Enhancement of dissolution rate and intestinal stability of candesartan cilexitil

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ABSTRACT

The objective of this work was to improve the dissolution rate of candesartan cilexitil, a poorly water soluble prodrug and to reduce its premature degradation in the intestinal lumen. Binary and ternary solid dispersions (SD) of the drug with Pluronic F68, Polyvinyl pyrrolidone (PVP), Hydroxypropyl Methylcellulose (HPMC) and Tween 80 were prepared using the solvent evaporation method. The dissolution rate of the drug was monitored and the prepared SD systems were characterized using thermal analysis and Fourier transform infrared (FTIR) spectroscopy. The stability of candesartan in extracted rabbit intestinal fluids was monitored. This was conducted in absence and presence of tested excipients. SD of the drug with PVP resulted in significant enhancement in the dissolution rate of drug even at the lowest drug to polymer weight ratio. Similarly, SD with HPMC showed enhanced dissolution rate. SD with Pluronic F68 showed promising dissolution enhancement but this was recorded at higher polymer concentrations. Formulation of ternary SD of the drug and PVP with either Pluronic or Tween 80 resulted in rapid drug dissolution. The enhanced dissolution was mainly due to amorphousization of the drug with possible contribution to the micelle formation as reflected from thermal analysis. Incubation of pure candesartan in intestinal fluid resulted in rapid degradation of the drug. This degradation was not affected by 0.1% Pluronic. In presence of Tween 80 the rate of drug degradation was reduced significantly with the efficacy of Tween 80 depending on its concentration. The study developed a system for enhanced dissolution rate of candesartan with better stability in the intestinal lumen.

INTRODUCTION

Candesartan, (±)1-{[(cyclohexyloxy) carbonyl]oxy} ethyl 2-ethoxy-1-{[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-yl]methyl}-1*H*-benzimidazole-7-carboxylate (Figure 1a), is an angiotensin II receptor blocker (ARB) (Detroja *et al.,* 2011). ARBs are widely used in the treatment of diseases like hypertension, heart failure, myocardial infarction and diabetic nephropathy. Candesartan cilexetil performs its action by antagonizing the action of angiotensin II. This can be achieved by blocking the angiotensin type-1 (AT1) receptor. Angiotensin II is the primary vasoactive hormone of the renin angiotensin-aldosterone system with effects that include vasoconstriction, stimulation of aldosterone secretion, and renal reabsorption of sodium (Husain *et al.,* 2011). Inhibition of such enzyme can have good impact on the blood pressure. Oral administration of candesartan shows low bioavailability, approximately 15% in humans (Surampalli *et al.,* 2015). Candesartan cilexetil is an ester prodrug of its active metabolite candesartan, which owns its therapeutic effect. The ester form of the drug was developed with the goal of increasing its partition coefficient with subsequent increase in membrane permeability. Accordingly, the prodrug is rapidly and completely bioactivated by carboxylesterase enzyme (ester hydrolysis) after absorption to produce the pharmacologically active form (Husain *et al.,* 2011). Premature degradation of this ester in the intestinal lumen can lead to significant reduction in the membrane permeability to the drug and hence the bioavailability. In vitro investigations revealed instability of the drug in the intestinal fluid.

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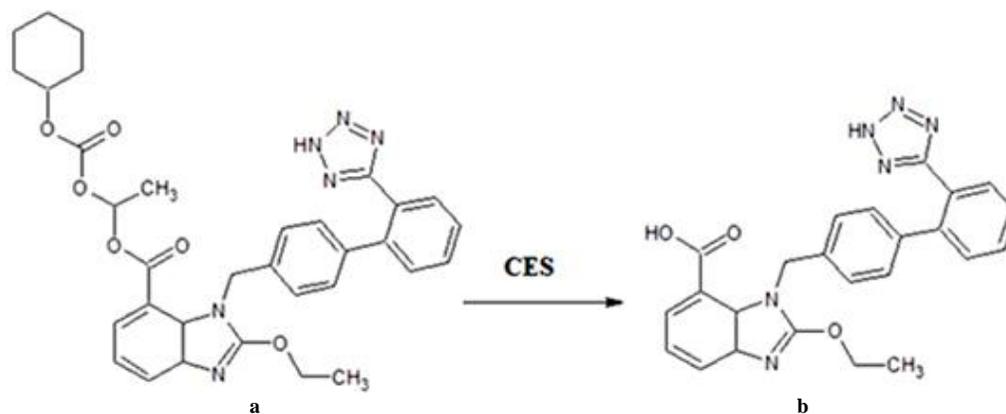
Fig. 1: Chemical structure of candesartan cilexetil (a) and its degradation product (b). modified after Borde et al., 2012. CES is Carboxylesterase enzyme.

This was evident both in human and dog intestinal fluids. This degradation was attributed to the enzymatic effect of carboxylesterases 1 and 2 (CES1 and CES2). These esterases are believed to exist in the human intestinal lumen suggesting a role of premature degradation of the prodrug in the reduced oral bioavailability of candesartan (Borde *et al.,* 2012; Laizure *et al.,* 2013). In addition to the premature degradation of the prodrug, its poor dissolution was shown to be another determining factor in reduced oral bioavailability (Surampalli *et al.,* 2015). Alternative strategies were attempted to enhance the dissolution rate and/or bioavailability of candesartan. These included formulation of solid lipid nanoparticles, nanosuspension or nanoemulsion (Nekkanti *et al.,* 2009; Detroja *et al.,* 2011; Gao *et al.,* 2011; Zhang *et al.,* 2012; and Kamalakkannan *et al.,* 2013). Other investigators employed the simple solid dispersion technique for the same purpose (Shaikh and Avachat, 2011; Devi *et al.,* 2014; Gurunath *et al.,* 2014; and Mehta *et al.,* 2014). However, none of these studies considered the effect of these strategies on the premature degradation of the prodrug in the intestinal lumen. Accordingly the objective of this work was to enhance the dissolution rate of the drug and to monitor the effects of dissolution enhancers on the stability of the prodrug in the intestinal lumen. To achieve these goals, solid dispersion technique was adopted using various excipients and rabbit intestinal fluid was used to monitor drug stability.

MATERIALS AND METHODS

Materials

Candesartan cilexetil was obtained as gift sample from Pharo Pharma, Borg Elarab, Alexandria, Egypt. Polyvinyl pyrrolidone (PVP) was purchased from Sigma Chemical Co., St Louis USA. Acetonitrile (HPLC grade) was obtained from Fisher Scientific UK, Loughborough, Leics UK. Poloxamer (Pluronic F68), hydroxypropyl methylcellulose E5 (HPMC E5), aerosil R200 (BET surface area 200 ±25m2/g, Loss on drying ≤ 1.5%, pH value 3.7-4.5) and Tween 20 were obtained from Sigma pharmaceutical company, Egypt. Tween 80, potassium dihydrogen phosphate, sodium hydroxide, sodium chloride, disodium hydrogen phosphate, potassium chloride and triethylamine (pharmaceutical grade) were purchased from El Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. All drug solutions were freshly prepared before use. Water was filtered through Millipore 0.45μm filter before used.

Chromatography

Drug analysis utilized high pressure liquid chromatography which employed a chromatographic system from Agilent technologies, 1260 infinity, DE, Germany. The system was comprised a quaternary pump (G1311C) and was equipped with a florescence detector (FLD 1260) and an automatic sampling system (TCC 1260). The whole system was under computer control. Chromatographic separation was performed on a BDS Hypersil C18 reversed phase column of 150 mm length and 4.6 mm internal diameter. The average particle size of the stationary phase of the column was 5 μm (Thermo scientific, USA). The mobile phase was prepared by mixing 0.02 M of potassium dihydrogen phosphate buffer (adjusted to pH 6) with acetonitrile and triethylamine at a ratio of 60:40:0.2 (Nekkanti *et al.,* 2010). The mobile phase was pumped isocratically at a rate of 1 ml/min at ambient temperature. The effluent was monitored by Flourimetric spectrophotometer (excitation wavelengths of 230 nm and emission wavelength 460 nm). The samples were loaded into the HPLC vials before injecting 30 μl into the HPLC. Chromatographic data analysis was accomplished using Agilent OpenLAB ChemStation software. The method was validated for linearity, selectivity, precision and lower limit of quantification (LOQ).

Preparation of solid dispersion

Table 1 presents the composition of the tested solid dispersion formulations. The solid dispersions were prepared by solvent evaporation method. The drug and the polymer were dissolved in a mixture of dichloromethane and ethanol (7:3). This involved adding the drug with stirring until complete solubility. The required amount of polymer was then added to the drug solution and the mixture was stirred to create a clear solution. The solvent was evaporated on a thermosated water bath at a temperature of 50°C. Aerosil was added gradually after removal of the solvent to produce free flowing mixture.

Table 1: The compositions of the tested formulations presented as weight ratios.

Formula	Drug	PluronicF68	PVP	HPMC	Tween 80	Aerosil 200
F1	33.33	33.33	0	0	0	33.33
F2	20	40	0	0	0	40
F3	11.11	44.44	0	0	0	44.44
F4	33.33	0	33.33	0	0	33.33
F5	25	0	50	0	0	25
F6	40	0	0	40	0	20
F7	25	0	0	50	0	25
F8	25	25	25	0	0	25
F9	25	0	25	0	25	25

PVP= Polyvinylpyrrolidone, HPMC = Hydroxy propyl methylcellulose

Determination of drug content and loading efficiency

Known amount of the solid dispersion formulation was dissolved in methanol with the aid of bath sonication. This was suitably diluted with the dissolution media before determination of the concentration of the drug in solution by HPLC. Experiments were done in triplicate. The drug content was calculated as a percentage of drug relative to the total weight of the formulation. The loading efficiency was calculated as the amount of drug recovered relative to the initial amount of drug added (expressed as percentage).

Characterization of solid dispersions
Fourier Transform Infrared (FTIR) spectroscopy

FTIR studies was conducted to investigate any interaction between the drug and the tested polymers. The studies utilized FTIR spectrophotometer (Bruker Tensor 27, Germany), working in potassium bromide diffuse reflectance mode for collection of IR data. The samples were dry mixed with potassium bromide (spectroscopic grade) before being compressed into disks using hydraulic press. The disks were subjected to FTIR scanning from 4000 to 400 cm^{-1}. Data analysis was performed using Opus IR, FT IR spectroscopy Software.

Differential Thermal Analysis (DTA)

Thermal analysis of the samples was performed on differential thermal analyzer (Shimadzu, DTA-60H, Japan). Samples of the drug loaded into aluminium pans which were crimped. The loaded samples were subjected to thermal analysis in the temperature range of 30–300°C at a heating rate of 10°C/min. The thermal behaviour was monitored under nitrogen flowing continuously at a rate of 20 ml/min. The data were collected and analyzed using Simultaneous DTA-TG software.

Dissolution Studies

The dissolution pattern of the drug was determined using the USP paddle method. The dissolution medium was selected according to the FDA reported dissolution media for candesartan cilexitil dose (32mg). This medium consisted of 0.7% polysorbate 20 in 0.05M phosphate buffer adjusted to pH 6.5. The medium was gently transferred into the dissolution vessel so as to minimize foaming of the medium during the experiment. The dissolution medium (900 ml) was equilibrated to and maintained at 37 ± 0.5^{o}C and the paddle speed was adjusted to 50 rpm. Dry samples equivalent to 32 mg of pure drug was added to a dissolution vessel. Samples (5ml) were collected at a predetermined time intervals (5, 10, 15, 30, 45, 60 and 90 min). These were immediately filtered through a 0.45 μm Millipore filter and analysed for the drug content by the validated HPLC method. The dissolution medium was replenished with fresh dissolution fluid to maintain a constant volume after each sample. Each dissolution test was performed in triplicate. The dissolution profiles were obtained from the plots of the cumulative amount dissolved as a function of time. These profiles were used to calculate the dissolution parameters which included the amount of drug dissolved in the first 5 minutes (Q5) and the dissolution efficiency (DE). The dissolution efficiency (DE) of a pharmaceutical dosage form is defined as the area under the dissolution curve between time points t1 and t2, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time, y100. It can be calculated by the following equation:

$$DE = \frac{\int_1^2 y\, dt}{y_{100} \times (t2 - t1)} \times 100$$

where y is the drug percent dissolved at time t (Khan and Rhodes, 1972; Khan, 1975; and Costa and Lobo, 2001).

In vitro stability study

The study protocol was approved by the ethical committee, College of Pharmacy, University of Tanta and was conducted according to Principles of Laboratory Animal Care. This study was performed so as to monitor the possibility for premature degradation of the prodrug in the intestinal fluids. To achieve this aim, freshly excised clean segments of rabbit small intestine (20cm each) and colon (5 cm each) were inverted. These were subjected to gentle extraction of the mucosal surface. The product of this procedure was dispersed in phosphate buffer saline containing known concentration of the drug which was dissolved alone or in presence of the tested pharmaceutical excipients. These dispersions were incubated in a thermostated water bath maintained at 37°C. Samples were collected at predetermined time intervals (0, 0.5, 1, 1.5, 2, 2.5 and 3 hours). The samples were immediately filtered through 0.2 μm syringe filter before determination of the drug content using the HPLC method. The sample (30μl) was injected without dilution into the previously described HPLC (See before). The percentage concentration of the drug remaining was calculated with reference to the initial drug concentration and was plotted as a function of time.

Statistical analysis

Data analysis employed Kruskal Wallis test with variation between formulations being compared by Tukey`s multiple comparison. This was performed using SPSS 20.

Table 2: The amount of drug released after 5 minutes (Q5), the dissolution efficiency, the drug content and drug loading efficiency of candesartan prepared in different formulation.

Formula	Q5min (%)	Dissolution efficiency (%)	Drug Content (%)	Loading efficiency (%)
Pure drug	12.7 (3.1)	41.8 (7)	-	-
F1	17.9 (2.1)	50 (5.5)	103 (1.4)	34.33 (1.4)
F2	21.3 (4)	57.6 (1.5)	94 (2.1)	18.8 (2.1)
F3	81.9 (2.8)	86.8 (1.5)	99 (0.8)	11(0.8)
F4	76.6 (0.2)	82 (1.2)	94 (2.5)	31.33 (2.5)
F5	73.7 (0.8)	80.1 (2.8)	101 (1.1)	25.25 (1.1)
F6	50.9 (1.8)	74.4 (5)	104 (3.1)	41.6 (3.1)
F7	67.4 (1.5)	82.2 (0.4)	100 (2.6)	25 (2.6)
F8	87.6 (2.8)	88.3 (0.9)	101 (2.8)	25.25 (2.8)
F9	72 (12.1)	83.8 (4)	95.3 (3.2)	23.83 (3.2)

Values between brackets are S.D. (n = 3).

RESULTS AND DISCUSSION

The technique used in solid dispersion was able to provide homogenous powdered mixtures. The drug loading efficiency in the solid dispersions ranged from 94-104% of the initial amount added (Table 2). These values exclude any possibility for segregation or degradation of drug during solid dispersion formation. The actual drug content in the formulation was used to take accurate dose of the drug in the dissolution studies (Table 2).

FTIR spectroscopy

The FTIR spectra of the drug, the polymers and their binary and ternary solid dispersions are shown in Figures 2 and 3. The FTIR spectrum of pure candesartan cilexitil showed the characteristic peaks which were noticed for N-H stretching at 3426 cm^{-1}, for aromatic C-H stretching at 2940 cm^{-1}, for aliphatic C-H stretching at 2862 cm^{-1}, for the two carbonyl groups at 1754 and 1720 cm^{-1}, for N-H bending at 1614 cm^{-1}, for the aromatic C-N stretching at 1353 cm^{-1}, for ether C-O stretching at 1036 cm^{-1} and for the aromatic C-H bending at 751 cm^{-1} (Figure 2). This spectrum correlates with the published spectra for the same drug (Reddy *et al.,* 2012; Gurunath *et al.,* 2014 ;and Surampalli *et al.,* 2015). The FTIR spectrum of pure Pluronic F68 showed the characteristic absorption bands at 3481 cm^{-1}, 2887 cm^{-1} and

1117 cm^{-1} corresponding to the OH stretching, C-H stretching and C-O stretching vibrations, respectively (Figure 2). This spectrum correlates with previously recorded one (El Maghraby and Alomrani, 2009). The FTIR spectrum of pure PVP-K30 (Figure 2) showed the characteristic absorption bands at 2955 cm^{-1} (C‑H aliphatic stretching), 1658 cm^{-1} (C=O stretching), 1495 cm^{-1} (C=C stretching) and 1288 cm^{-1} (C-N stretching). This is in good agreement with previous research on the same polymer (Gurunath *et al.,* 2014). The FTIR spectrum of pure HPMC showed the characteristic absorption bands at 3461cm^{-1} which corresponds to the OH stretching. It also revealed the characteristic band for the aliphatic C-H stretching at 2933 cm^{-1} and that of the aliphatic C-O stretching at 1121 cm^{-1} (Figure 2). Similar spectrum was recorded by other investigators (El Maghraby and Alomrani, 2009, Sekharan *et al.,* 2011). The FTIR spectrum of pure Tween 80 (Figure 3) showed characteristic peaks at 3449 cm^{-1} (OH stretching), 2924 cm^{-1} (aliphatic C-H stretching), 1736 cm^{-1} (C=O stretching), and 1110 cm^{-1} (aliphatic C-O stretching). This spectrum is similar to that recorded by other investigators (Kura *et al.,* 2014). With respect to the FTIR spectrum of aerosil 200 the spectral features reflected its chemical structure which was revealed as a distinct absorption band at 3448 cm^{-1} for the OH group which results from the hydrogen bonding between silica oxygen and water of crystallization or moisture. The absorption band at around 1637cm^{-1} is due to H—O—H bending vibration.
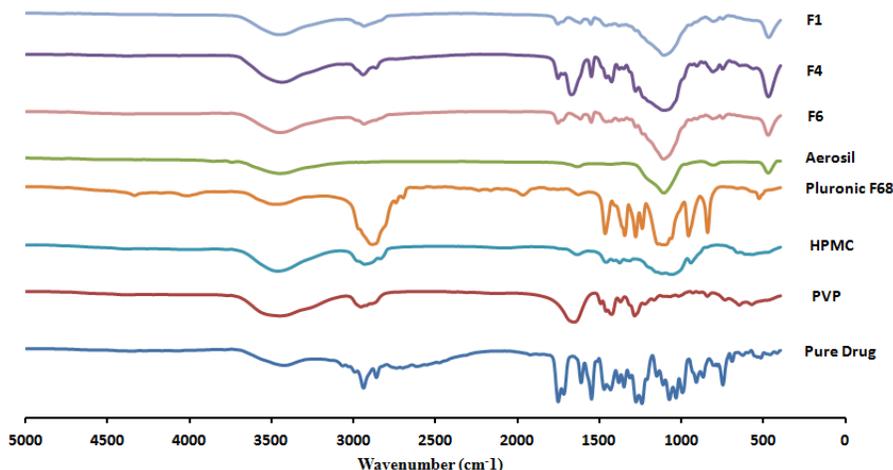
Fig. 2: FTIR spectra of pure drug, polyvinyl pyrrolidine (PVP), hydroxypropyl methylcellulose (HPMC), Pluronic F68, aerosil 200 and their binary solid dispersions. Formulation details are in Table 1.
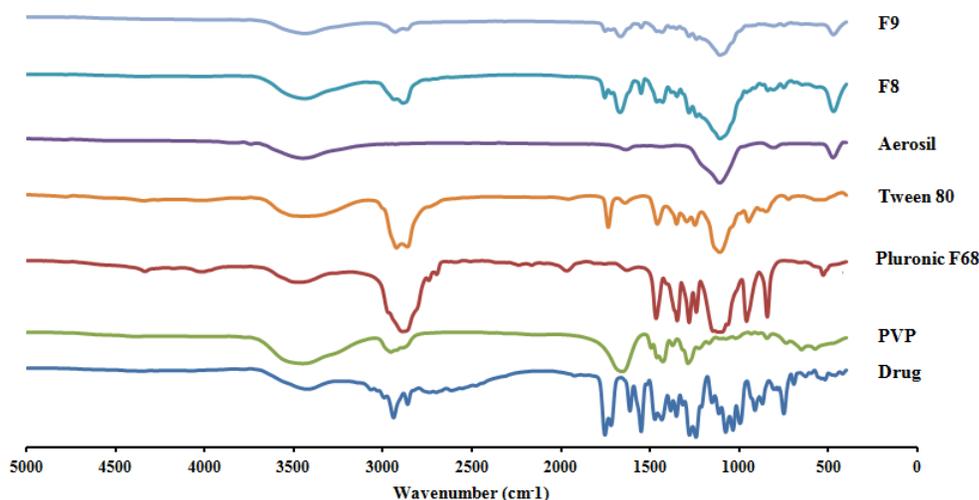
Fig. 3: FTIR spectra of pure drug, polyvinyl pyrrolidine (PVP), Pluronic F68, Tween 80, aerosil & their ternary solid dispersions. Formulation details are in Table 1.

The SiO symmetric stretching vibration, the asymmetric SiO stretching and SiO bending vibrations were recorded at 1110, 810 and 474 cm^{-1}, respectively (Figures 2 and 3). These features are in good agreement with the published spectrum for the same material (El-Gizawy et al., 2015). The FTIR spectra of binary and ternary solid dispersions reflect spectral features similar to the sum of the spectra of the components of the solid dispersion. This eliminates the possibility of interaction between the drug and the tested excipients (Figures 2 and 3).

Differential thermal analysis (DTA)

The thermal behavior of the drug, the polymers and their binary and ternary solid dispersions was monitored using DTA. Figure 4 shows examples of the DTA traces. The DTA trace of pure drug showed a sharp endothermic peak at 171.49°C with an enthalpy of 4.36J/gm. This peak is due to the melting of the drug indicating that the drug is highly crystalline. This endotherm is followed by an exothermic peak at 186.88°C which can be attributed to the thermal decomposition of the drug. The recorded thermal behavior of the unprocessed drug is comparable with the literature findings (Nekkanti et al., 2009; Detroja et al., 2011; Gurunath et al., 2014; and Sezgin-Bayindir et al., 2015).

The thermogram of pure Pluronic F68 reflected its melting transition which was recorded as single sharp endothermic peak at 60.21°C (Figure 4a). This thermogram is similar to the previously reported for the same material (Fathy and El-Badry, 2003; El Maghraby and Alomrani, 2009; and He et al., 2011).

The thermogram of pure PVP showed a very broad endothermic peak in the temperature range of 31.5 - 129.8°C. This broad endotherm can be attributed to the evaporation of water and is characteristic for the amorphous PVP (Figure 4b). Similar thermogram was recorded for PVP (Marin et al., 2002; Gurunath et al., 2014; Liu et al., 2014; and Gorajana et al., 2015).

Pure HPMC showed a broad endothermic peak was noticed. This peak has an onset of 32.2°C and endset of 128.06°C (Figure 4c). This endotherm reflects the release of the adsorbed moisture. Similar thermal pattern was recorded by other investigators and was similarly explained (Barakat et al., 2008; El Maghraby and Alomrani, 2009; and Mesnukul et al., 2009).

For pure aerosil, the thermogram did not show any endothermic peaks which is expected with such amorphous material (Figure 4a). Similar findings were found by other investigators (El-Gizawy et al., 2015).

Formulation of the drug as binary solid dispersion with Pluronic F68 resulted in a significant reduction in the Tm of the melting transition of the drug with significant reduction in the enthalpy of the transition. This effect was noticed at lower ratios of the polymer with the endothermic peak disappearing completely in case of 1:4 weight ratio of the drug to Pluronic F68 (Figure 4a). This effect can be attributed to the formation of amorphous structure of the drug, solubility of the drug in melted polymer which melts at low melting point or formation of eutectic mixture. It should be noted that the recorded thermal behavior of the drug in solid dispersion was associated with broadening of the endothermic peak of Pluronic F68 reflecting reduction of cooperativity of the transition and indicating the dispersion of drug molecules within the matrix structure of the polymer. Similar thermal changes were recorded for the solid dispersion of Pluronic F68 with other drugs (El Maghraby and Alomrani, 2009).

Preparation of the drug in the form of binary solid dispersions with different weight ratios of PVP abolished the main endothermic peak of the drug. This effect was recorded with all of the tested solid dispersion systems (Figure 4b). This suggests possible transformation of the drug from crystalline to amorphous form. It is important to highlight the existence of an exothermic peak at about 247°C. This peak strengthens with increasing the weight ratio of PVP. This exotherm can be attributed to the decomposition of the solid dispersion system. Amorphousization of candesartan was recorded after solid dispersion formation with PVP (Gurunath et al., 2014).
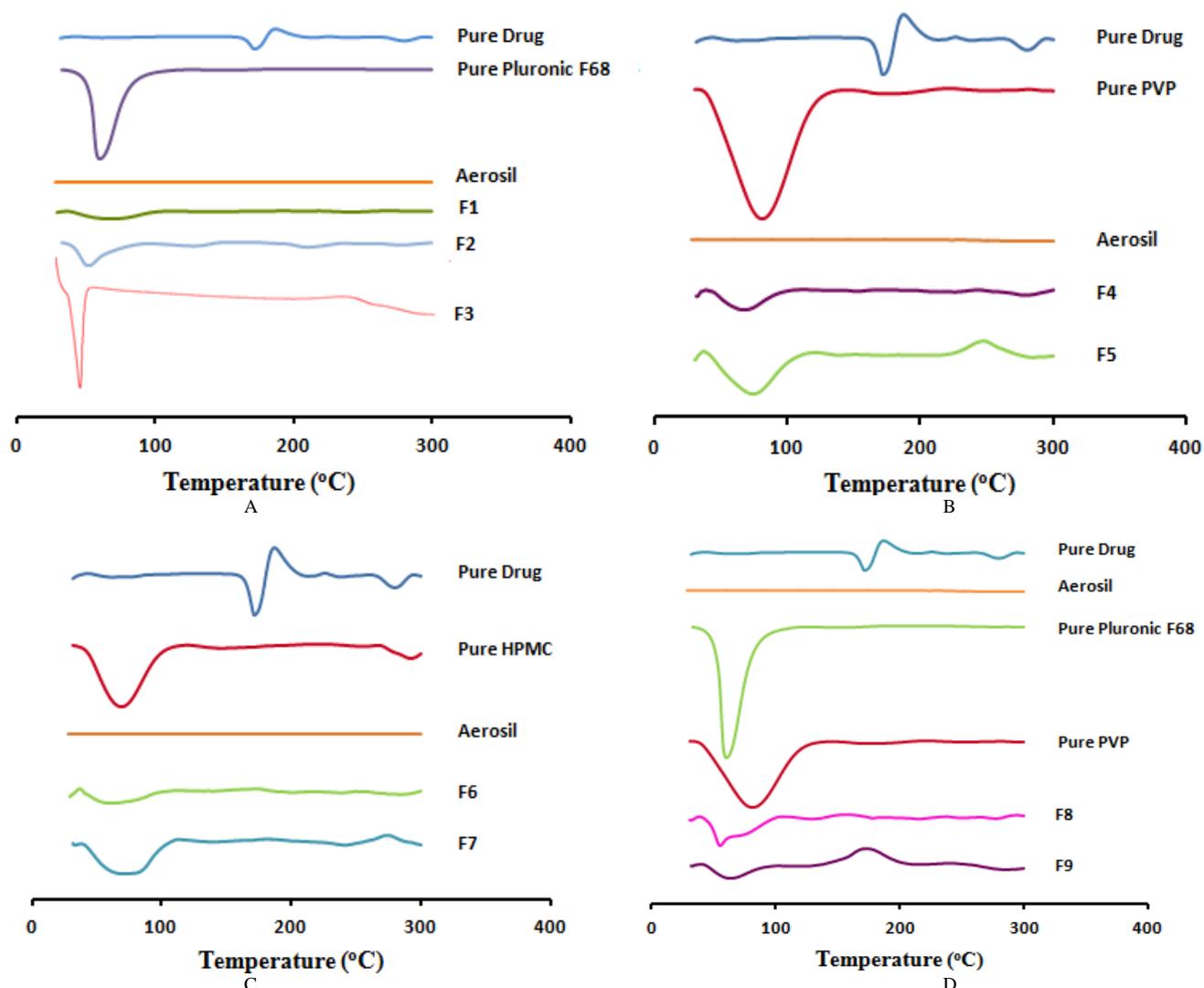
Fig. 4: DTA traces of pure candesartan and its binary and ternary solid dispersions with different polymers. Formulation details are in Table 1.

The thermal behaviour of the drug after formation of binary solid dispersion with HPMC was similar to that recorded in case of solid dispersions with PVP (Figure 4c). This again suggests transformation of the drug into amorphous structure with subsequent decomposition of the solid dispersion at high temperature value. Solid dispersions of HPMC with other lipohpilic drugs showed the same pattern (El Maghraby and Elsergany, 2014).

The prepared ternary solid dispersions in presence of PVP with either Pluronic F68 or Tween 80 also revealed the transformation of the crystalline pure drug into the amorphous form where the characteristic endothermic peak of the pure drug disappeared (Figure 4d). In the Tween 80 containing system, an exotherm was recorded at 173°C which can be attributed to the decomposition of the components of the ternary system.

Dissolution studies

The dissolution profiles of the drug and the prepared solid dispersions are shown in Figure 5. The calculated dissolution parameters are presented in Table 2. The dissolution profile of the pure unprocessed drug revealed erratic and slow dissolution (Figure 5). The drug liberated only 13% of the dose in the first 5 minutes. The total amount of drug dissolved in 90 minutes was just over 55%. The calculated DE was only 41.8% (Table 2). This poor dissolution behaviour reflects the hydrophobicity of the drug. The dissolution pattern was recorded by other investigators (Devi *et al.,* 2014). Preparation of binary solid dispersions of the drug with Pluronic F68 increased the dissolution rate of the drug but the effect was noticed at the highest tested ratio of the polymer (1:4; drug/Pluronic F68) (F3) (Figure 5).

At this weight ratio the solid dispersion resulted in significant increase in the dissolution parameters (P < 0.05). It was able to liberate 81.9% of the labelled drug in the first 5 minutes with the overall dissolution efficiency reaching 86.8%. The increase in the dissolution rate can be due to the wetting effect of the polymer which was indicated from rapid dispersion of the dry formula in the dissolution medium which differs from the unprocessed drug which tends to float due to its hydrophobicity.
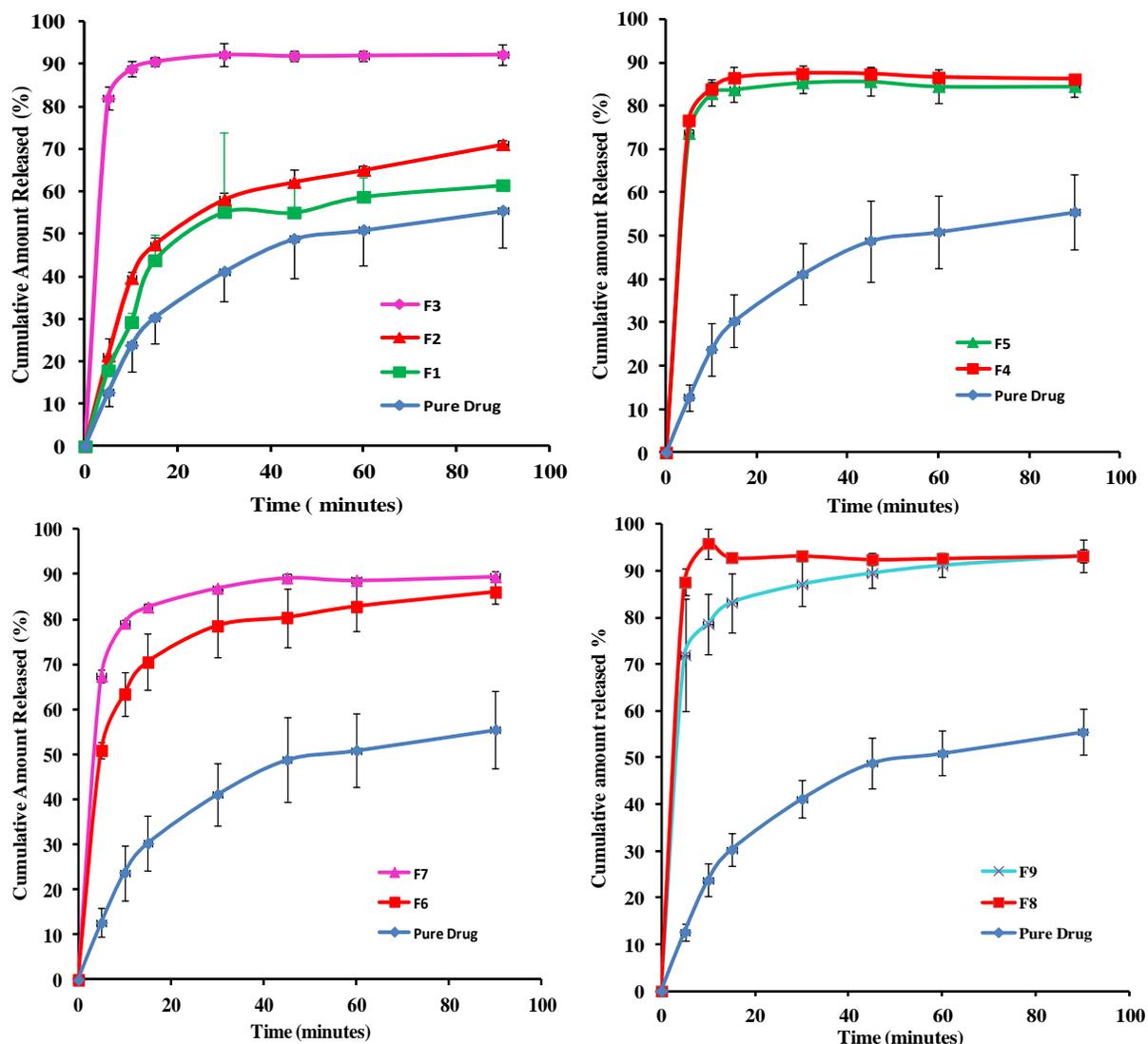
Fig. 5: The dissolution profiles of candesartan in pure state, binary and ternary solid dispersion with different polymers. Formulation details are in Table 1.

Another possible explanation for the enhanced dissolution after solid dispersion formation with Pluoronic F68 is modification of the crystalline structure of the drug as revealed from the thermal analysis results. The ability of Pluronic F68 to self-aggregate forming micelles and liquid crystalline phases was suggested as another mechanism for enhanced dissolution in these systems (Devi *et al.,* 2014). Successful dissolution enhancement was recorded for different lipophilic drugs after solid dispersion formation with Pluronic F68 (El Maghraby and Alomrani, 2009; El Maghraby and Elsergany, 2014; Emami *et al.,* 2014; and Yan *et al.,* 2014).

Formulation of binary solid dispersions of the drug with PVP K30 resulted in significant increase in the dissolution rate of the drug compared to the unprocessed powder (Figure 5). This enhancement in the dissolution rate was shown with the lowest tested concentration of PVP (at 1:1 weight ratio with drug) (F4). Further increase in the ratio of PVP did not result in significant

change in the drug release pattern (Figure 5). The amount of the drug dissolved in the first 5 minutes (Q5) was 76.6% and 73.7% for solid dispersions containing the drug with PVP at weight ratios of 1:1 and 1:2, respectively (Table 2). The calculated dissolution efficiency values were 82% and 80.1% for the same systems, respectively. This indicates that PVP is effective even at the lowest tested concentration. The mechanism of enhanced dissolution after solid dispersion formation of the drug with PVP can be mainly attributed to the change in the crystalline structure of the drug which was evident from the thermal analysis data which reflected the amorphousization of the drug in presence of PVP. The wetting effect of PVP was also suggested by other investigators (Karavas *et al.,* 2007; Emami *et al.,* 2014; Ha *et al.,* 2014; and Meka *et al.,* 2015). Finally, in case of HPMC the binary solid dispersions resulted in significant increase in the dissolution rate with effect starting to be significant at 1:1 (drug: polymer, weight ratio) (F6) (Figure 5). At this ratio the formulation liberated almost 50% of

the drug in the first 5 minutes with a DE of 74.4% (Table 2). The dissolution rate increased with increasing polymer concentration with more than 64% of the drug being released in the first 5 minutes in case of solid dispersion containing the drug with the polymer at 1:2 weight ratio (F7) with the overall dissolution efficiency reaching 82.2% (Table 2). The mechanism of improvement in the dissolution parameters of the drug after solid dispersion formation with HPMC can be due to the formation of amorphous structure of the drug. This is confirmed from the thermal analysis results. The wetting effect of the polymer can be considered as another mechanism for enhanced dissolution as indicated from rapid dispersion of the powdered formulation. Binary solid dispersions of other poorly water soluble drugs with HPMC was previously prepared and showed an increase in the dissolution profile of the tested drugs (Boghra *et al.,* 2011; Ha *et al.,* 2015).

Comparing the tested polymers with respect to enhancing drug dissolution, PVP was the most efficient. This conclusion was based on the fact that PVP produced the greatest enhancement in drug dissolution at the lowest weight ratio with the drug compared with other polymers at the same weight ratio. Taking into consideration that the mechanism of enhanced dissolution after solid dispersion with PVP is mainly based on the change in the crystalline structure of the drug, it was decided to explore ternary systems of the drug with PVP and micelle forming materials. This can employ dual mechanisms for dissolution enhancement while maintaining the potential enzymatic inhibitory function of the micelle forming material. Thus ternary solid dispersions were prepared using the drug with PVP and Pluronic F68 or Tween 80. With respect to the ternary system containing Pluronic F68 the recorded dissolution profile showed rapid dissolution pattern with the dispersion liberating more than 87% of the labelled drug (Figure 5). The overall dissolution efficiency of this ternary system was 88.3% (Table 2). Replacing Pluronic F68 with Tween 80 produced a ternary system which was able to liberate 72% of the drug in the first 5 minutes with the overall dissolution efficiency reaching 83.8% (Figure 5 and Table 2). These results reflected the potential of ternary system formation for enhanced dissolution. Other investigators recorded an increase in the dissolution rate of a poorly soluble drug using a ternary system containing the drug with PVP K30 and Pluronic F68 (Ha *et al.,* 2014). Tween 80 was used in a ternary solid dispersion system by Akbari to enhance dissolution of spironolactone (Akbari *et al.,* 2015). Synergistic dissolution enhancement was recorded after preparation of ternary solid dispersion system of other poorly water soluble drugs (Rashid *et al.,* 2015; Yan *et al.,* 2014).

Effect of micelle forming material on the enzymatic stability of Candesartan

To probe the possibility of premature degradation of the prodrug in the intestinal lumen, the drug stability was assessed in the fluids extracted from rabbit intestine. The stability was monitored both in presence and absence of the selected excipients. Incubation of pure drug solution in the small intestinal fluids

resulted in rapid degradation of the drug with more than 82 % of the drug being degraded in small intestinal fluids after 30 minutes. The same pattern was shown in the colonic fluids in which the drug lost more than 87% of its potency in the first 30 minutes (Figure 6).
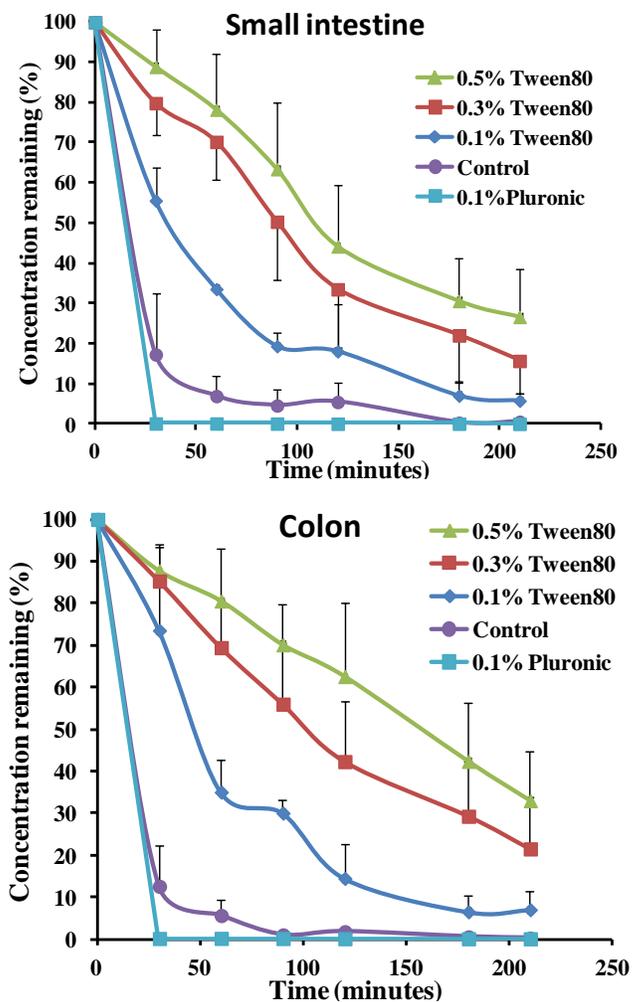
Fig. 6: Amounts remaining of candesartan cilexitil after incubation in the fluids extracted from rabbit intestine both in absence (control) and presence of Pluronic F68 or Tween 80.

These results highlight the existence of premature degradation of the prodrug in the intestinal lumen. Similar presystemic intestinal hydrolysis was recorded for candesartan cilexitil by other investigators (Nishimuta *et al.,* 2014). Premature degradation was reported for another prodrug after incubation in fluids extracted from rabbit intestine (Bali *et al.,* 2015). The recorded degradation can be attributed to the effect of carboxylesterases. These carboxylesterases are known to exist in several organs including the intestine. These enzymes can significantly reduce the bioavailability of substrate drug (Schiel *et al.,* 2007; Laizure *et al.,* 2013). This premature degradation can result in liberation of the parent compound which is less lipophilic compared with the corresponding prodrug with subsequent reduction in the membrane permeability of the drug. This can explain the poor bioavailability of candesartan cilexitil.

Candesartan cilexetil is suggested to be a substrate for both CES1 and CES2 (Borde *et al.,* 2012; Laizure *et al.,* 2013). This suggestion was based on the recorded degradation of the drug in presence human and dog intestinal fluids with the drug preserving its structure in absence of enzymes (Borde *et al.,* 2012).

Incubation of the drug in the small intestinal fluid in presence of 0.1% Pluronic F68 did not enhance the stability of candesartan compared with the corresponding pure drug solution. The same trend was obtained in case of colonic fluids (Figure 6).

Incubation of the drug in the intestinal fluids in presence of 0.1% Tween 80 resulted in significant increase in the stability of the drug compared with the corresponding pure solution. In presence of such concentration of Tween 80 the drug retained about 55% of its potency compared to 17% remaining in its absence after 30 minutes. The same trend was recorded after incubation in colonic fluids with the drug retaining 74% of its potency in the first 30 minutes. These results showed a promising stabilizing effect of Tween 80. Accordingly the efficacy of Tween 80 was tested at higher concentrations. Increasing the concentration of Tween 80 to 0.3% resulted in further increase in the drug stability with the drug retaining 80 and 85 % of its potency after 30 minutes incubation in small intestinal and colonic fluids, respectively. The drug retained 70.2% and 69.4% of its potency in the same fluids after 1 hour and retained 50.4% and 56% after 1.5 hours (Figure 6). Further increase in the concentration of Tween 80 to 0.5% was associated with stability enhancement of candesartan celixitil after incubation in intestinal fluids. The amount drug remaining after 30 minutes was 88.9 and 87.8% in case of small intestinal and colonic fluids, respectively. The efficacy of Tween 80 was concentration dependent with the stability of the drug increasing at higher concentrations of Tween 80 (Figure 6). This finding may suggest enzyme inhibitory effect of Tween 80 as a mechanism for stabilization of the drug in intestinal fluids. Tween 80 was previously shown to inhibit the carboxlesterase activity (Zhang *et al.,* 2014). Another possible explanation may depend on micelle formation with subsequent entrapment of significant concentrations of the lipophilic drug within the micelles. This can protect the drug from being attacked by the enzyme. In micellar solution there is an equilibrium between the free drug and drug in the micelle with the concentration of the free drug reducing with increasing concentrations of the surfactant. Accordingly, Tween is expected to be more effective as stabilizer at higher concentrations which correlates with the recorded results in the current study. Similar stability enhancement was recorded for ester-based produg in presence of cremophore RH4 which is another micelle forming material (Bali *et al.,* 2015).

CONCLUSION

Solid dispersion formation of candesartan with PVP, HPMC or Pluronic F68 resulted in significant enhancement in the dissolution rate of the drug. The dissolution enhancement was retained in case of ternary solid dispersion of the drug with PVP with either Pluronic or Tween 80. The enhanced dissolution was mainly due to change in the crystalline structure of the drug after solid dispersion formation. The drug was shown to be subject to premature degradation in the intestinal fluid. This degradation was significantly inhibited in presence of Tween 80. The stability enhancing effect of Tween 80 is concentration dependent with the surfactant becoming more effective at higher concentration.

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