EFFECT OF BINARY AND TERNARY SOLID DISPERSIONS ON THE *IN VITRO* DISSOLUTION AND IN-SITU RABBIT INTESTINAL ABSORPTION OF GLICLAZIDE

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ABSTRACT

Solid dispersion technique is widely used to improve the dissolution rate of drugs. Most investigators relied on the in-vitro characterization and considered the enhanced dissolution as an indication of improved bioavailability. The current study investigated the effects of binary and ternary solid dispersions of gliclazide with polyethylene glycol 6000 (PEG 6000) and/or pluronic F68 (PL F68) on the dissolution of gliclazide. The study also investigated the intestinal absorption in presence of solid dispersion components. The latter employed the in-situ rabbit intestinal perfusion technique. Preparation of binary solid dispersion with PEG 6000 or PL F68 significantly enhanced the dissolution rate compared to pure drug. The ternary solid dispersion of gliclazide with both polymers resulted in rapid drug dissolution with most drug being released in the first five minutes. The intestinal perfusion indicated the possibility of complete drug absorption from the small intestine. This, together with slow dissolution of pure drug suggested that the absorption of gliclazide is dissolution rate limited. The presence of PEG 6000 did not alter the intestinal absorption but PL F68 showed a trend of enhanced intestinal absorption of the drug. Ternary solid dispersion can thus provide rapid absorption due to rapid dissolution and potential increase in intestinal permeability.

Keywords: Gliclazide, intestinal absorption, permeability, P-glycoprotein, *in situ.*

INTRODUCTION

Rapid intestinal absorption of oral hypoglycemic drug is required to avoid rapid increase in the concentration of blood glucose after meals (Hong *et al*., 1998). Gliclazide is an oral hypoglycemic agent which belongs to the second generation sulfonyl urea drugs. It is useful for treatment of type II diabetes mellitus (Harrower, 1994). It is one of the most commonly used oral hypoglycemic agents for which good tolerability has been recorded with low record of secondary failure (Mailhot, 1993; Palmer and Brogden, 1993). Unfortunately, the drug suffers from slow and variable absorption. Previous studies showed variable absorption of gliclazide after oral administration with the time required to reach the maximum plasma concentration (t_{max}) ranging from 2 to 8 hours (Palmer and Brogden, 1993). The slow absorption of drugs can be due to slow dissolution and or poor membrane permeability. For gliclazide, the variable absorption was attributed at least to its hydrophobicity which resulted in poor dissolution (Hong *et al*., 1998). Alternative techniques have been utilized to solve this problem. These included suspending the drug in polyethylene glycol before loading in soft gelatin capsules (Hong, 1998). However, this study recorded only a reduction in the t_{max} with no increase in the maximum drug concentration in the plasma or in the AUC. Others employed the inclusion complexation with cyclodextrins as a tool to increase the dissolution rate (Aggarwal and Singh, 2002; Abou Auda *et al*., 2006). These studies also showed reduced t_{max} but no correlation was recorded between the dissolution results and the hypoglycemic effect or the AUC. This suggested a possible role for membrane permeability in limiting the oral absorption of gliclazide. Other attempts employing complexation with different cyclodextrins were reported but the authors concentrated only on the enhanced in vitro dissolution without monitoring the bioavailability or at least the intestinal permeability of the prepared complexes (Moyano and Arias-Blanco, 1997; Winters and York, 1997; Shewale and Fursule, 2008). In addition, no net intestinal absorption was recorded for gliclazide after monitoring the intestinal absorption in rats. This study suggested a role for P-glycoprotein efflux pump transporter (P-gp) in limiting the intestinal absorption of the drug (Al-Salami *et al*., 2008).

In situ micronization in presence of hydrophilic additives was recently employed to enhance the dissolution of gliclazide (Varshosaz and Talari, 2008; Talari and Varshosaz, 2009). Again, the investigators monitored the in vitro dissolution characteristics of the micronized gliclazide.

Solid dispersions with polyethylene glycols (PEGs) were also adopted to enhance the in vitro dissolution of gliclazide. These studies employed PEG 4000, PEG 6000 or PEG 8000 and recorded enhanced in vitro dissolution (Biswal *et al*., 2008; Patil and Gaikwad, 2009). These

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investigations did not evaluate the bioavailability or at least the intestinal permeability of the drug from solid dispersion.

In vitro and *in vivo* evaluation of fast dissolving microparticles of a class II drug (poorly soluble highly permeable drug) revealed an enhanced in vitro dissolution. However, this was not associated with enhanced oral bioavailability. The authors concluded that enhanced dissolution of class II drugs should not be taken as an indicator of enhanced bioavailability as this is not always the case (Wong and Kellaway, 2006). This interesting finding highlights the need of evaluating the oral bioavailability or at least the intestinal absorption of drugs after formulation with the dissolution enhancers. These investigations will ensure that the solubilizing excipients will not reduce the intestinal absorption of the given drug. In vitro compatibility may not be enough to indicate the suitability of the excipients.

Accordingly, the main objective of this study is to investigate the effect of binary and ternary dispersion systems of gliclazide with PEG 6000 and pluronic F68 (PL F68) on the in vitro dissolution and the intestinal absorption of gliclazide. PL F68 was employed in this study as it belongs to a class of non-ionic surfactants which has very low toxicity with a potential for enhancing the solubility and intestinal absorption of drugs (Brusewitz *et al*., 2007; El Maghraby and Alomrani, 2009). In addition, PL F68 Pluronic F68 was previously suggested as a possible inhibitor of the intestinal efflux (Huang, 2008).

MATERIALS AND METHODS

Materials

Gliclazide was generously donated by Tabouk Pharmaceutical Co., Tabouk, Saudi Arabia, polyethylene glycol 6000 (PEG 6000) was obtained from Winlab Laboratory Chemicals, Leicestershire, UK. Pluronic F68 (PL F68) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol (HPLC grade), acetonitrile (HPLC grade), were purchased from BDH, England. Isopropanol (HPLC grade) was obtained from central drug house, CDH®, New Delhi, India. Other chemicals and reagents were of analytical grade.

Methods of solid dispersions

Solid dispersions containing binary and ternary systems have been prepared by fusion method (Patil and Gaikwad, 2009), according to the drug-polymer ratios presented in table 1. The preparation involved melting the polymers at about 65°C. The drug was added to the molten polymer and mixed well before immediate cooling while mixing. The resulting solid dispersion was grinded before passing through a 355µm sieve.

Table 1: Composition of the tested formulations

Formulation	Gliclazide	PEG 6000	Pluronic F68
B1	1	1	0
B2	1	2	0
B3	1	3	0
B4	1	0	1
B5	1	0	2
B6	1	0	3
T1	1	1	1
T2	1	2	2

Differential scanning calorimetry

Differential scanning calorimeter (DSC) was utilized for characterization of the prepared samples. Samples thermograms (gliclazide, PEG 6000, PL F68, binary and ternary solid dispersions) were obtained by using a differential scanning calorimetry (DSC-60, Shimadzu, Japan). Solids equivalent to approximately 2.8 mg of gliclazide were loaded into aluminum pans and crimped by aluminum lids by a crimper. The DSC trace of each sample was recorded under nitrogen at a heating rate of 10°C/min, covering temperature ranges of 25-220°C. The instrument was calibrated with an indium standard. Data analysis was conducted using the TA-60WS thermal analysis software.

The following parameters were calculated:
Tm = transition midpoint.
Enthalpy (ΔH) = the area under the transition peak normalized to the sample weight.

Fourier transform infrared spectroscopy

The Fourier transform infrared (FTIR) was used to investigate any gliclazide-polymer interaction. FTIR spectra of gliclazide, PEG 6000, PL F68 and their binary and ternary solid dispersions were recorded using FTIR spectrophotometer (NICOLET 380 FTIR, Thermo Fisher Scientific, Madison, WI, USA). Samples were mixed with potassium bromide (spectroscopic grade), the mixture then compressed into disks by hydraulic press. The disks were loaded into FTIR holder and scanned from 4000 to 400 cm^{-1}. The data were analyzed using TQ analyst software.

X-ray diffraction

The crystalline nature of gliclazide in pure or solid dispersion state was investigated using automated Rigaku Ultima IV, X-ray Diffractometer. The X-ray data were collected, at room temperature, using 2theta scan axis and continuous scan mode with scan speed of 0.5 deg/min and scan range of 3.0-60.0 deg.

Determination of drug dissolution

Dissolution test was conducted for the pure drug, the binary and ternary solid dispersions. The tests employed the USP XXIV method 2 (paddle method) dissolution

apparatus. The dissolution medium was 0.1 N HCl (pH 1.2) which was maintained at a temperature of 37°C with a paddle speed of 100 rpm. Powdered samples equivalent to 80 mg gliclazide were added to the dissolution vessels while stirring. Samples (5 ml) were taken at 0, 5, 10, 15, 30, 45, 60, 90 and 120 min (the withdrawn volume was replaced with dissolution medium at each time interval). These samples were filtered through 0.45 μm filters, discarded the first 2 ml of the filtrate, and the samples were assayed (UV spectrophotometry at 227nm) for glicalzide content (appropriate dilution with the dissolution medium was employed for samples their absorbance showed readings above calibration limits). The dissolution profile of the dissolved gliclazide for each sample was constructed by plotting the cumulative amount of gliclazide dissolved (demonstrated as % of the total amount of gliclazide added) against time. The dissolution efficiency (DE) was obtained from the area under the curve of the dissolution profile using the nonlinear trapezoidal rule and demonstrated as a percentage of the area of the rectangle described by 100% dissolution in the same time (El Maghraby and Alomrani, 2009).

In situ intestinal perfusion studies

Perfusion solutions containing 20 μg/ml of the drug were prepared in 0.9% w/v aqueous sodium chloride. This was achieved by dissolving 20mg of the drug or its equivalent from the solid dispersion in 1000ml of the solvent with the aid of sonication. All solutions were freshly prepared before each perfusion experiment.

The study protocol was conducted according to the published principles of laboratory animal care and was approved by the King Saud University ethical committee. The study employed 9 male albino rabbits weighing 3.2 ± 0.3 kg.

The protocol for the preparation of the isolated intestinal segments for the *in situ* perfusion experiments were illustrated in details previously (Osman and El Maghraby, 2006). Briefly, the rabbits were fasted overnight before the experiment. The animal was anesthetized by intramuscular injection (IM) of ketamine HCl. This was administered in two doses each of 45 mg/kg at 15 minutes interval and when necessary a third dose of 25 mg/kg was injected. Chloropromazine HCl was used as muscle relaxant (two doses of 2 mg/kg given I.M at 15 min interval, given before the anesthetic). The rabbit was then laid on its back on a warm pad and the hair on the abdominal area was removed before making a midline abdominal incision of 6-8 cm. To cannulate the jejuno-ileum part, the proximal end of jejunum was tied off and cannulated with a 3-way stopcock cannula. The desired length (30 cm) was then measured and the distal end was cannulated with L-shaped glass cannula. The segment was washed by perfusing warm normal saline (37°C) through the segment. For the colon cannulation, fifteen cm (15 cm) was measured as a desired length between the proximal end which was tied off immediately after the ampulla coli and the distal end. The segment was cannulated as before after removing the solid fecal debris. Warm normal saline (37°C) was perfused for complete cleaning.

The isolated segments were kept moist and warm by frequent spraying of warm normal saline (37°C) over a cotton pad covering the intestine.

The perfusion solutions were separately perfused at a flow rate of 0.27 ml/min using a controlled rate perfusion pump (Harvard-22 Apparatus, Millis, MA, USA). After the lag time, the effluent samples were collected at 10-minutes intervals for 120 minutes. The volume of the effluent was measured to determine the intestinal net water flux and the effluent concentrations were corrected accordingly.

At the end of the experiment the animal was sacrificed. The intestinal segments under study were removed and an exact the length of each was measured. This length was used for estimation of the membrane transport parameters (Osman and El Maghraby, 2006).

Chromatography

The perfusate samples were centrifuged for 5 minutes to precipitate any mucus debris before loading into the HPLC.

The samples were analyzed for drug content using HPLC analysis. This employed a high pressure liquid chromatograph (Waters™ 600 controller, USA) equipped with Waters™ 486, Tunable Absorbance Detector and Waters™ 717 Plus Autosampler. The whole system was under computer control. Gliclazide HPLC assay method was performed on a reversed phase column 15 cm X 3.9 mm (i.d.) C_{18}, μ Bondapak™, Waters, with an average particle size of 10 μm. The mobile phase comprised 40mM aqueous potassium dihydrogenphosphate (pH 4.6), acetonitrile and isopropyl alcohol (4:5:1, v/v) (Hong *et al*., 1998). Investigated samples (30 μl) were injected into the HPLC. The mobile phase was pumped at rate of 1.2 ml/min, at ambient temperature and samples were detected by UV detector at 227 nm.

Analysis of the intestinal perfusion data

The data analysis will be presented briefly here and the reader is referred to the details which were reported previously (Osman and El Maghraby, 2006). Corrections were made for the outflow drug concentration for the net water flux. For each perfusate sample, the ratio between the corrected concentration at the outflow $\{C_{(out)}\}$ and that at the inflow $\{C_{(in)}\}$ was measured. The average of the ratios between the outflow- and -inflow concentration for

the samples collected at time intervals of 70 till 120 min was considered as the steady-state ratio. This ratio at steady-state is given by the following equation (Osman and El Maghraby, 2006).

$$\{ C_{(out)} / C_{(in)} \}_{ss} = \exp^{-(PeA / Q)} \quad (1)$$

"Where A is the effective surface area (cm^2), Pe is the apparent permeability coefficient (cm/min), and Q is the average flow rate within the intestinal segment (ml/min)." Rearrangement of equation **(1)** allows the calculation of the permeability-area product (PeA):

$$PeA = - Q . \ln (C_{(out)} / C_{(in)})_{ss} \quad (2)$$

The term (PeA) was normalized to the length of the intestinal segment. This is important to facilitate the comparison of the permeability of segments that have different lengths.

The fraction absorbed can be calculated as follow:

$$Fa = 1 - \{(C_{(out)})/(C_{(in)})\}_{ss} = 1 - \exp^{-(PeA/Q)} \quad (3)$$

Where $\{C_{(out)}/(C_{(in)})\}_{ss}$ is the fraction remaining after solution has passed through the intestinal segment.

The anatomical reserve length (ARL) is an important parameter related to the concept of absorption through the intestine. The ARL is defined as the intestinal length that remained after completion of the absorption (Osman and El Maghraby, 2006) and is given by:

$$ARL = (L^*) - (l^*) \quad (4)$$

Where, L* is the longest intestinal length accessible for absorption and l* is the intestinal length available for absorption to complete (arbitrary taken as the length required for 95% absorption).

In order to explore the effect of water flux on the absorption of the drugs through the intestinal membrane the absorptive clearance was plotted against the net water flux (after normalizing to the segment length at steady state). The slope of the plot represents the convective absorption (paracellular pathway) and the intercept represents the diffusive absorption (transcellular pathway) for the different intestinal segments.

RESULTS

Solid state characterization of the binary and ternary systems

Solid state characterization of binary and ternary solid dispersions employed DSC, FT-IR and X-ray diffraction. Figs. 1-3 show examples of the DSC profile of gliclazide, PEG 6000 and PL F68 alone and as binary and ternary solid dispersions. The thermodynamic parameters (T_m and transition enthalpy) of the drug are presented in table 2. The DSC trace of pure gliclazide showed a single sharp endothermic peak at 170.8°C (figs. 1-3). DSC trace profile of pure PEG 6000 showed a sharp endothermic peak at 62.9°C (fig. 1). Preparation of binary solid dispersions of gliclazide with PEG produced significant reduction in both Tm and enthalpy of the characteristic peak of gliclazide with the peaks becoming broader compared to the pure gliclazide (table 2). The reduction of the Tm and enthalpy depended on the proportion of PEG with the reduction being significant with increasing the proportion of the PEG in the solid dispersion (fig. 1 and table 2).

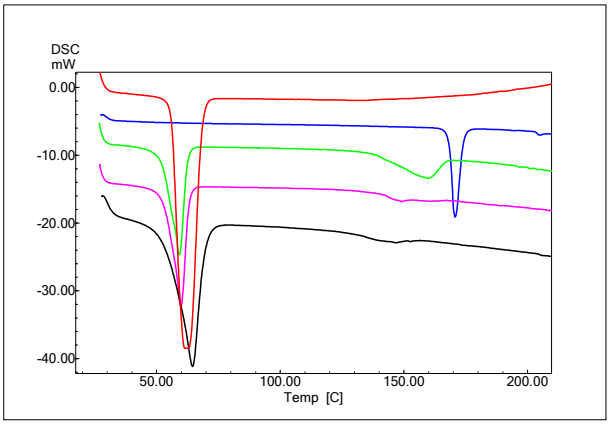

Fig. 1: Examples of the DSC traces of gliclazide, polyethylene glycol (PEG 6000) and their binary solid dispersions. The traces from top to bottom are pure PEG 600, pure gliclazide, gliclazide-PEG 6000 (1:1), gliclazide-PEG 6000 (1:2), and gliclazide-PEG 6000 (1:3), respectively.

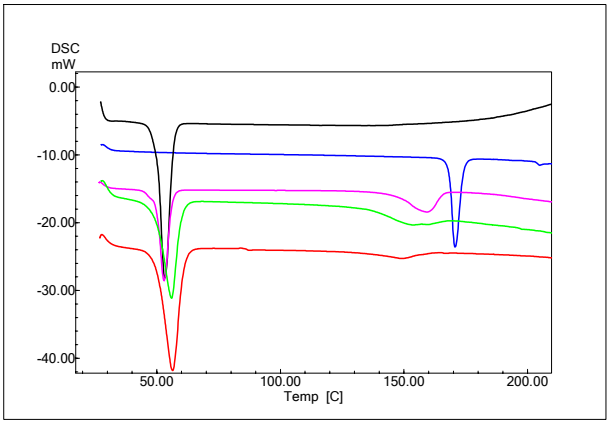

Fig. 2: Examples of the DSC traces of gliclazide, pluronic F68 (PL F68) and their binary solid dispersions. The traces from top to bottom are pure PL, pure gliclazide, gliclazide- PL F68 (1:1), gliclazide- PL F68 (1:2) and gliclazide- PL F68 (1:3), respectively.

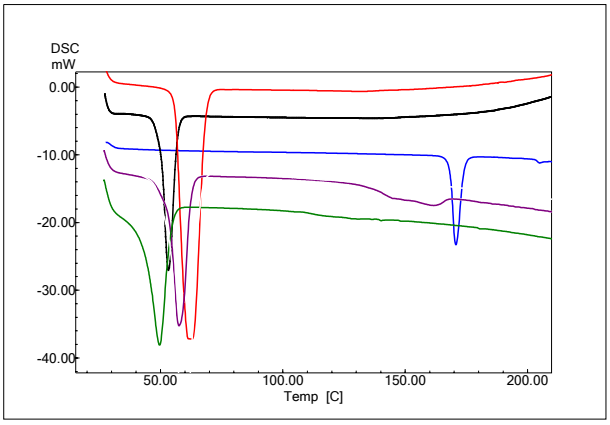

Fig. 3: Examples of the DSC traces of gliclazide, polyethylene glycol (PEG 6000), pluronic F68 (PL) and their ternary solid dispersions. The traces from top to bottom are pure PEG, pure PL, pure gliclazide, gliclazide-PEG-PL (1:1:1), and gliclazide-PEG-PL (1:2:2), respectively.

The DSC trace of pure PL F68 showed a clear endothermic peak at 53.3 °C (*Figure 2*). Incorporation of gliclazide with PL F68 (binary solid dispersion) resulted in a significant reduction and broadening in the Tm of gliclazide compared to its profile in the pure state. The reduction and broadness of the endothermic peak were gradually increased with increasing the concentration of PL F68. The reduction in the Tm was also associated with a significant reduction in the enthalpy (fig. 1 and table 2).

Preparation of ternary solid dispersion of gliclazide with PEG 6000 and PL F68 at a weight ratio of 1:1:1, produced a DSC trace showing very broad endothermic peak of the drug with the Tm and the enthalpy being reduced significantly compared to that obtained in cases of pure drug or the corresponding binary solid dispersions. Increasing the proportions of the polymers resulted in complete disappearance of the endothermic peak of the drug as in case of formulation T2 (1:2:2; drug: PEG: PL) (fig. 3 and table 2).

Fig. 4 shows representative FTIR spectra for gliclazide, PEG 6000, PL F68 and their binary and ternary solid dispersions. The spectrum of pure gliclazide showed the characteristic peaks of gliclazide which were recorded at 3273, 1708, 1350 and 1164 cm^{-1}. The absorption band at 3273 cm^{-1} can be attributed to the amine groups. The sharp peak which was recorded at 1708 cm^{-1} is ascribed to carbonyl group (C=O) of the drug. The asymmetric and symmetric stretching peaks observed at 1350 cm^{-1} and 1164 cm^{-1}, respectively can be assigned for the sulphonyl group.

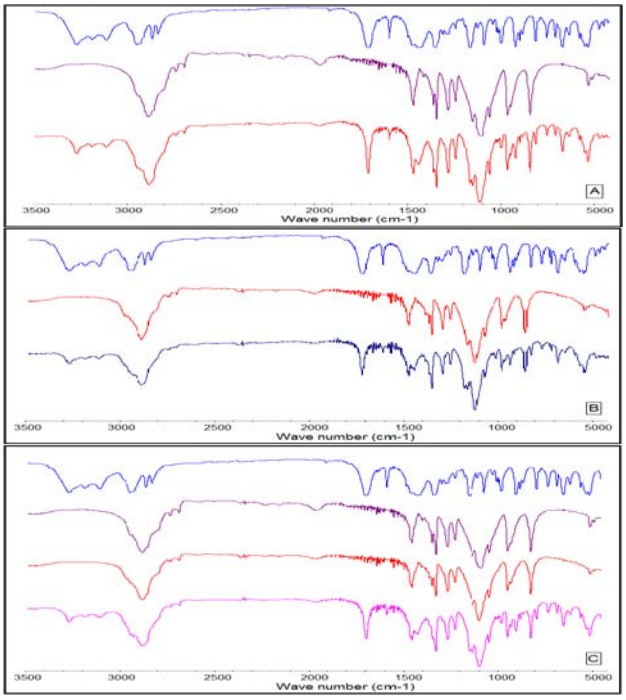

Fig. 4: Examples of the FTIR spectra of gliclazide, PEG 6000, PL F68 and their binary and ternary solid dispersions. The spectra from top to bottom are pure gliclazide, pure PEG 6000, and binary solid dispersion (1:2), respectively (A); pure gliclazide, pure PL F68, and binary solid dispersion (1:2), respectively (B); pure gliclazide, pure PEG 6000, pure PL F68, and ternary solid dispersion (1:1:1), respectively (C).

Table 2: The melting transition parameters and the dissolution efficiency of gliclazide binary and ternary solid dispersions

Formulation	Tm (°C)	Enthalpy (J/g)	Dissolution efficiency (%)
Pure drug	170.8 (0.7)	139.0 (11.9)	41.0 (3.8)
B1	157.9 (1.8)	88.7 (8.7)	72.4 (3.6)
B2	136.2 (17.0)	29.4 (10.8)	74.3 (13.2
B3	140.7 (8.6)	21.7 (4.6)	88.4 (10.0)
B4	161.0 (2.4)	95.4 (12.1)	81.8 (7.8)
B5	157.7 (6.6)	60.8 (2.7)	87.9 (2.7)
B6	143.6 (4.3)	50.4 (11.9)	88.9 (3.1)
T1	157.7 (6.6)	60.8 (2.7)	83.9 (7.0)
T2	No peak	No peak	93.9 (1.7)

Values between brackets are S.D. (n = 3). Formulation details are in table 1.

The PEG 6000 spectrum (fig. 4A) showed characteristic peaks at 3425 cm^{-1}, 1109 cm^{-1}, and 2889 cm^{-1} which belong to OH, C-O, and CH groups (stretching mode), respectively. As for PEG, the FTIR spectrum of pure PL F68 revealed the distinguish absorption bands at 3503, 2884 and 1114 cm^{-1} which can be attributed to OH, CH and C-O groups (the stretching vibrations), respectively (fig. 4B). The recorded spectra for the binary and ternary solid dispersions of the drug with the tested polymers revealed the specific peaks of the drug which were recorded in the same position.

The X-ray diffraction pattern of gliclazide, PEG 6000, PL F68 and their binary and ternary solid dispersions are shown in *Figure 5*. The X-ray diffraction pattern of gliclazide showed crystalline morph characterized by numerous peaks which were recorded at 2θ values of 10.59°, 14.98°, 17.21°, 18.15°, 22.07°, 25.42°, 26.25°, 26.75° and 29.51° (*Figure 5*). The diffraction patterns of pure PEG 6000 and pure PL F68 revealed similar crystalline pattern with the main peaks being detected at 2θ values of 19.41, 23.34. Preparation of gliclazide in the form of binary or ternary solid dispersion affected the intensity of the diffraction peaks with the effect being significant in cases of the peaks recorded at 2θ values of 25.42°, 26.25°, 26.75° and 29.51°. The peak recorded at 22.07° overlapped with that of the polymers. The extent in reduction in the intensity of peaks increased with increasing the proportions of the polymer in the binary and ternary systems.

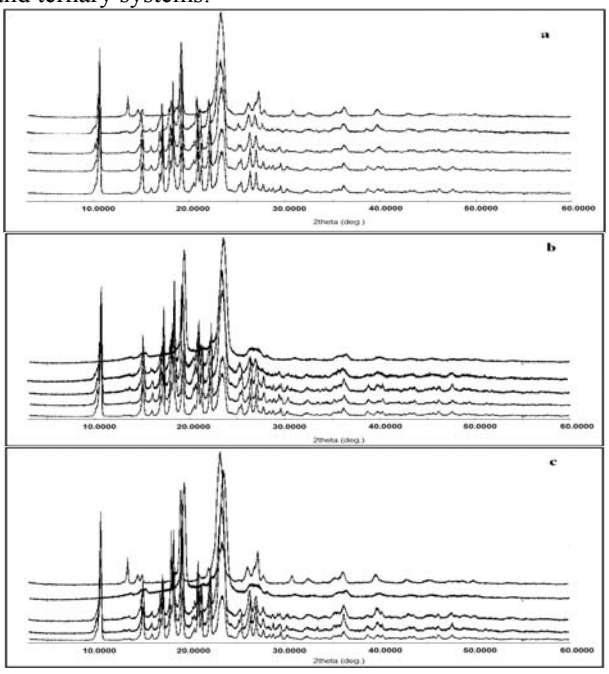

Fig. 5: X-ray diffraction patterns of gliclazide, PEG, PL F68 and their binary and ternary solid dispersions. The patterns from bottom to top are (a) pure gliclazide, B1, B2, B3 and pure PEG 6000; (b) pure gliclazide, B4, B5, B6 and pure PL F68; pure gliclazide, T1, T2, pure PL F68, and pure PEG 6000, respectively.

Dissolution studies

Fig. 6 shows the dissolution profiles of gliclazide in pure state or in binary and ternary solid dispersions. The calculated DE% values are presented in table 2. The dissolution results revealed poor and slow dissolution of pure gliclazide. Formulation of gliclazide as binary solid dispersion with either PEG or PL F68 resulted in significant enhancement of gliclazide dissolution. This is revealed from the significant increase in the DE% compared to the pure drug (table 2). The DE% of the drug was increased by increasing the proportion of PEG or PL F68 in the binary system. The data revealed a trend of higher efficiency for PL F68 compared to that recorded in case of PEG systems. This was evident when comparing the corresponding binary systems with respect to their dissolution efficiencies (table 2). Similar dissolution pattern was recorded for the drug with PEG (Shavi *et al*., 2010).

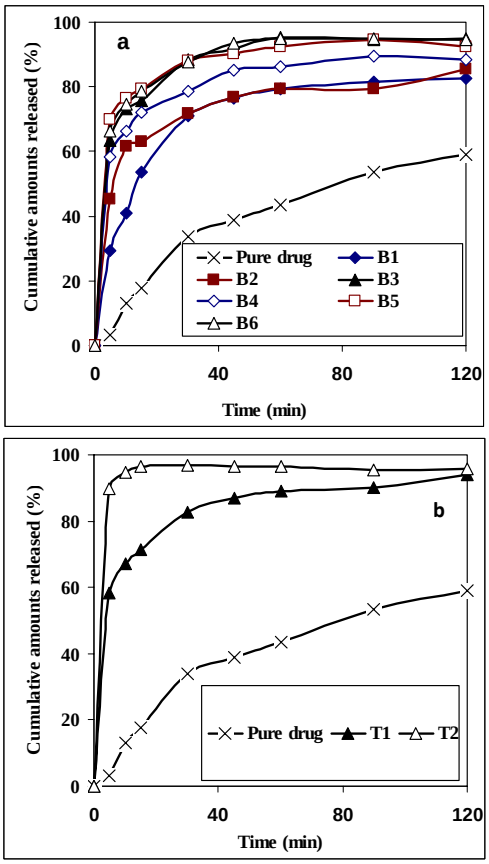

Fig. 6: The dissolution profiles of gliclazide from pure powder or from binary (a) and ternary (b) solid dispersions with PEG 6000 and or PL F68 (formulation details are in table 1).

Preparation of ternary solid dispersion enhanced the dissolution of the drug compared to the pure drug. The ternary solid dispersion T2 (1:2:2; drug : PEG : PL F68) resulted in an ideal dissolution profile with more than 89% of the drug being released in the first 5 minutes. The

Table 3: The in situ rabbit intestinal membrane transport parameters of gliclazide in absence or presence of PEG 6000 alone or with PL F68.

Parameters	Jejunoileum		
	Pure drug	B2	T2
PeA (ml/min)	0.215 (0.074)	0.182 (0.048)	0.258 (0.011)
PeA/L (ml/min.cm)	0.0066 (0.0098)	0.0064 (0.0022)	0.0085 (0.0008)
Fa (%)	53.2 (13.3)	48.0 (8.9)	61.7 (0.4)
L95 (cm)	131.9 (29.5)	142.4 (36.5)	98.0 (8.0)
ARL (cm)	48.1 (29.5)	37.6 (36.5)	81.9 (8.0)
	Colon		
PeA (ml/min)	0.075 (0.041)	0.072 (0.034)	0.111 (0.09)
PeA/L (ml/min.cm)	0.0087 (0.0031)	0.0090 (0.0022)	0.0115 (0.007)
Fa (%)	22.6 (11.6)	26.9 (9.5)	30.9 (21.4)
L95 (cm)	168.3 (115)	135 (54)	116.8 (68.5)
ARL (cm)	-153 (115)	-120 (54)	-102 (68.5)

Values between brackets are S.D., n = 3.

recorded dissolution efficiency with this formulation was higher than that obtained with binary solid dispersions containing the same proportion of either PEG or PL F68 (table 2).

In situ rabbit intestinal absorption of gliclazide

Table 3 presents the membrane transport parameters of gliclazide which were recorded after in situ rabbit intestinal perfusion of drug solutions prepared from pure drug, drug-PEG solid dispersion (formulation B2) or drug-PEG- PL F68 ternary solid dispersion (formulation T2). The membrane transport parameters of pure drug solution through jejunoileum indicated complete absorption of the drug from this segment. This is evidenced by the recorded value of the L95 which was 131.9 cm (table 3). This was revealed further from the positive value of the ARL. Comparing the recorded values of the absorptive clearance per unit length in case of jejunoileum with recorded after perfusion of the colon there was no significant difference between the recorded values ($p > 0.05$). The length required for complete drug absorption from the colon was 168.3 cm. The ARL was negative (table 3).

To investigate the absorption pathway, the effect of water flux on the absorptive clearance was monitored (fig. 7). The linear regression of these plots indicted that the slope is significantly different from zero. The intercept was also significantly different from zero. The colonic absorption showed the same pattern.

Perfusion of the drug in presence of PEG 6000 produced similar membrane transport parameters as those recorded in absence of PEG (table 3). Considering the data in fig. 7, the effect of water flux on the absorptive clearance was the similar to that recorded after perfusion of pure drug.

In case of the ternary system, which comprised the drug with both PEG and PL F68 (T2), co-perfusion of the drug with both polymers resulted in a clear trend of increased intestinal permeability of the drug compared to that recorded in cases of pure drug or drug-PEG system. For the jejunoileum the absorptive clearance was increased by 30%, the anatomical reserve length was increased by more than 33 cm (table 3). The same trend was recorded in case of colonic absorption. Monitoring the effect of water flux on the absorptive clearance of the drug in this case revealed a role for both transcellular and paracellular absorption with the former dominating (fig. 7).

DISCUSSION

Solid state characterization of binary and ternary solid dispersions employed DSC, FT-IR and X-ray diffraction. The DSC profile of pure gliclazide showed a single sharp endothermic peak which had thermodynamic parameters similar to that recorded by other researchers (Biswal *et al*., 2008; Talari *et al*., 2009). DSC trace profiles of pure PEG 6000 or pure PL F68 were comparable to those recorded in previous studies with each of them showing clear endotherm (Biswal *et al*., 2008; El Maghraby and Alomrani, 2009). Preparation of binary solid dispersions of the drug with PEG 6000 or PL F68 reduced the Tm and enthalpy of the endothermic peak of the drug with the peak becoming broader. This effect was clearer in case of ternary solid dispersion with the endotherm disappearing in case of the ternary system containing higher proportion of the polymers. These findings imply the possibility of formation of complex between the drug and any of the polymers, loss of the crystalline structure of the drug, solubility of the drug in the polymer or eutectic mixture formation. To verify this, FTIR and X-ray diffraction studies were conducted.

The recorded FTIR spectra of pure gliclazide, PEG 6000 and PL F68 showed the characteristic peaks of each material. These peaks were identical to those recorded in previous studies (Shah and Amin, 2007; Biswal *et al*.,

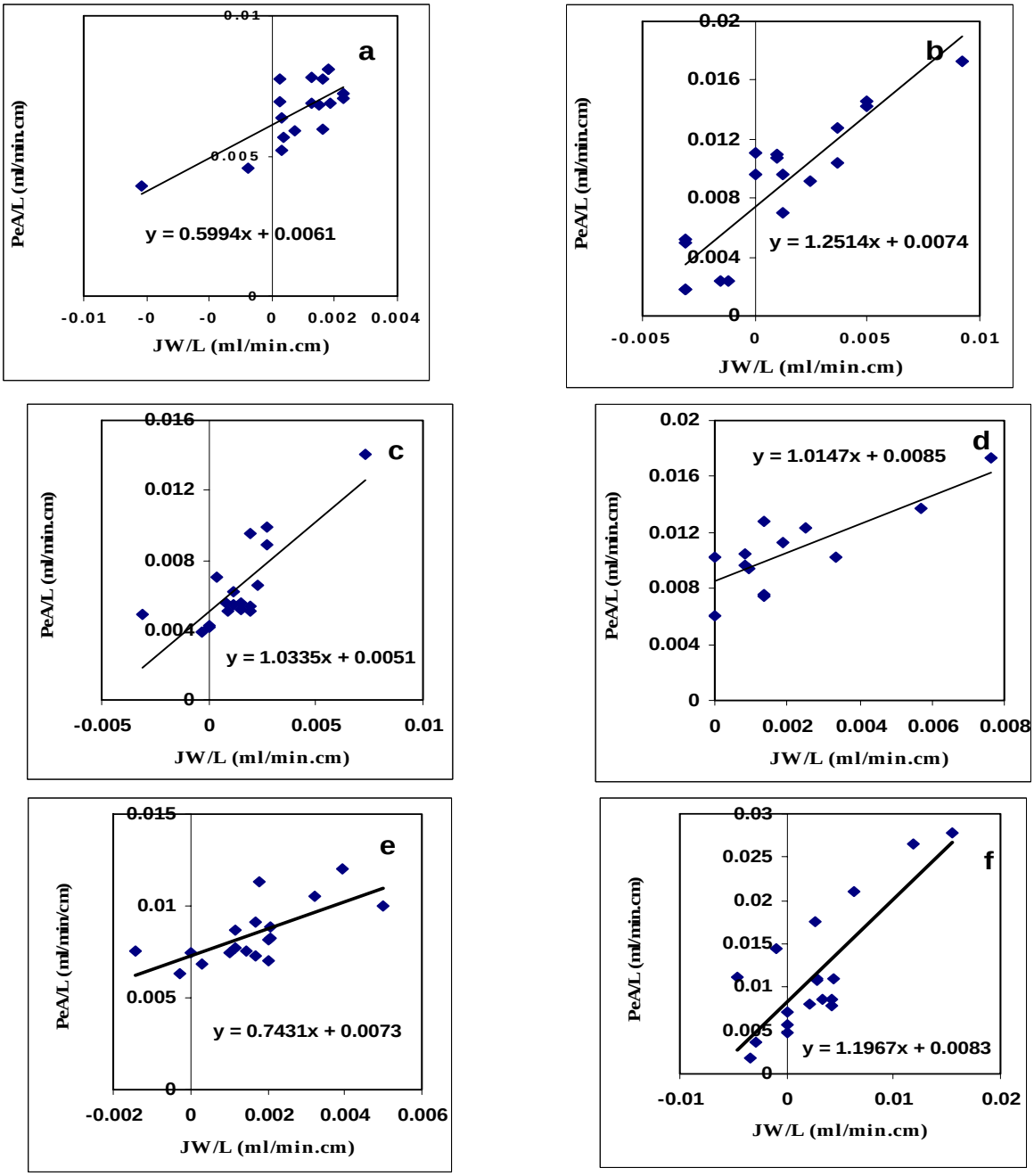

Fig. 7: The absorptive clearance of gliclazide versus water flux through the jejunoileum (left) and colon (right). The data obtained after perfusion of pure drug (a-b), formulation B2 (c-d) or formulation T2 (e-f).

2008; Patil and Gaikwad, 2009). Taking the available function groups of PEG and PL F68 into consideration, there may be a possibility for hydrogen bonding between the hydroxyl group of polymers and carbonyl group of gliclazide and/or hydrogen bonding between hydrogen atom of the NH group of gliclazide and oxygen atom of the polymers. Accordingly, any interaction will be observed as a change in C=O, S=O and NH groups vibrations in the gliclazide spectrum profile. The recorded spectra for the binary and ternary solid dispersions of the drug with the tested polymers revealed the specific peaks of the drug suggesting the absence of any interaction between the drug and the PEG or PL F68. This excludes any chemical interaction between the drug and the tested polymers. Similar findings were recorded for the gilclazide-PEG 6000 solid dispersion (Shavi *et al*., 2010). The X-ray diffraction pattern of pure gliclazide was similar to that recorded by other researchers (Biswal *et al*., 2008). Preparation of gliclazide in the form of binary or ternary solid dispersion affected the intensity of the diffraction peaks of the drug. The reduction in the intensity of the peaks when considered with the recorded

reduction in the Tm and enthalpy of the endothermic peak of the drug (*Table II*) can be explained on basis of reduced quality of the crystalline structure of the drug or presence of the drug in the form of microcrystalline dispersion in the polymers. Similar explanation was reported for gliclazide solid dispersions with PEG (Biswal *et al*., 2008).

The dissolution results revealed poor and slow dissolution of pure gliclazide. This was attributed to the hydrophobicity of the drug and can at least explain the variable and slow absorption of gliclazide (Hong *et al*., 1998). Formulation of gliclazide as binary or ternary solid dispersion increased gliclazide dissolution compared to the pure drug. The most important feature of the ternary solid dispersion formulation, T2 was the rapid dissolution behaviour. Alternative mechanisms have been suggested for enhanced dissolution of drug from solid dispersion. These include reduction in the size of crystal, lack of crystalline drug aggregates, and/or conversion of drug from crystalline state to amorphous one (Ford, 1986). Improved wettability of the hydrophobic drug by amphiphilic compounds is another alternative. Amphiphilic molecules can modify the surface properties of particles which will result in a reduction of the contact angle with water or by formation of hydrophilic film around the particles (Mooter and Augustijns, 1998). The enhanced dissolution of gliclazide from PEG solid dispersion was mainly attributed to the formation of microcrystalline dispersion of the drug (Biswal *et al*., 2008). This explanation is supported with the recorded DSC and X-ray data. For PL F68, the enhanced wettability is the most likely option. Eutectic mixture formation was also suggested for PL F68 solid dispersion with other drugs (El Maghraby and Alomrani, 2009). The rapid dissolution obtained in case of the ternary system can be thus explained on the basis that both polymers enhance the dissolution by different mechanisms which provide a chance for synergism upon combination.

The in situ rabbit intestinal absorption of gliclazide was monitored to determine the membrane transport parameters of the drug and to investigate any possible absorption enhancing or retarding effects of the tested polymers. The membrane transport parameters of pure drug solution through jejunoileum indicated complete absorption of the drug from this segment. This is evidenced by the recorded value of the L95 which was shorter than the total length of the segment. Further evidence is also indicated from the positive value of the ARL. The recorded value of the absorptive clearance per unit length in case of the colon was close to that obtained in case of jejunoileum ($p > 0.05$). However, the length required for complete drug absorption from the colon was longer than the actual colon length. Thus the ARL was negative. The negative value of the ARL is an indication for incomplete absorption from such segment (Osman and El Maghraby, 2006). The overall data obtained after perfusion of the pure drug indicates that the small intestine is the main site of absorption of gliclazide and complete absorption can be achieved through it. Taking this into consideration with the recorded poor dissolution of the pure drug, it could be concluded that bioavailability of the drug is dissolution rate limited.

The effect of water flux on the absorptive clearance of the drug (fig. 7) revealed possible role for both paracellular and transcellular pathways. The former was revealed from linear regression of the absorptive clearance versus water flux plots which indicted that the slope is significantly different from zero (Osman and El Maghraby, 2006). The latter was indicated from the intercept of the line which was also significantly different from zero. Taking the value of the intercept (fig. 7) into consideration with the recoded value for the PeA/L (table 3), the transcellular pathway can be considered as the main mechanism operating. This comment was based on the fact that the intercept represent the transport in absence of any water flux. The colonic absorption showed the same pattern.

Perfusion of the drug in presence of PEG 6000 did not affect the membrane transport parameters of the drug with the drug permeating through the same permeation pathways. This confirms further the inertness of PEG. However, perfusion of the drug solution in presence of both PEG and PL F68 (ternary system, T2) resulted in a clear trend of increased intestinal permeability of the drug compared to that recorded in cases of pure drug or drug-PEG system. The recorded trend indicates a potential penetration enhancing effect for PL F68. However, higher concentrations of PL F68 may be required to achieve significant effect. The mechanism of absorption enhancing effect of PL F68 can be attributed to inhibition of P-glycoprotein efflux pump (Huang *et al*., 2008). In addition, being surfactant, it can perturb the intestinal membrane with the result that the intestinal permeability of drugs is increased.

CONCLUSION

Preparation of solid binary and ternary solid dispersion of gliclazide with PEG and or PL F68 significantly enhanced the dissolution rate of the drug compared to the pure drug. The ternary system containing the drug with PEG and PL (1 : 2 : 2) was the most effective with most of the drug being released in the first few minutes. No interaction between the drug and the polymers was recorded. There was only a trend for an absorption enhancing effect of PL F68 at the tested concentration.

ACKNOWLEDGMENT

The authors acknowledge the Research Center, College of Pharmacy, King Saud University for sponsoring this work (Grant number: C.P.R.C.238). The authors also extend

gratitude to TABUK Pharmaceutical Mfg. Co. for providing the researchers with free sample of gliclazide, Mr. Kamal Altayeb and Mr. Osama Bader-Edeen for their technical support.

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