: time to maximum concentration; C_{max}: maximum concentration; and $AUC_{0–24}$: area under the curve of blood concentration vs. time from t= 0 to 24 h afteradministration. Values are expressed as mean ±SD (n=6)

4. CONCLUSION

From these observations of highly enhanced TMC dissolution and improved pharmacokinetic behavior, TMC loaded ternary SD in PEG 6000 and MC could be considered as a promising formula to improve the therapeutic potential of TMC.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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COMPETING INTERESTS

The authors state no conflicts of interest and have received no funding for the research or in the preparation of this manuscript.

REFERENCES

1. Amidon GL, Lennernäs 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(3):413–420.
2. Purvis T, Mattucci ME, Crisp MT, Johnston KP, Williams RO 3rd. Rapidly dissolving repaglinide powders produced by the ultra-rapid freezing process. AAPS PharmSciTech. 2007;8(3):E58.
3. Sinha S, Ali M, Baboota S , Ahuja A, Kumar A, Ali J. Solid dispersion as an approach for bioavailability enhancement of poorly water-soluble drug ritonavir. AAPS PharmSciTech. 2010;11(2):518-527 doi: 10.1208/s12249-010-9404.

4. Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci. 2003;18(2):113-120.
5. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy. J Pharm Pharmacol. 2004;56(7):827-840.
6. Uekama K, Hirayama F, Irie T. Cyclodextrin Drug Carrier Systems. Chem Rev. 1998;98 (5):2045-2076.
7. Jung JY, Yoo SD, Lee SH, Kim KH, Yoon DS, Lee KH. Enhanced solubility and dissolution rate of itraconazole by a solid dispersion technique. Int J Pharm. 1999;187(2):209-218.
8. Chiou WL, Riegelman S. Pharmaceutical applications of solid dispersion systems. J Pharm Sci. 1971;60(9):1281-1302.
9. Tachibana T, Nakamura A. A method for preparing an aqueous colloidal dispersion of organic materials by using water-soluble polymers: dispersion of β-carotene by polyvinyl pyrrolidone. Colloid and Polymer Science. 1965;203:130-133.
10. Sugimoto I, Kuchiki A, Nakagawa H.Stability of Nifedipine-polyvinylpyrrolidone coprecipitate. Chem Pharm Bull (Tokyo). 1981;29(6):1715-1723.
11. Law SL, Lo WY, Lin FM, Chaing CH. Dissolution and absorption of nifedipine in polyethylene glycol solid dispersion containing phosphatidylcholine. Int J Pharm, 1992;84:161-166.
12. Rojas-Oviedo I, Retchkiman-Corona B, Quirino-Barreda CT, Cárdenas J, Schabes-Retchkiman PS. Solubility enhancement of a poorly water soluble drug by forming soliddispersions using mechanochemical activation. Indian J Pharm Sci. 2012;74(6):505-511. doi: 10.4103/0250-474X.110576.
13. Javadzadeh Y, Adibkia K, Bozorgmehr Z, Dastmalchi S. Evaluating retardation and physicochemical properties of co-ground mixture of Na- diclofenac with magnesium stearate. Powder Tech. 2012;218:51-56
14. Physicians' Desk Reference,47th ed. Montvale, NJ: Medical Economics Co; 1993;1126.
15. Monteagudo E, gandola Y, Gonzalez L, Bregni C, Carlucci AM.Development, characterization, and in vitro evaluation of Tamoxifen microemulsions. J Drug Deliv. 2012;2012:1-11.
16. Reddy L H, Vivek K, Bakshi N, Murthy R.S.R Tamoxifen citrate loaded solid lipid nanoparticles (SLN™): preparation, characterization, in vitro drug release, and pharmacokinetic evaluation. J Pharm Dev Tech. 2006;11(20):167-177.
17. Sugimoto M, Okagaki T, Narisawa S, Koida Y, Nakajima K. Improvement of dissolution characteristics and bioavailability of poorly water-soluble drugs by novel cogrinding method using water-soluble polymer. Int J Pharm. 1998;160(1):11-19.
18. Khan KA. The concept of dissolution efficiency. J Pharm Pharmacol. 1975;27(1):48-49.
19. Borgna JL, Rochefort H. High-affinity binding to the estrogen receptor of [3H] 4-hydroxytamoxifen, an active antiestrogen metabolite. Mol Cell Endocrinol. 1980;20(1):71-85.
20. Shin SC, Choi JS, Li X. Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with quercetin in rats. Int J Pharm. 2006;313(1-2):144-149.
21. Gao S, Singh J. In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J Control Release. 1998;51(2-3):193-199.
22. Rai VK, Dwivedi H, Yadav NP, Chanotiya CS, Saraf SA. Solubility enhancement of miconazole nitrate: binary and ternary mixture approach. Drug Dev Ind Pharm. 2013; Jun 19: *(In press)* doi:10.3109/03639045.2013.801487.
23. Sikarra D, Shukla V, Kharia AA, Chatterjee DP. Techniques for solubility enhancement of poorly soluble drugs: An overview. J Med Pharm Allied Sci. 2012;1:1-22.

24. Jagabeesh HG, Deviv K. Tamoxifen loaded poly (ε- caprolactone) based injectable microspheres for breast cancer. Int J Pharm Pharm Sci. 2010;2(4):189-195.
25. Pathan IB, Setty CM. Enhancement of transdermal delivery of tamoxifen citrate using nanoemulsion vehicle. Int J PharmTech Res. 2011;3(1):287-297.
26. Marin MT, Margarit MV, Salcedo, GE. Characterization and solubility study of solid dispersions of flunarizine and polyvinylpyrrolidone. IL Farmaco. 2002;57:723-727.
27. Gamberini MC, Baraldi C, Tinti A, Palazzoli F, Ferioli V. Vibrational study of tamoxifen citrate polymorphism. J Mol Structure. 2007;840(1-3):29-37.
28. Kojima T, Onoue S, Katoh F, Teraoka R, Matsuda Y, Kitagawa S, Tsuhako M. Effect of spectroscopic properties on photostability of tamoxifen citrate polymorphs Int J Pharm. 2007;336(2):346-51.
29. Onoue S, Sato H, Ogawa K, Kawobata Y, Mizumoto T, Yuminoki K et al. Improved dissolution and pharmacokinetic behavior of cyclosporine A using high-energy amorphous solid dispersion approach. Int J Pharm. 2010;399:94-101. doi: 10.1016/j.ijpharm.2010.08.007.
30. Lien EA, Solheim E, Lea OA, Lundgren S, Kvinnsland S, Ueland PM. Distribution of 4-hydroxy-N-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. Cancer Res. 1989;49(8):2175-2183.
31. Buckley MM, Goa KL. Tamoxifen. A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. Drugs. 1989;37(4):451-490.

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