

Full Length Research Paper

Formulation and evaluation of binary and ternary solid dispersions of domperidone by solvent evaporation method

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First-pass metabolism affects many oral medications and limits the attainment of their therapeutic level. It can be bypassed by administering buccal dosage forms that allow systemic drug absorption via buccal mucosa. Drugs formulated as buccal medicaments should have an acceptable solubility in saliva. Numerous technologies had been experimented to increase the aqueous solubility of poorly water-soluble drugs e.g. solid dispersion technique. This technique is efficient for improving the solubility and dissolution rate of hydrophobic drugs and consequently improving their bioavailability. Domperidone is an antiemetic drug that undergoes extensive first-pass metabolism, having poor solubility in saliva and poor bioavailability. This study aimed to improve the aqueous solubility of domperidone at pH simulating saliva by preparing multicomponent solid dispersions using different carriers by solvent evaporation method. *In vitro* dissolution studies showed enhanced dissolution rates of all prepared systems with release kinetics approaching Higuchi model. Ternary solid dispersion (SD) of 1:9:0.25 drug/polyvinylpyrrolidone K30/pluronic F-127, respectively, achieved the highest dissolution rate. Physicochemical characterization of this SD using differential scanning calorimetry, Fourier-transform infrared spectroscopy, powder X-ray diffraction and scanning electron microscopy indicated the presence of an interaction between domperidone and polyvinylpyrrolidone K30 with evidence of drug amorphization that might be responsible for the enhanced dissolution rate.

Key words: Domperidone, polyvinylpyrrolidone K30, pluronic F-127, solvent evaporation method, multicomponent solid dispersions, physicochemical characterization.

INTRODUCTION

First-pass metabolism is the most popular disadvantage of the orally administered drugs where this pathway affects drug bioavailability (Waite and Keenan, 2010). Alternative non-enteral routes of administration can overcome this metabolic pathway allowing the systemic drug absorption, thereby increasing its bioavailability and decreasing metabolite production e.g. sublingual, rectal, inhalation, intravenous, intramuscular and transdermal routes (Rose and Golan, 2008).

For a drug to be absorbed, it should have an acceptable solubility at the absorption site. Since many drugs discovered by the technological innovation of combinatorial chemistry are poorly water-soluble entities, it is often difficult to adopt them as candidates for pharmaceutical preparations (Ohara et al., 2005). Therefore, several techniques were developed to improve the aqueous solubility of these drugs. The most popular approach is the incorporation of the active hydrophobic component into

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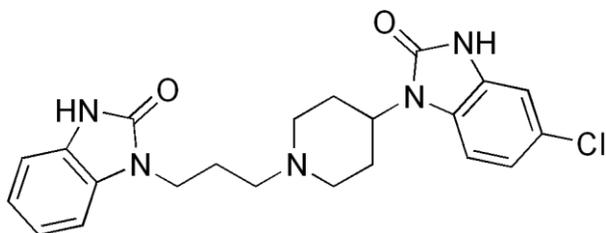


Figure 1. Chemical structure of domperidone.

solid dispersions (Farizon et al., 2013), inclusion complexes (Sreenivasa et al., 2012), inert lipid vehicles (Mirza et al., 2010), surfactant dispersion (Jagdale et al., 2012), self-emulsifying formulations (Zhao et al., 2012), dry emulsions (Ge et al., 2008) and niosomes (Samyuktha and Vedha, 2011).

Chiou and Riegelman (1971) define solid dispersion systems as "the dispersion of one or more active ingredients in an inert carrier matrix at solid state". Solid dispersions can be prepared by different methods using different water-soluble carriers. These solid systems exhibit enhanced solubility and dissolution rate compared to the plain drug that may be attributed to the molecular/colloidal dispersion of drug in mixture, absence of aggregation of drug particles, particle size reduction, improved wettability and dispersability and polymorphic transformation of drug crystals (Chiou and Riegelman, 1971; Leuner and Dressman, 2000; Dua et al., 2009). Enhancement of solubility may contribute directly to the improved bioavailability of poorly water-soluble drugs.

Domperidone (DMP), the model drug of this research, is an antiemetic drug that has the chemical structure of 5-Chloro-1-[1-[3-(2-oxobenzimidazol-1-yl)propyl]-4-piperidyl] benzimidazol-2-one (Figure 1). It is described as a peripheral antidopaminergic drug that is mainly used as an antiemetic for the treatment of nausea and vomiting of various etiologies. DMP has low systemic bioavailability of about 13 to 17% of the orally administered dose due to the extensive hepatic and intestinal metabolism (Rose, 2004). Different attempts were performed to improve DMP solubility and hence its bioavailability. For example, Patel et al. (2011) examined the solubility enhancement of domperidone using different carriers e.g. PEG 4000, PEG 6000 and Myrj 52 by melt granulation technique. In addition, multicomponent inclusion complexes of DMP were prepared using native cyclodextrin, cyclodextrin derivatives, hydroxypropyl cellulose, citric acid and other polymers by kneading method resulting in almost 92 to 100% of domperidone released after 5 to 60 min (Ghodke et al., 2009; Swami et al., 2010; Chavan et al., 2011).

The objective of the present study was to improve the solubility and dissolution rate of DMP in phosphate buffer of pH 6.8 by the formulation of solid dispersions (SDs). This pH was selected to simulate salivary pH that ranges from 5.5 to 7.0 (Hildegard et al., 2007) in order to incorporate

the prepared solid dispersion later into buccal dosage forms. These SDs were prepared by solvent evaporation method using different water-soluble carriers in different weight ratios. *In vitro* dissolution studies were performed to select the best formula that in turn would be physicochemically characterized by differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD) and scanning electron microscopy (SEM). To survey more precisely the mechanism of drug release from the optimized SDs, their release data were fitted to zero order, first order and Higuchi kinetic model.

MATERIALS AND METHODS

Domperidone was given as a gift from Delta Pharma Company for Pharmaceutical Industries, Cairo, Egypt. Dichloromethane was purchased from Fisher Scientific LTD, Leicestershire, UK. Polyvinylpyrrolidone K30 was supplied by Himedia laboratories PVT, LTD, Mumbai, India. Methanol AR, monobasic potassium hydrogen phosphate, sodium hydroxide pellets and urea were obtained from EL Gomhouria Co., Cairo, Egypt. Anhydrous calcium chloride and pluronic- F127 were supplied by sigma-aldrich Inc., Missouri, USA. Polyethylene glycol 8000 was purchased from Scharlau Chemie, S.A., Barcelona, Spain. Hydroxypropyl methylcellulose E50 LV was supplied by LOBA Chemie PVT, LTD, Mumbai, India. All other ingredients were of analytical grade.

Phase solubility studies

An excess amount of DMP was added to 20 ml carrier solutions ranging in concentration from 1% to 5% w/v prepared in phosphate buffer solution that was adjusted at pH 6.8 using 0.2 M sodium hydroxide solution in a series of 50 ml stoppered glass bottles. The prepared suspensions were shaken at 25°C for 7 days in Julabo thermostatically controlled shaking water bath (Julabo SW 20C, Osaka, Japan). After equilibrium being achieved, aliquots were withdrawn, filtered through 0.45 µm syringe filters (0.45 PTFE, Thermo Scientific Chromacol, Leicestershire, UK) and assayed spectrophotometrically at wavelength of 284 nm using Shimadzu UV/VIS spectrophotometer (UV- 1650 PC, Shimadzu Corporation, Kyoto, Japan). DMP content was determined using the regression equation of the standard curve that was developed in the same medium. Blank solutions were performed in the same concentrations of the respective carriers in pH 6.8 phosphate buffer solution. In addition, the solubility of DMP alone was also determined by the same procedure mentioned earlier (Shinde et al., 2010).

To investigate the effect of the auxiliary substances e.g. PL F-127, HPMC E50 LV and PEG 8000 on DMP solubility, the previously mentioned solubility phase study was performed using phosphate buffer solution containing 5% w/v PVP K30 and increasing consecration of PL F-127 (ranging from 2 to 4.5% w/v), HPMC E50 LV and PEG 8000 (ranging from 0.5 to 2% w/v).

Preparation of SDs by solvent evaporation method

To prepare SDs of DMP with PEG 8000, urea and PVP K30 in weight ratios of 1:1, 1:5 and 1:9; an appropriate amount of carrier was added to a solution of DMP in methanol and dichloromethane (1:1 v/v). This solution was stirred on a magnetic stirrer (1200, Jenway, Staffordshire, UK) for 2 h at room temperature and maintained in open trays for at least 12 h to allow slow evaporation of solvent

(Khan et al., 2000). After drying overnight, solid residue was scratched, dried in a vacuum oven for 24 h at room temperature, pulverized and sieved using Tongxin 45-mesh sieve (TX Tongxin, Henan, China).

Powdered samples were stored in closed containers away from the light and humidity and kept in a desiccator containing anhydrous calcium chloride as a dehydrating agent until further evaluation. SDs containing DMP, PVP K30 and PL F-127 in weight ratios of 1:9:0.125, 1:9:0.25 and 1:9:0.5 were prepared as mentioned earlier. SDs containing DMP, PVP K30, HPMC E50 LV or PEG 8000 in weight ratios of 1:9:2.25, 1:9:4.5 and 1:9:9 were similarly prepared.

Preparation of physical mixtures (PMs)

PMs were prepared by simple trituration of the drug and carriers with their respective weight ratios in a porcelain mortar for 5 min. PMs were sieved and stored as mentioned earlier until use (Gill et al., 2010).

Determination of drug content uniformity of the prepared systems

Powdered samples equivalent to 10 mg of DMP were accurately weighed, dissolved in 50 ml of phosphate buffer (pH 6.8) and stirred on a magnetic stirrer for 15 min. These solutions were filtered through 0.45 μm syringe filters, diluted and assayed spectrophotometrically at wavelength of 284 nm for DMP content.

In vitro dissolution studies

In vitro dissolution studies of plain DMP, SDs and PMs were performed using dissolution USP apparatus II (rotating paddle) (SOTAX AT7 smart, Allschwil, Switzerland). The dissolution medium consisted of 500 ml of phosphate buffer (pH 6.8). The stirring speed was 100 rpm and temperature was maintained at $37 \pm 0.5^\circ\text{C}$. Powdered samples of each preparation equivalent to 10 mg of DMP were sprinkled on the surface of the dissolution medium. At the appropriate time intervals for a period of 60 min, 3 ml aliquots were withdrawn from the dissolution medium through 0.45 μm syringe filters and replaced with an equivalent amount of fresh medium to keep the volume constant. Concentrations of DMP were determined spectrophotometrically at wavelength of 284 nm. Each experiment was carried out in triplicates to determine the mean and the standard deviation.

The dissolution profiles were evaluated according to four parameters: (i) initial dissolution rate (IDR) that was calculated as the percentage of drug dissolved over the first 15 min/min; (ii) percentage of drug dissolved after 2 min (PD_2); (iii) percentage of drug dissolved after 10 min (PD_{10}) and (iv) dissolution efficiency ($\text{DE}_{60\%}$) parameter after 60 min (Sammour et al., 2006). Only PD_2 data are shown since they were statistically analyzed using SPSS[®] computer software program (version 16.0, SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) test was performed to investigate the significant difference between the tested carriers and their effects on the PD_2 at 95% confidence limit.

Kinetic studies

To survey more precisely the mechanism of drug release from the optimized SDs; their release data were fitted to zero order, first order and diffusion controlled kinetic equations (Schwartz et al., 1968).

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of the pure drug, optimized ternary SD, its PM and their individual components were obtained using JASCO FTIR spectrophotometer (FTIR 4100, JASCO, Essex, UK) operated with potassium bromide disc technique. FTIR analysis was performed using a pressure of 6 to 8 tons, die size of 13 mm, scanning range of 400 to 4000 cm^{-1} and resolution of 1 cm^{-1} .

Differential scanning calorimetry (DSC)

DSC analysis was performed using Shimadzu differential scanning calorimeter (DSC-50, Shimadzu Corporation, Kyoto, Japan). Samples (1.5 to 2.5 mg) were heated in a hermetically sealed aluminum pans at a temperature ranged from 30 to 300 $^\circ\text{C}$ and constant rate of 10 $^\circ\text{C min}^{-1}$ under a nitrogen purge (30 ml/min).

Powder X-ray diffraction (PXRD)

PXRD patterns were obtained using XGEN X-ray powder diffractometer (XGEN 4000, Scintage Inc., California, USA) supplied with $\text{CuK}\alpha$ radiation. Diffractograms were run at a scanning rate of 1.8 $^\circ \text{min}^{-1}$ and the scanning scope was over a range of 2θ angle from 0 to 80 $^\circ$ at room temperature.

A relationship was established between some representative peak heights in the diffraction patterns of the ternary systems and those of a reference substance (that is, plain drug). This relationship was translated into the following equation that calculates the relative degree of crystallinity (RDC) in order to monitor crystallinity improvement at a designated 2θ value:

$$\text{RDC} = \text{Isam}/\text{lref}$$

where Isam is the peak height of the sample under investigation and lref is the peak height for the reference substance (that is, plain drug) at the same angle of the highest intensity (Ryan, 1986; Bahti et al., 2012).

Scanning electron microscopy (SEM)

SEM was carried out using JEOL Electron Probe Microanalyzer (JXA-840A, JEOL, Tokyo, Japan) to study the morphological characteristics of the optimized ternary SD and its PM compared to pure DMP. The selected samples were mounted on double-sided adhesive tape. Gold coating was applied on the surface of particles before examination to render the surface electroconductive.

RESULTS AND DISCUSSION

Phase solubility studies

After UV scanning of DMP in phosphate buffer (pH 6.8), the maximum absorption of DMP in such medium was at a wavelength of 284 nm which in accordance with what was found by Chavan et al. (2012). Figure 2 shows the effect of different carriers (PVP K30, urea and PEG 8000) on the solubility of DMP in phosphate buffer pH 6.8 at $25 \pm 0.5^\circ\text{C}$ according to the phase solubility technique established by Higuchi and Connors (1965). Correlation coefficients (R^2) were 0.9875, 0.9969 and 0.9447 for phase solubility diagrams of DMP with PVP K30, urea

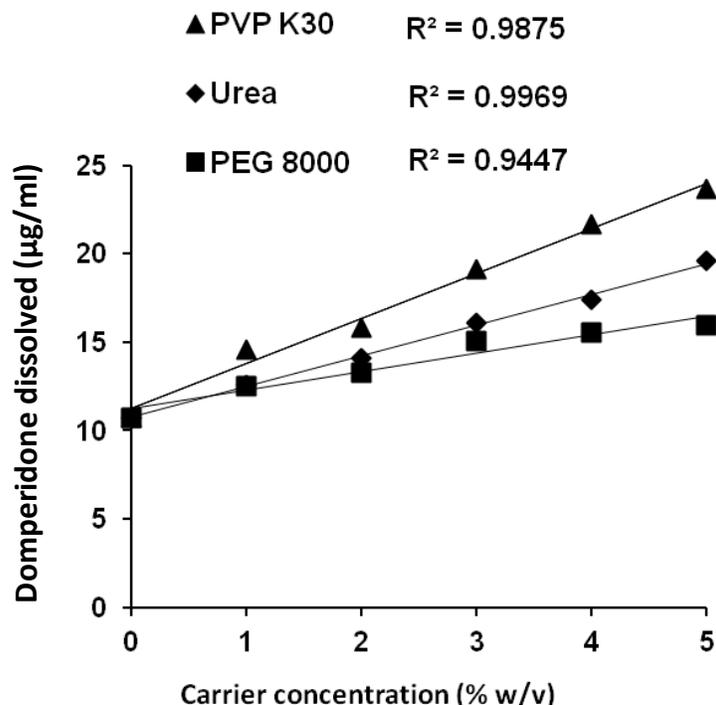


Figure 2. Phase solubility diagram of DMP in phosphate buffer pH 6.8 at $25\pm 0.5^\circ\text{C}$ in the presence of increased concentrations of PVP K30, urea and PEG 8000.

and PEG 8000, respectively. The solubility of DMP was found to be $10.73\ \mu\text{g/ml}$ and linearly increased as the carrier concentration was increased suggesting the features of an A_L -type solubility phase diagram.

At 5% w/v of PVP K30, urea and PEG 8000, DMP solubility increased by 2.20, 1.83 and 1.48 folds, respectively (Table 1). Consequently, these carriers can be ranked according to their effect on increasing DMP solubility as $\text{PVP K30} > \text{urea} > \text{PEG 8000}$. The increment of drug solubility can be explained by solubilization effect of carriers, their improving influence on drug wettability and through the formation of soluble complexes between hydrophobic drug and hydrophilic carrier (Bhole and Patil, 2009; Shah et al., 2012).

The phase solubility diagram obtained for DMP in 5% w/v PVP K30 solutions and increased concentrations of PL F-127, HPMC E50 LV and PEG 8000 is as shown in Figure 3. The addition of other polymers along with 5% w/v PVP K30 resulted in increasing drug solubility from $23.73\ \mu\text{g/ml}$ in the presence of 5% w/v PVP K30 alone up to $33.70\ \mu\text{g/ml}$ at 4% w/v PL F-127, $26.37\ \mu\text{g/ml}$ at 1% w/v HPMC E50 LV and $29.09\ \mu\text{g/ml}$ at 1% w/v PEG 8000. This might be due to the higher improvement of drug wettability and dispersibility compared to the effect of single polymer. Furthermore, the addition of PL F-127 reduced the interfacial tension between the hydrophobic drug and dissolution medium resulting in enhancing the wettability of drug particles (Dumortier et al., 2006). Higher concentration of these polymers led to a decrement

of drug solubility due to the increased viscosity of the diffusion boundary layer adjacent to the dissolving surface. Previous expectation was confirmed by the dissolution data of ternary systems.

The apparent stability constant of the resulted complexes could not be calculated since the exact drug/polymer stoichiometric ratio was not known.

Drug content uniformity of the prepared systems:

The drug content ranged from 9.80 to 53.63% and from 7.00 to 99.25% for PMs and SDs, respectively (Table 2). The drug content percent of ternary SDs was found to be within the pharmacopoeia limit (85 to 115%) (European Pharmacopoeia, 2011) indicating the effective impact of ternary polymers on drug dispersion.

In vitro dissolution studies

Dissolution profiles of the prepared systems are demonstrated in Figures 4 to 9 and the statistically analyzed PD_2 data are presented in Table 3.

It was evident that the pure drug exhibited a slow dissolution even after 60 min where the percentage of drug dissolved after 60 min only reached about $6.54\pm 2.66\%$ that could be related to the hydrophobicity, poor wettability and/or agglomeration of DMP particles resulting

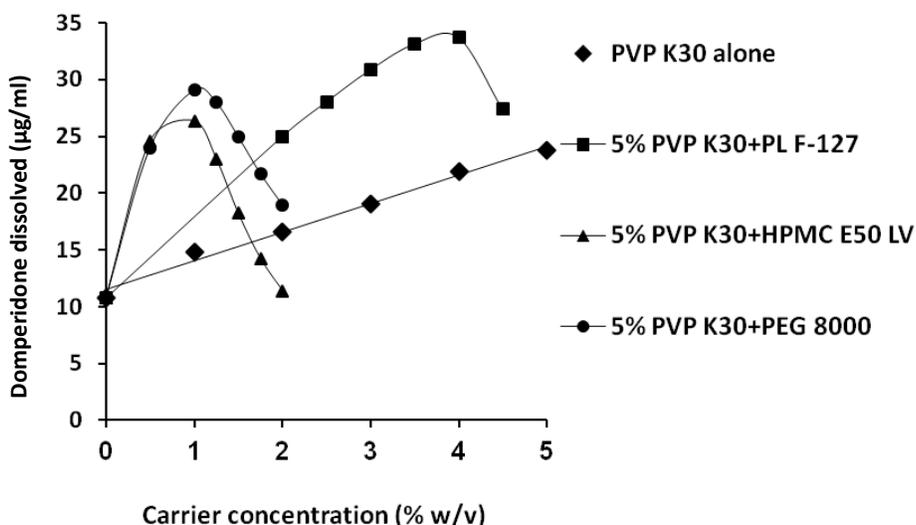


Figure 3. Phase solubility diagram of DMP in phosphate buffer pH 6.8 at $25\pm 0.5^\circ\text{C}$ in the presence of 5% w/v PVP K30 and increased concentrations of PL F-127, HPMC E50 LV and PEG 8000.

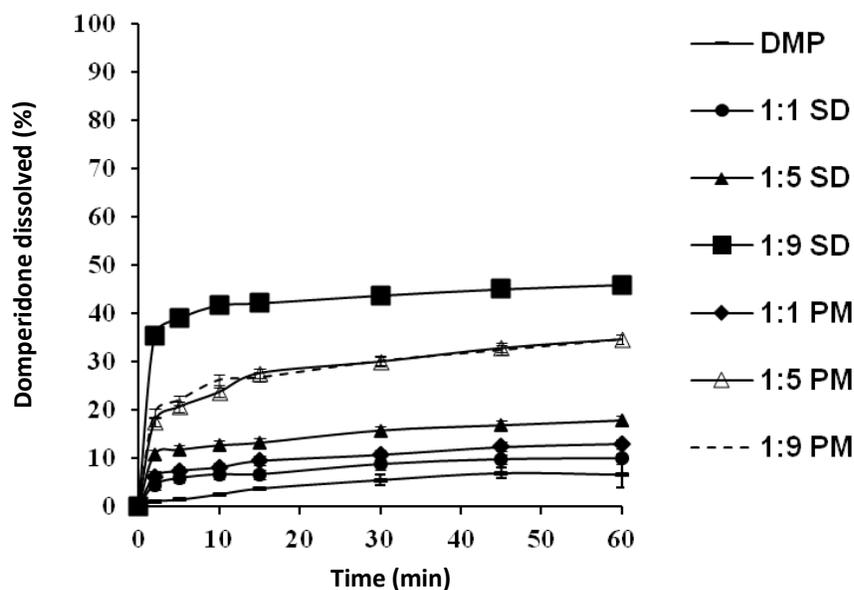


Figure 4. Dissolution profiles of domperidone from different domperidone/PEG 8000 solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at $37\pm 0.5^\circ\text{C}$.

in floating of drug powder on the surface and consequently hindering its dissolution. On the contrary, PMs as well as SDs immediately sank to the bottom of the dissolution vessels.

All carriers had significant effects on PD_2 where the P value was less than 0.05. As general observations, the dissolution rate of DMP from all PMs was higher than that of the pure drug. The increased dissolution rate might be attributed to the increased wettability and dispersibility of

DMP where the dry mixing brought the drug in close contact with the hydrophilic carrier (Gauri et al., 2011). Similarly, all SDs showed enhanced dissolution rate as compared to pure DMP that might be due to the effect of hydrophilic carriers on drug wettability. Other explanations were related to the solubilization, molecular/colloidal dispersion of drug in the mixture and reduction in the drug crystallinity (that is, polymorphic transformation of drug crystals) that were obtained via the formulation of

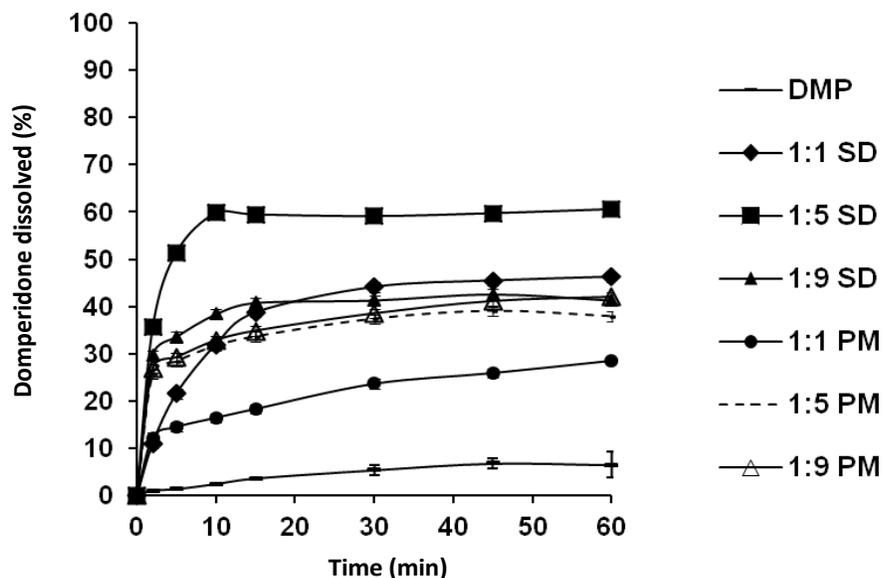


Figure 5. Dissolution profiles of domperidone from different DMP/urea solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at $37\pm 0.5^\circ\text{C}$.

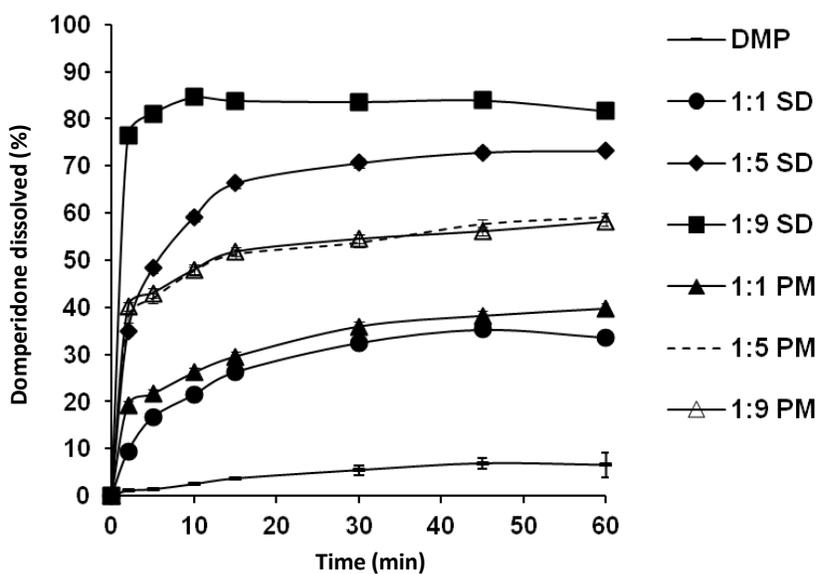


Figure 6. Dissolution profiles of domperidone from different DMP/PVP K30 solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at $37\pm 0.5^\circ\text{C}$.

formulation of solid dispersions (Akiladevi and Basak, 2010; Muralidhar et al., 2010; Prasad et al., 2010).

Binary solid dispersions

Domperidone/PEG 8000 systems

PD_2 was significantly enhanced by increasing PEG 8000

concentration in all drug/PEG 8000 systems ($p < 0.05$) till it reached the highest value for 1:9 SD where PD_2 was 35.32 ± 0.71 (Table 3 and Figure 4).

Domperidone/Urea systems

As shown in Table 3 and Figure 5, PD_2 of drug/urea SDs was significantly increased by increasing urea concentration

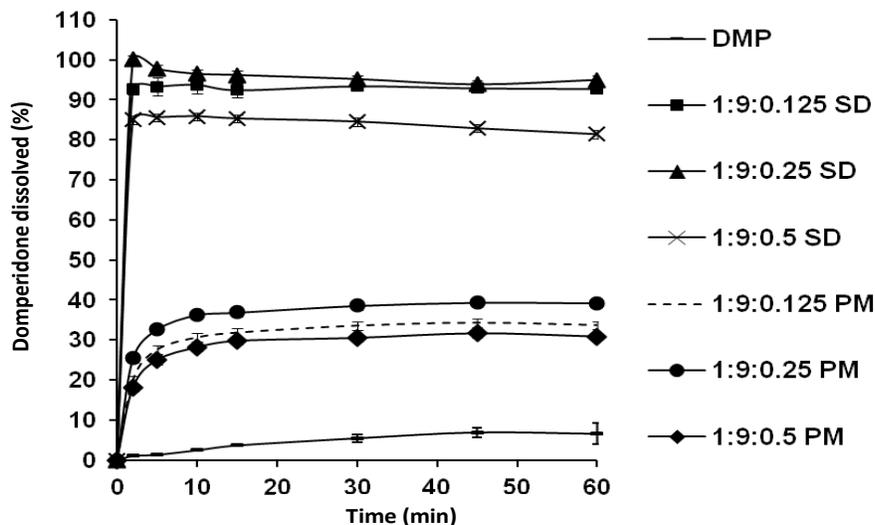


Figure 7. Dissolution profiles of DMP from different DMP/PVP K30/PL F-127 solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at $37\pm 0.5^\circ\text{C}$.

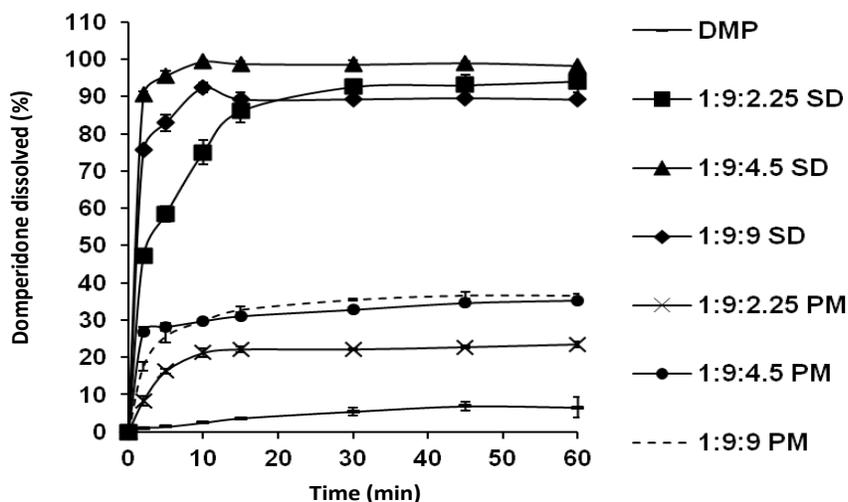


Figure 8. Dissolution profiles of DMP from different DMP/PVP K30/HPMC E50 LV solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at $37\pm 0.5^\circ\text{C}$.

concentration up to 1:5 weight ratio ($p < 0.05$) where PD_2 was 35.55 ± 0.31 . After this particular ratio, further increase of urea concentration (that is, 1:9 SD) resulted in a significant decrement of DMP dissolution rate ($p < 0.05$) where PD_2 of 1:9 SD was 29.75 ± 1.22 . This might be due to the long time that was consumed by the higher amount of carrier to dissolve (Arora et al., 2010).

Domperidone/PVP K30 systems

According to the *in vitro* dissolution studies, SD of 1:9 DMP/PVP K30 had the highest significant dissolution rate

($p < 0.05$) compared to other SDs where its PD_2 value was 76.37 ± 0.23 (Table 3 and Figure 6). Therefore, this formula was selected to be reformulated as ternary systems using additional water-soluble carriers e.g. PL F-127, HPMC 50 LV and PEG 8000 in different weight ratios by solvent evaporation method.

Ternary solid dispersions

Domperidone/PVP K30/Pluronic F-127 systems

Ternary systems containing PL F-127 showed significant

Table 1. Solubility diagram data of DMP in solutions of different carriers at 25±0.5°C.

| Parameter | PVP K30 ^c | Urea | PEG 8000 ^d |
|---------------------------------|----------------------|----------------|-----------------------|
| Phase solubility diagram type | A _L | A _L | A _L |
| Solubility (µg/ml) ^a | 23.64 | 19.62 | 15.93 |
| Solubility factor ^b | 2.20 | 1.83 | 1.48 |

^aSolubility of DMP in the presence of 5% w/v carrier concentration.

^bSolubility factor=total solubility of DMP in the presence of 5% w/v carrier concentration/intrinsic solubility of DMP. ^cPolyvinylpyrrolidone K30 and ^dPolyethylene glycol 8000.

Table 2. Drug content uniformity of different DMP systems.

| Formula | Weight ratio | Drug content (%) | |
|---|--------------|------------------|-----------------|
| | | PM ^e | SD ^f |
| DMP ^a /PEG 8000 ^a | 1:1 | 9.80 | 7.00 |
| | 1:5 | 27.81 | 15.01 |
| | 1:9 | 28.01 | 42.22 |
| DMP/Urea | 1:1 | 18.61 | 39.42 |
| | 1:5 | 34.62 | 60.83 |
| | 1:9 | 37.02 | 41.82 |
| DMP/PVP K30 ^b | 1:1 | 30.62 | 26.61 |
| | 1:5 | 53.63 | 67.23 |
| | 1:9 | 52.43 | 85.04 |
| DMP/PVP K30/PL F-127 ^c | 1:9:0.125 | 32.42 | 93.85 |
| | 1:9:0.25 | 37.42 | 97.85 |
| | 1:9:0.5 | 30.22 | 86.64 |
| DMP/PVP K30/HPMC E50 LV ^d | 1:9:2.25 | 23.01 | 89.04 |
| | 1:9:4.5 | 31.82 | 99.25 |
| | 1:9:9 | 33.62 | 91.25 |
| DMP/PVP K30/PEG 8000 | 1:9:2.25 | 28.61 | 85.04 |
| | 1:9:4.5 | 27.81 | 85.84 |
| | 1:9:9 | 27.41 | 96.25 |

enhanced dissolution behaviors ($p < 0.05$) by increasing the concentration of PL F-127 reaching maximum PD_2 at weight ratio of 1:9:0.25 DMP/PVP K30/PL F-127 SD (PD_2 was 100.08 ± 1.66) (Table 3 and Figure 7). This might be due to the surfactant property and the great hydrophilicity of PL F-127 resulting in a reduction of the interfacial tension between DMP and dissolution medium, surface availability for rapid dissolution and hence greater wettability of the drug (Patil et al., 2010).

Higher concentration of PL F-127 led to a significant decrease in the percentage of drug dissolved ($p < 0.05$). This might be related to the gelling property of PL F-127 at higher concentration which increases the viscosity of

the diffusion boundary layer adjacent to the dissolving surface e.g. PD_2 was 18.14 ± 1.42 for the PM of 1:9:0.5 DMP/PVP K30/PL F-127 and 84.98 ± 0.46 for the respective SD (Park et al., 2003).

Domperidone/PVP K30/HPMC 50 LV systems

Increasing the concentration of HPMC E50 LV up to a certain level resulted in significant enhanced dissolution rate of the drug ($p < 0.05$) (Table 3 and Figure 8). For example, PD_2 values were 26.95 ± 1.10 and 90.51 ± 0.83 for PM and SD of 1:9:4.5 DMP/PVP K30/HPMC E50 LV,

Table 3. Percentage of drug dissolved after 2 min (PD₂) in phosphate buffer pH 6.8 of different DMP systems at 37±0.5°C (mean±SD, n=3).

| DMP ^a | | | | 1.07 ± 0.23 | | | |
|---------------------------|-----------------|-----|------------|--|-----------|------------|-------------|
| Binary systems | | | | Ternary systems | | | |
| DMP/PEG 8000 ^b | PM ^c | 1:1 | 6.20 ±0.20 | DMP/PVP K30/PL F-127 ^f | 1:9:0.125 | 20.08±0.64 | |
| | | 1:5 | 17.61±0.20 | | PM | 1:9:0.25 | 25.41±1.51 |
| | | 1:9 | 19.43±0.31 | | | 1:9:0.5 | 18.14±1.42 |
| | SD ^d | 1:1 | 4.47±0.42 | | | 1:9:0.125 | 92.71±1.45 |
| | | 1:5 | 10.74±1.72 | | SD | 1:9:0.25 | 100.08±1.66 |
| | | 1:9 | 35.32±0.71 | | | 1:9:0.5 | 84.98±0.46 |
| DMP/Urea | PM | 1:1 | 12.27±1.03 | DMP/PVP K30/HPMC E50 LV ^g | 1:9:2.25 | 8.34±1.29 | |
| | | 1:5 | 25.68±0.31 | | PM | 1:9:4.5 | 26.95±1.10 |
| | | 1:9 | 26.88±0.64 | | | 1:9:9 | 17.61±1.11 |
| | SD | 1:1 | 11.00±2.43 | | | 1:9:2.25 | 47.29±0.64 |
| | | 1:5 | 35.55±0.31 | | SD | 1:9:4.5 | 90.51±0.83 |
| | | 1:9 | 29.75±1.22 | | | 1:9:9 | 75.64±0.72 |
| DMP/PVP K30 ^e | PM | 1:1 | 19.21±1.00 | DMP/PVP K30/PEG 8000 | 1:9:2.25 | 12.27±1.17 | |
| | | 1:5 | 37.75±2.53 | | FM | 1:9:4.5 | 21.74±0.31 |
| | | 1:9 | 40.15±1.52 | | | 1:9:9 | 11.14±2.60 |
| | SD | 1:1 | 9.40±0.20 | | | 1:9:2.25 | 82.04±3.29 |
| | | 1:5 | 34.95±0.31 | | SD | 1:9:4.5 | 88.64±0.40 |
| | | 1:9 | 76.37±0.23 | | | 1:9:9 | 88.24±1.83 |

^aDomperidone; ^bPolyethylene glycol 8000; ^cPhysical mixture; ^dSolid dispersion; ^ePolyvinylpyrrolidone K30; ^fPluronic F-127 and ^gHydroxypropyl methylcellulose E50 LV.

respectively.

HPMC is a hydrophilic swellable polymer that is responsible for the formation of highly viscous gelatinous barrier diffusion layer at the interface of drug and dissolution medium (Sarkar et al., 2012). Accordingly, further increment of HPMC concentration up to 1:9:9 weight ratio of drug/PVP K30/HPMC E50 LV resulted in a significant decrease in the dissolution rate of PM and SD ($p < 0.05$) where the drug was released slowly from such matrix by diffusion process (Yogesh et al., 2007; Roni et al., 2011b). For example, PD₂ values were 17.61±1.11 and 75.64±0.72 for PM and SD of 1:9:9 weight ratio, respectively.

Domperidone/PVP K30/PEG 8000 systems

As presented in Table 3 and Figure 9, ternary systems containing PEG 8000 as a second polymer showed a significant increment of PD₂ of DMP up to 1:9:4.5 weight ratio of drug/PVP K30/PEG 8000 ($p < 0.05$). In case of PM, PD₂ of 1:9:9 SD was significantly lower than that of 1:9:4.5 SD ($p < 0.05$). The explanation of this phenomenon

might be due to the formation of viscous boundary layer around the drug particles leading to a decrement of DMP dissolution rate (Deshmukh and Jain, 2012). Compared to 1:9:4.5 SD, PD₂ of 1:9:9 SD was decreased with no significant difference between them ($p > 0.05$).

Regarding the *in vitro* dissolution data, it was obviously indicated that drug/carrier ratio was one of the main factors controlling the dissolution performance of the prepared systems (Mehanna et al., 2010). One-way ANOVA (statistical analysis) of PD₂ of different SDs revealed that ternary SD of 1:9:0.25 DMP/PVP K30/PL F-127 exhibited the most significant enhanced PD₂ compared to other SDs ($p < 0.05$). Therefore, this ternary SD would be physicochemically characterized by FTIR, DSC, PXRD and SEM analysis.

Kinetics studies

Changing the drug/carrier ratio has an effect on the mechanism of drug release from its different systems. Treatment of the data according to both zero and first order kinetics gave correlation coefficients lower than

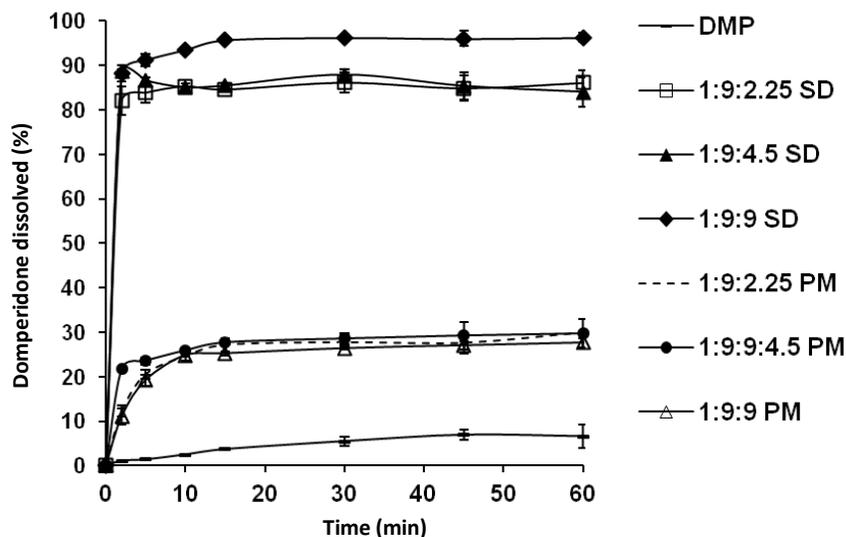


Figure 9. Dissolution profiles of domperidone from different DMP/PVP K30/PEG 8000 solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at $37\pm 0.5^\circ\text{C}$.

those obtained from Higuchi kinetics. Comparing the correlation coefficients (R^2) of the different models of release kinetics indicated that the release of DMP from all prepared systems approaching Higuchi model of the release kinetics, that is, diffusion was the release mechanism of the drug from all systems. Table 4 shows the kinetics data of DMP released from the optimized SDs according to zero order, first order and diffusion (Higuchi) models. For example, R^2 of 1:5 DMP/urea SD was 0.9556 after being calculated according to Higuchi model. Similarly, R^2 values of 1:9 DMP/PVP K30 and 1:9:0.25 DMP/PVP K30/PL F-127 solid dispersions were in accordance with Higuchi model where they were 0.9848 and 0.9523, respectively Table 5.

Fourier-transform infrared spectroscopy (FTIR)

In order to get indication on the feasible interaction of the drug with the studied PVP K30 and PL F-127, FTIR analysis was employed (Figure 10). The FTIR spectrum of plain DMP was characterized by N-H stretching at 3119.3 cm^{-1} , asymmetric C-H stretching at 2939.95 cm^{-1} , symmetric C-H stretching at 2820.38 cm^{-1} , N-H deformation at 1697.05 cm^{-1} , aromatic C-H stretching at 3022.87 cm^{-1} , C=C at 1622.02 cm^{-1} and N=C stretching peak at 1485.88 cm^{-1} . The spectrum of PVP K30 showed C-H stretching band at 2953 cm^{-1} , C=O band at 1666.20 cm^{-1} and a very broad endothermic band at $3048\text{--}3750\text{ cm}^{-1}$ that was related to the presence of water confirming the broad endotherm detected later in DSC study. FTIR spectrum of PL F-127 is characterized by principal absorption peaks of aliphatic C-H stretching at 2886.92 cm^{-1} , in-plane O-H bend at 1355.71 cm^{-1} and C-O stretching

at 1110.8 cm^{-1} .

The FTIR spectra of the optimized ternary SD and PM showed the disappearance of N-H stretching peak of DMP with slight shifting of PVP carbonyl band from 1666.20 to 1664.27 cm^{-1} and 1662.34 cm^{-1} for PM and SD, respectively. This might indicate an intermolecular hydrogen bonding between =NH group of DMP and the C=O band of PVP in the drug-polymer systems (Tantishaiyakul et al., 1996; Ran et al., 2012).

Differential scanning calorimetry (DSC)

As shown in Figure 11, DSC thermogram of DMP presents a sharp endothermic peak at 243.43°C corresponding to the melting point of the drug. A broad endothermic peak corresponding to PVP K30 was observed at 80.15°C that might be attributed to the loss of water from the hygroscopic PVP K30. Pluronic F-127 has an endothermic peak at 57.39°C related to its melting point.

The DSC thermograms of SD and PM showed a disappearance of the drug peak. The absence of DMP endotherm in PM suggested the dissolution of the crystalline drug particles within the molten polymer due to the heating phase during analysis. In case of SD, the absence of DMP endotherm might be due to the formation of solid dispersion of the drug in the presence of water-soluble polymer where the drug could be transformed into an amorphous state. This amorphousness might be related to the intermolecular hydrogen bonding between DMP and PVP K30 and/or loss of drug mobility where the drug was entrapped in polymer after evaporation of solvent (Roni et al., 2011a; Shah et al., 2012).

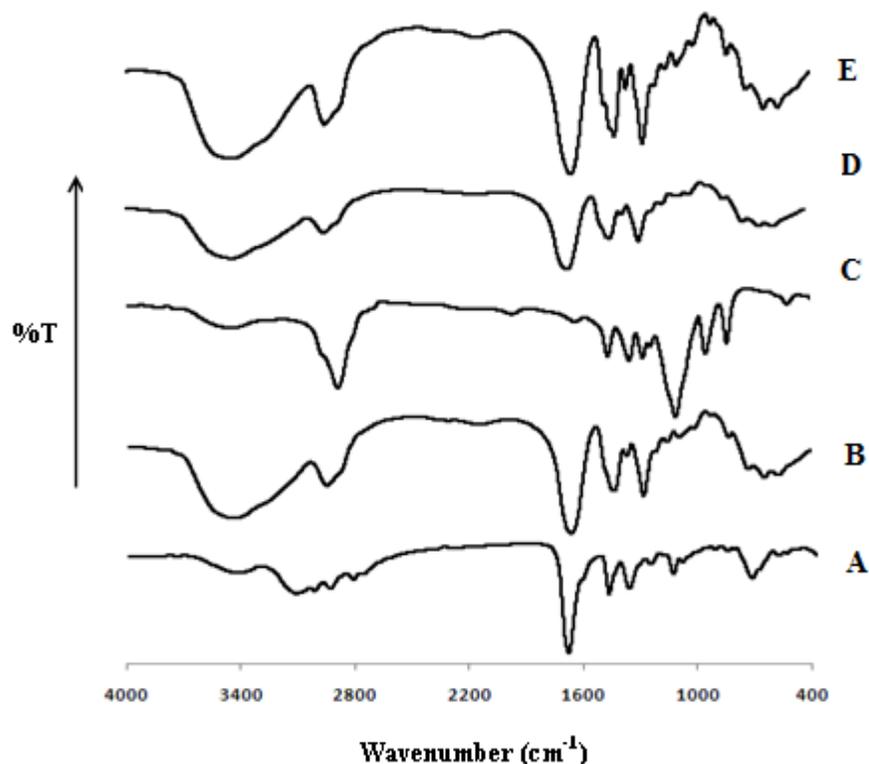


Figure 10. FTIR spectra of (A) pure domperidone (DMP), (B) polyvinyl pyrrolidone K30 (PVP K30), (C) pluronic F-127 (PL F-127), (D) physical mixture of 1:9:0.25 DMP/PVP K30/PL F-127, and (E) solid dispersion of 1:9:0.25 DMP/PVP K30/PL F-127.

Powder X-ray diffraction (PXRD)

Figure 12 shows the PXRD patterns of DMP solid systems. The diffraction spectrum of pure DMP shows its crystalline nature that was demonstrated by numerous sharp, highly intense and less diffused peaks. These peaks were observed at 2θ values of 9.22, 11.77, 13.90, 14.88, 15.53, 19.00, 19.75, 22.58, 24.76, 28.98, 31.47 and 42.61° in finger print regions referring to its crystallinity. A hollow pattern with no diffraction peaks was recorded for PVP K30 indicating its amorphous state. The diffraction spectrum of PL F-127 shows two characteristic peaks at 2θ values of 19.07 and 23.24° indicating the crystalline nature of PL F-127.

The position of characteristic peaks of the crystalline polymer was not changed in PM and SD suggesting no change of its polymorph. PXRD patterns of the ternary PM and SD exhibited 'halo' shaped diffractograms characterizing the amorphous material since the reflexes did not return to the base line. Furthermore, broadening of DMP peaks and reduction of their intensities were observed suggesting the conversion of crystalline DMP to partially disordered molecules (Shah et al., 2012).

Peak height of DMP at 22.6° 2θ was selected to calculate the RDC of DMP, best ternary PM and ternary

SD. When pure DMP was considered as a reference sample, a significant decrement in crystallinity of the characterized ternary systems was observed ($p < 0.05$). RDC values were 1, 0.17 and 0.14 for pure DMP, ternary PM and ternary SD, respectively indicating the amorphousness of DMP and the formation of SD as previously investigated by PXRD patterns.

Scanning electron microscopy (SEM)

SEM micrographs that reveal the surface morphology of scanned samples at 1000X are shown in Figure 13. SEM micrograph of pure DMP shows crystalline particles of rather irregular shape and size (Figure 13A), while the SEM micrograph of PM reveals more identified cotton-shaped powder with crystalline dusts of DMP deposit on the surface (Figure 13B). SD appeared in the form of irregular particles in which the original crystalline morphology of DMP disappeared and small lumps of amorphous pieces of irregular size were present (Figure 13C). This result could be attributed to dispersion of the drug in the polymer matrix confirming the findings based on PXRD patterns. The change in structure might be one of the causes for the increased dissolution rate.

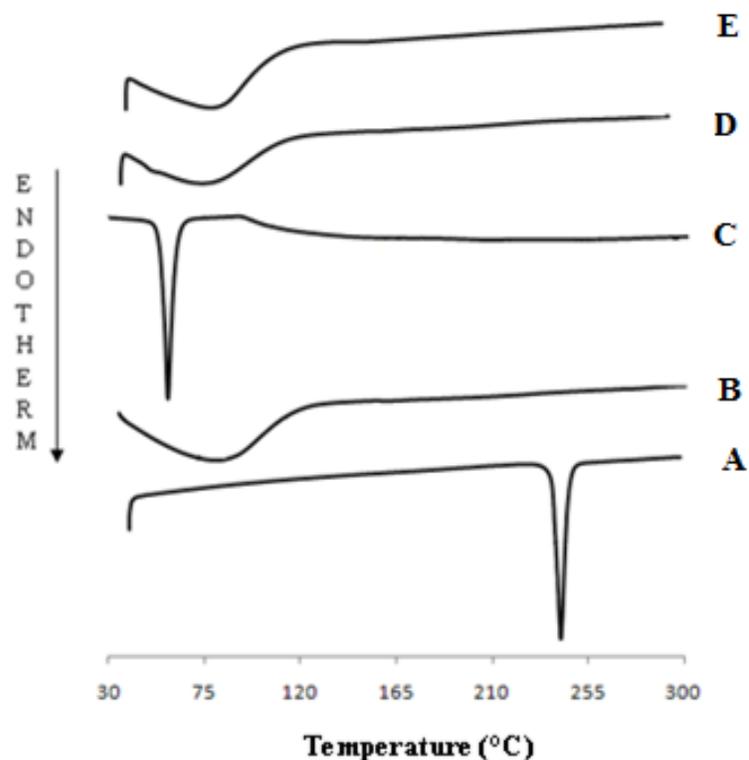


Figure 11. DSC thermograms of (A) pure domperidone (DMP), (B) polyvinyl pyrrolidone K30 (PVP K30), (C) pluronic F-127 (PL F-127), (D) physical mixture of 1:9:0.25 DMP/PVP K30/PL F-127 and (E) solid dispersion of 1:9:0.25 DMP/PVP K30/PL F-127.

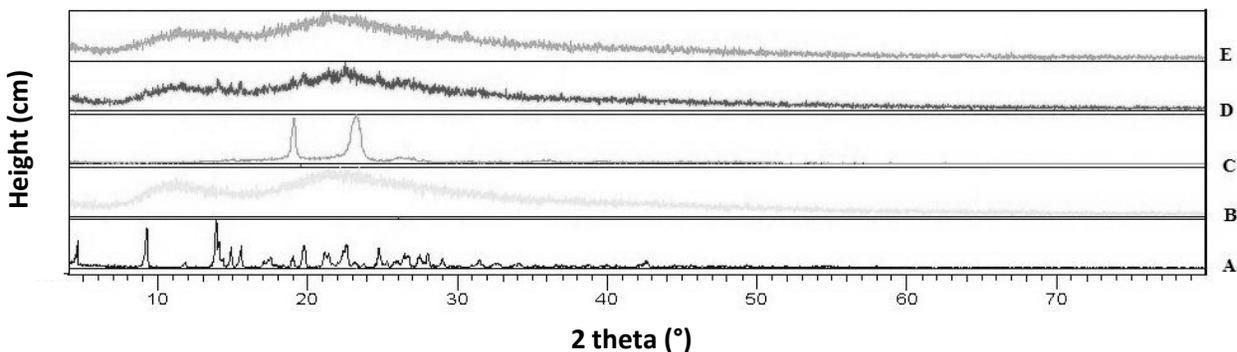


Figure 12. PXRD patterns of (A) pure domperidone (DMP), (B) polyvinyl pyrrolidone K30 (PVP K30), (C) pluronic F-127 (PL F-127), (D) physical mixture of 1:9:0.25 DMP/PVP K30/PL F-127, and (E) solid dispersion of 1:9:0.25 DMP/PVP K30/PL F-127.

Conclusion

This study demonstrated the possibility of improving DMP solubility and dissolution performance by the formulation of solid dispersions. The binary solid dispersion of 1:9 DMP/PVP K30 achieved the highest significant percentage of drug dissolved after 2 min compared to all binary systems. This weight ratio was selected to formulate ternary

solid systems by incorporating other water-soluble carriers. Ternary SD of 1:9:0.25 DMP/PVP K30/pluronic F-127 achieved approximately 100% drug dissolved over the first 2 min. Treatment of dissolution data according to zero, first order and Higuchi model resulted in correlation coefficient values subjected to diffusion release kinetics. *In-vitro* dissolution studies, FTIR, DSC, PXRD and SEM analysis revealed the amorphization of DMP and the

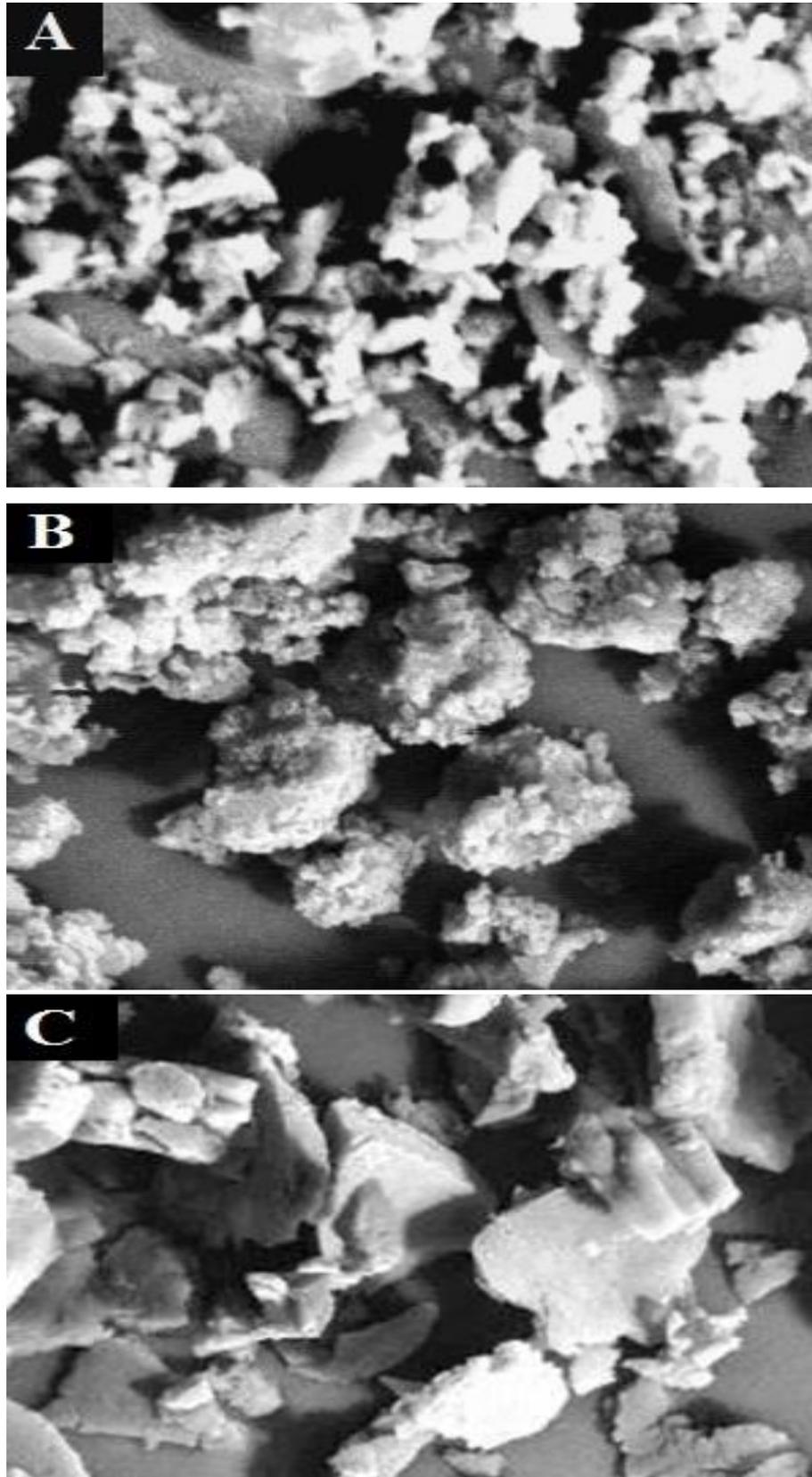


Figure 13. SEM microphotographs of (A) pure domperidone (DMP), (B) physical mixture of 1:9:0.25 DMP/Polyvinyl pyrrolidone K30/Pluronic F-127, and (C) solid dispersion of 1:9:0.25 DMP/Polyvinyl pyrrolidone K30/Pluronic F-127.

Table 4. Kinetics data of DMP release from the optimized solid dispersions.

| Order of release | | Zero order | First order | Higuchi |
|--------------------------------------|--------------|----------------|----------------|----------------|
| Formulae | Weight ratio | R ² | R ² | R ² |
| DMP ^a | pure | 0.9732 | 0.9756 | 0.9888 |
| DMP/PEG 8000 ^b | 1:9 | 0.9543 | 0.9603 | 0.9887 |
| DMP/Urea | 1:5 | 0.9003 | 0.9346 | 0.9556 |
| DMP/PVP K30 ^c | 1:9 | 0.9470 | 0.9709 | 0.9848 |
| DMP/PVP K30/PL F-127 ^d | 1:9:0.25 | 0.8955 | 0.8958 | 0.9523 |
| DMP/PVP K30/HPMC E50 LV ^e | 1:9:4.5 | 0.9572 | 0.9862 | 0.9901 |
| DMP/PVP K30/PEG 8000 | 1:9:4.5 | 0.9446 | 0.9259 | 0.9835 |

^aDomperidone; ^bPolyethylene glycol 8000; ^cPolyvinylpyrrolidone K30; ^dPluronic F-127; and ^eHydroxypropyl methylcellulose E50 LV.

Table 5. Relative degree of crystallinity (RDC) values of domperidone/polyvinylpyrrolidone K30/Pluronic F-127 systems at a degree of 2θ= 22.6°.

| Formula | RDC ^a at 2θ=22.6° |
|---|------------------------------|
| DMP ^b | 1 |
| PM ^c | |
| DMP/PVP K30 ^d /PL F-127 ^e 1:9:0.25 | 0.17 |
| SD ^f | |
| DMP/PVP K30/PL F-127 1:9:0.25 | 0.14 |

^aRelative degree of crystallinity; ^bDomperidone; ^cPhysical mixture; ^dPolyvinylpyrrolidone K30; ^ePluronic F-127 and ^fSolid dispersion.

formation of intermolecular hydrogen bond between the drug and PVP K30 that might be responsible for dissolution enhancement.

REFERENCES

- Akiladevi D, Basak S (2010). Dissolution enhancement of paracetamol by solid dispersion technique. *J. Pharm. Res.* 2(12): 2846-2849.
- Arora SC, Sharma PK, Irchhaiya R, Khatkar A, Singh N, Gagoria J (2010). Development, characterization and solubility study of solid dispersion of cefpodoxime proxetil by solvent evaporation method. *Int. J. Chem. Tech. Res.* 2(2):1275-1280.
- Bhati LK, Tiwari G, Tiwari R, Kumar V (2012). Enhancement of complexation efficiency of meloxicam using binary and ternary solid systems: Formulation and considerations. *Am. J. Drug Dis. Dev.* 2(1):17-31.
- Bhole PG, Patil VR (2009). Enhancement of water solubility of felodipine by preparing solid dispersion using poly-ethylene glycol 6000 and poly-vinyl alcohol. *Asian J. Pharm.* 3(3):240-244.
- Chavan BA, Mali KK, Dias RJ (2012). Formulation and evaluation of melt-in-mouth tablets of domperidone containing multicomponent inclusion complex. *Int. J. Pharm. Pharm. Sci.* 4(1):71-75.
- Chavan BA, Mali KK, Dias RJ, Kate LD (2011). Solid state characterization of multicomponent inclusion complex of domperidone with β-cyclodextrin, polyvinyl pyrrolidone and citric acid. *Der Pharmacia Lettre* 3(5):281-290.
- Chiou WL, Riegelman S (1971). Pharmaceutical applications of solid dispersion systems. *J. Pharm. Sci.* 60: 1281-1302.
- Deshmukh KR, Jain SK (2012). Development of aceclofenac mouth dissolving tablets using solid dispersion technique: In-vitro evaluation. *Ind. J. Pharm. Edu. Res.* 46(2):97-104.
- Dua K, Pabreja K, Sharma VK, Singh UV, Ramana MV (2009). Solid dispersion technology. (<http://saffron.pharmabiz.com/article/detnews.asp?articleid=51592§ionid=46>).
- Dumortier G, Grossiord JL, Agnely F, Chaumeil JC (2006). A review of poloxamer 407 pharmaceutical and pharmacological characteristics. *Pharm. Res.* 23:2709-2728.
- European Pharmacopoeia (2011). *Pharmaceutical Technical Procedures.* 1:253-323.
- Farizon F, Eloy JDO, Donaduzzi CM, Mitsui ML, Marchetti JM (2013). Dissolution rate enhancement of loratadine in polyvinylpyrrolidone K-30 solid dispersions by solvent methods. *Powder Tech.* 235:532-539.
- Gauri N, Aditi L, Shikha A, Dubey PK (2011). Solubility enhancement of a poorly aqueous soluble drug ketoprofen using solid dispersion technique. *Der Pharmacia Sinica* 2(4):67-73.
- Ge Z, Zhang XX, Gann L, Gan Y (2008). Redispersible, dry emulsion of lovastatin protects against intestinal metabolism and improves bioavailability. *Acta Pharmacol. Sin.* 29(8):990-997.
- Ghodke DS, Nakhat PD, Yeole PG, Naikwade NS, Magdum CS, Shah RR (2009). Preparation and characterization of domperidone inclusion complexes with cyclodextrin: Influence of preparation

- method. *Iran. J. Pharm. Res.* 8(3):145-151.
- Gill B, Kaur Tk, Kumar S, Gupta GD (2010). Formulation and evaluation of glimepiride solid dispersion. *Asian J. Pharma.* 4(3):212-218.
- Higuchi T, Connors KA (1965). Phase-solubility techniques. *Adv. Anal. Chem. Instr.* 4: 117-210.
- Hildegrad M, Wendtner S, Korting HC (2007). pH and skin care. Berlin: ABW Wissenschaftsverlag GmbH, pp. 22-23.
- Jagdale SC, Kuchekar BS, Sharma SN, Patil SA (2012). Solubility enhancement of poorly soluble drug febuxostat by melt granulation technique. *Int. J. Pharm. Res. Dev.* 4(6):318-323.
- Khan MA, Karnachi AA, Agarwal V, Vaithiyalingam SR, Nazzal S, Reddy IK (2000). Stability characterization of controlled release coprecipitates and solid dispersions. *J. Cont. Rel.* 63:1-6.
- Leuner C, Dressman J (2000). Improving drug solubility for oral delivery using solid dispersions. *Eur. J. Pharm. Biopharm.* 50:47-60.
- Mehanna MM, Motawaa AM, Samaha MW (2010). In sight into tadalafil - block copolymer binary solid dispersion: Mechanistic investigation of dissolution enhancement. *Int. J. Pharm.* 402:78-88.
- Mirza S, Miroshnyk I, Habib MJ, Brausch JF, Hussain MD (2010). Enhanced dissolution and oral bioavailability of piroxicam formulations: Modulating effect of phospholipids. *Pharm.* 2:339-350.
- Muralidhar S, Rao GD, Nizami SA, Reddy TK, Reddy SR (2010). Enhancement of dissolution rate and anti-inflammatory potential of celecoxib using solid dispersion technique. *J. Adv. Pharm. Res.* 1:74-81.
- Ohara T, Kitamura S, Kitagawa T, Terada K (2005). Dissolution mechanism of poorly water- soluble drug from extended solid dispersion system with ethyl cellulose and hydroxypropyl methylcellulose. *Int. J. Pharm.* 302:95-102.
- Park YJ, Yong CS, Kim, HM, Rhee JD, Oh YK, Kim CK, Choi HG (2003). Effect of sodium chloride on the release, absorption and safety of diclofenac sodium delivered by poloxamer gel. *Int. J. Pharm.* 263:105-111.
- Patel K, Prad RJ, Bajpai M (2011). Enhancement of dissolution rate of domperidone using melt granulation technique. *Der Pharmacia Lett.* 3(2):25-33.
- Patil SB, Shete DK, Narade SB, Surve SS, Khan ZK, Bhise SB, Pore YV (2010). Improvement in the dissolution profile of diacerein using a surfactant-based solid dispersion technique. *Drug Discov. Ther.* 4(6):435-441.
- Prasad KA, Narayanan N, Rajalakshmi G (2010). Preparation and evaluation of solid dispersion of terbinafine hydrochloride. *Int. J. Pharm. Sci. Rev. Res.* 3(1):130-134.
- Ran Z, Fei W, Ming C, Hongkun Y, Lanxiang S, Yongliang Z (2012). Preparation and evaluation of solid dispersion of asiatic acid with PVP K30. *Digest. J. Nanomat. Biostruc.* 7(3):1015-1020.
- Roni MA, Dipu MH, Kibria G, Rahman H, Rony MDR, Jalil RU (2011a). Dissolution enhancement of poorly soluble carbamazepine by using polymeric solid dispersions. *Int. J. Pharm. Sci. Res.* 2(1):49-57.
- Roni MA, Islam S, Kibria G, Sadat SMA, Rony R, Rahman H, Jalil RU (2011b). Effects of poloxamer and HPMC on the dissolution of clonazepam-polyethylene glycol solid dispersions and tablets. *Indian J. Pharm. Educ. Res.* 45(2):139-144.
- Rose HS, Golan DE (2008). Pharmacodynamics. In: Golan DE, Tashjian AR, Armstrong EJ, Armstrong AW (eds) *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Baltimore: Lippincott Williams & Wilkins. pp. 19-30.
- Rose S (2004). *Gastrointestinal and Hepatobiliary Pathophysiology*, 2nd ed. North Carolina: Hayes Barton Press. pp. 507-535.
- Ryan JA (1986). Compressed pellet X-ray diffraction monitoring for optimization of crystallinity in lyophilized solids: Imipenem-cilastatin sodium case. *J. Pharm. Sci.* 75(8):805-807.
- Sammour OA, Hammad MA, Megrab NA, Zidan AS (2006). Formulation and optimization of mouth dissolve tablets containing rofecoxib solid dispersion. *AAPS Pharm. Sci. Tech.* 7(2):1-9.
- Samyuktha RB, Vedha HBN (2011). Niosomal formulation of orlistat: Formulation and in-vitro evaluation. *Int. J. Drug Dev. Res.* 3(3):300-311.
- Sarkar R, AL-Hossain M, Islam S, Faroque ABM (2012). Effect of hydrophilic swellable polymers on dissolution rate of atorvastatin using simple physical mixing technique. *Ind. J. Novel Drug. Del.* 4(2):130-138.
- Schwartz JB, Simonelli AP, Higuchi WI (1968). Drug release from wax matrices. I. Analysis of data with first-order kinetics and with the diffusion-controlled model. *J. Pharm. Sci.* 57(2):274-277.
- Shah S, Joshi S, Lin S, Madan PL (2012). Preparation and characterization of spironolactone solid dispersions using hydrophilic carriers. *Asian J. Pharm. Sci.* 7(1):40-49.
- Shinde SS, Patil SS, Mevekari FI, Satpute AS (2010). An approach for solubility enhancement: Solid dispersion. *Int. J. Adv. Pharm. Sci.* 1:299-308.
- Sreenivasa RK, Iqbal MM, Shirse P (2012). Preparation and evaluation of cyclodextrin inclusion complexes of water insoluble drug-glimipiride. *Int. J. Res. Pharm. Biomed. Sci.* 2(1):428-434.
- Swami G, Koshy MK, Pandey M, Saraf SA (2010). Preparation and characterization of Domperidone- β -cyclodextrin complexes prepared by kneading method. *Int. J. Adv. Pharm. Sci.* 1:68-74.
- Tantishaiyakul V, Kaewnopparat N, Ingkawatornwong S (1996). Properties of solid dispersions of piroxicam in polyvinylpyrrolidone K-30. *Int. J. Pharm.* 1:59-68.
- Waite M, Keenan J (2010). *CPD for Non-Medical Prescribers: A Practical Guide*. Oxford: Blackwell Publishing Ltd, pp. 97-100.
- Yogesh R, Rajshree M, Mayur S, Jolly S (2007). Effect of hydrophilic swellable polymers on dissolution enhancement of carbamazepine solid dispersions studied using response surface methodology. *AAPS Pharm. Sci. Tech.* 8(2):1-11.
- Zhao G, Duan J, Xie Y, Lin G, Luo H, Li G, Yuan X (2012). Effects of solid dispersion and self-emulsifying formulations on the solubility, dissolution, permeability and pharmacokinetics of isorhamnetin, quercetin and kaempferol in total flavones of *Hippophae rhamnoides* L. *Drug Dev. Ind. Pharm.* 1-9 (Ahead of Print) (doi: 10.3109/03639045.2012.699066).