African Journal of Pharmacy and Pharmacology

Volume 8 Number 3, 22 January, 2014
ISSN 1996-0816

Academic Journals

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Vol. 8(3), pp. 66-80, 22 January, 2014
DOI: 10.5897/AJPP2013.1401
ISSN 1996-0816 © 2014 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

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

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Accepted 20 February, 2013

First-pass metabolism affects many oral medications and limits the attainment of their therapeutic level. It can be bypassed by administrating 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, physiochemical characterization.

INTRODUCTION

First-pass metabolism is the most popular disadvantage of the orally administrated 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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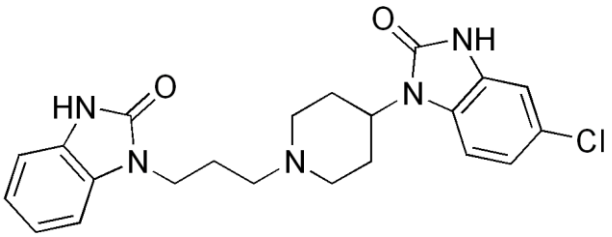

Figure 1. Chemical structure of domperidone.

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 by 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-oxobenzimidazolin-1-yl)propyl]-4-piperidyl} benzimidazolin-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 administrated 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 (Hildegrad 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±0.5°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 (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°C and constant rate of 10°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 CuKα radiation. Diffractograms were run at a scanning rate of 1.8° min^{-1} and the scanning scope was over a range of 2θ angle from 0 to 80° 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:

RDC = Isam/Iref

where Isam is the peak height of the sample under investigation and Iref 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±0.5°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

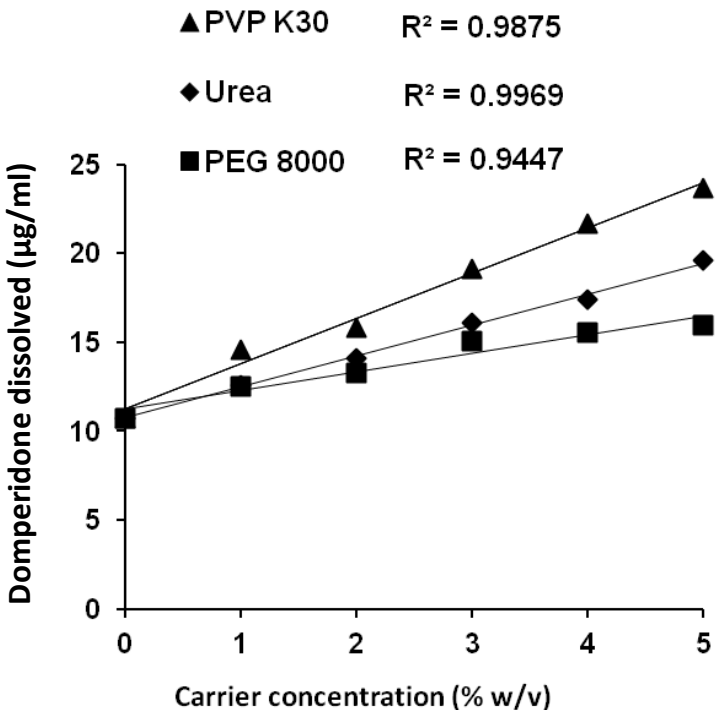

Figure 2. Phase solubility diagram of DMP in phosphate buffer pH 6.8 at 25±0.5° C in the presence of increased concentrations of PVP K30, urea and PEG 8000.

and PEG 8000, respectively. The solubility of DMP was found to be 10.73 µg/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 PVP K30> urea> 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 µg/ml in the presence of 5% w/v PVP K30 alone up to 33.70 µg/ml at 4% w/v PL F-127, 26.37 µg/ml at 1% w/v HPMC E50 LV and 29.09 µg/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±2.66% that could be related to the hydrophobicity, poor wettability and/or agglomeration of DMP particles resulting

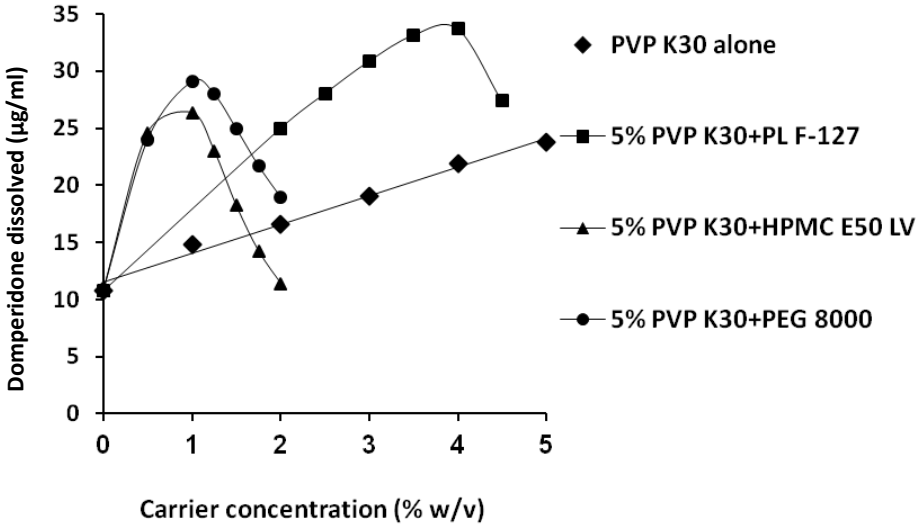

Figure 3. Phase solubility diagram of DMP in phosphate buffer pH 6.8 at 25±0.5° C in the presence of 5% w/v PVP K30 and increased concentrations of PL F-127, HPMC E50 LV and PEG 8000.

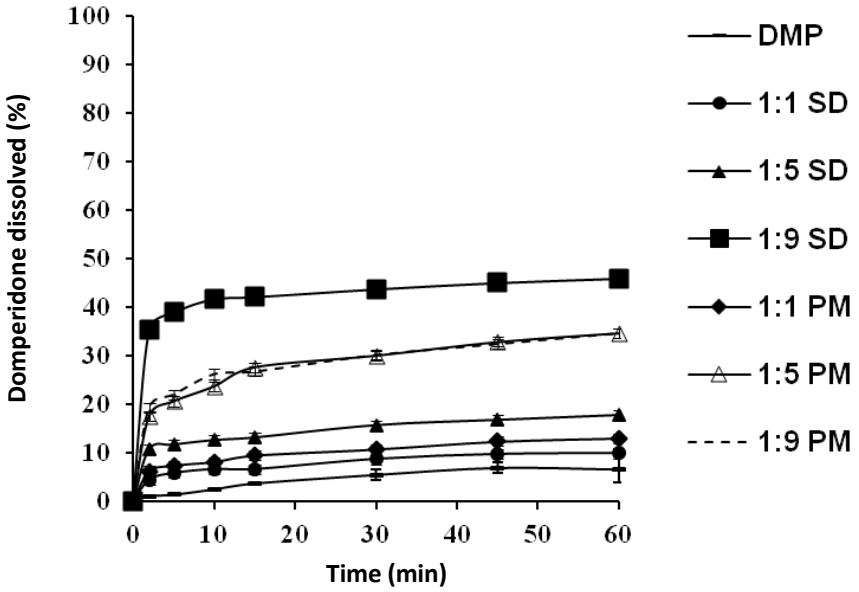

Figure 4. Dissolution profiles of domperidone from different domperidone/PEG 8000 solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at 37±0.5°C.

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

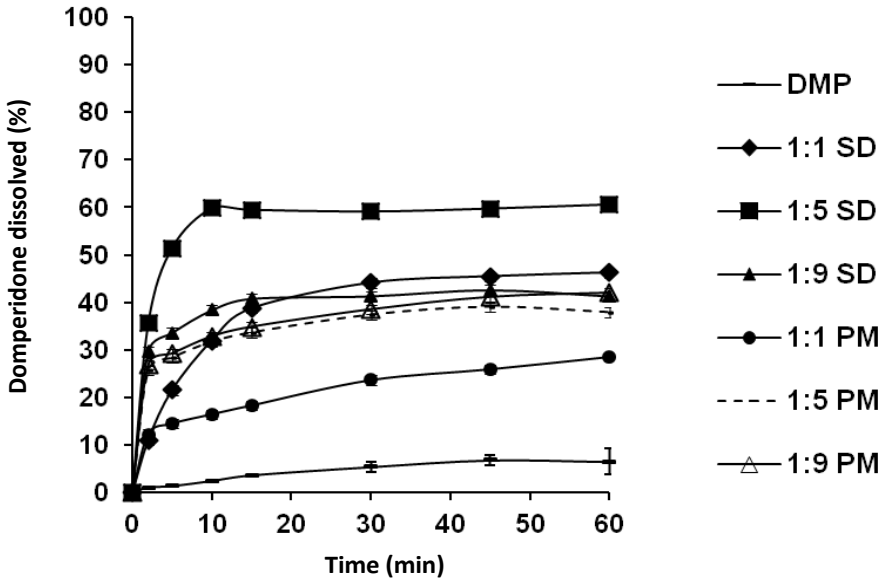

Figure 5. Dissolution profiles of domperidone from different DMP/urea solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at 37±0.5°C.

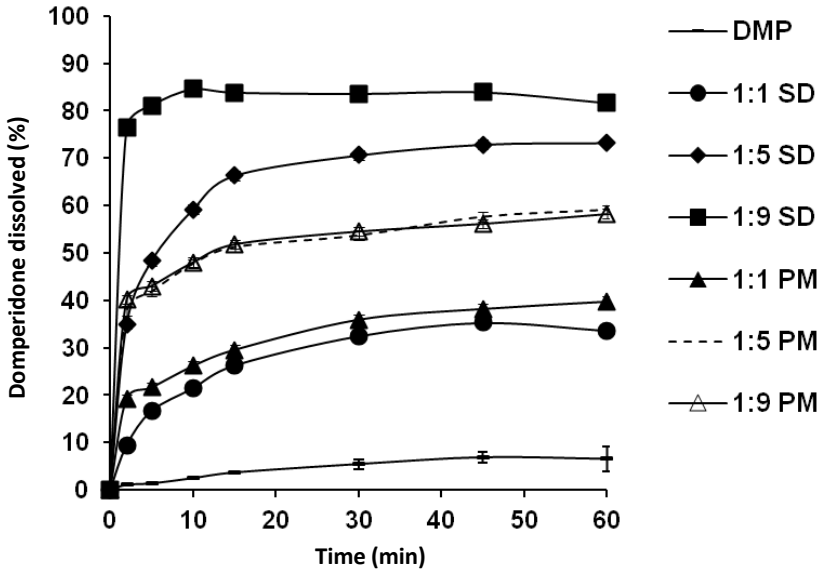

Figure 6. Dissolution profiles of domperidone from different DMP/PVP K30 solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at 37±0.5°C.

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

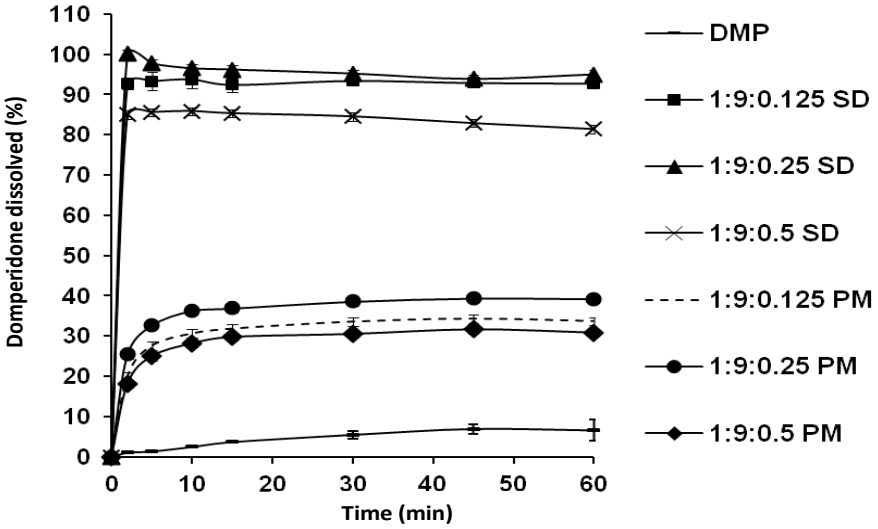

Figure 7. Dissolution profiles of DMP from different DMP/PVP K30/PL F-127 solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at 37±0.5°C.

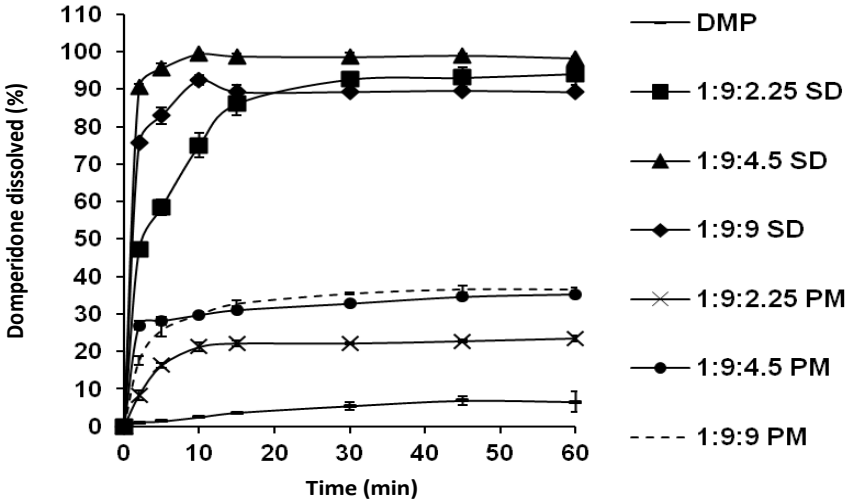

Figure 8. Dissolution profiles of DMP from different DMP/PVP K30/HPMC E50 LV solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at 37±0.5°C.

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

[a]Solubility of DMP in the presence of 5% w/v carrier concentration. [b]Solubility factor=total solubility of DMP in the presence of 5% w/v carrier concentration/intrinsic solubility of DMP. [c]Polyvinylpyrrolidone K30 and [d]Polyethylene 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 (PD_2) 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]	PM	1:9:0.125	20.08±0.64
		1:5	17.61±0.20			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		SD	1:9:0.125	92.71±1.45
		1:5	10.74±1.72			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]	PM	1:9:2.25	8.34±1.29
		1:5	25.68±0.31			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		SD	1:9:2.25	47.29±0.64
		1:5	35.55±0.31			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	FM	1:9:2.25	12.27±1.17
		1:5	37.75±2.53			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		SD	1:9:2.25	82.04±3.29
		1:5	34.95±0.31			1:9:4.5	88.64±0.40
		1:9	76.37±0.23			1:9:9	88.24±1.83

[a]Domperidone; [b]Polyethylene glycol 8000; [c]Physical mixture; [d]Solid dispersion; [e]Polyvinylpyrrolidone K30; [f]Pluronic F-127 and [g]Hydroxypropyl 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, PD_2 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 PD_2 of DMP up to 1:9:4.5 weight ratio of drug/PVP K30/PEG 8000 ($p<0.05$). In case of PM, PD_2 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, PD_2 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 PD_2 of different SDs revealed that ternary SD of 1:9:0.25 DMP/PVP K30/PL F-127 exhibited the most significant enhanced PD_2 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

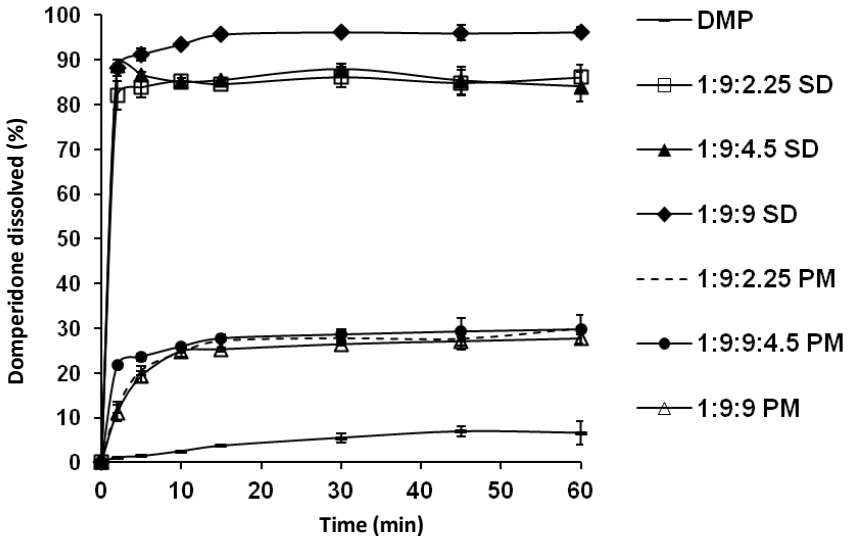

Figure 9. Dissolution profiles of domperidone from different DMP/PVP K30/PEG 8000 solid dispersion (SD) and physical mixture (PM) systems in phosphate buffer pH 6.8 at 37±0.5°C.

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 3048-3750 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).

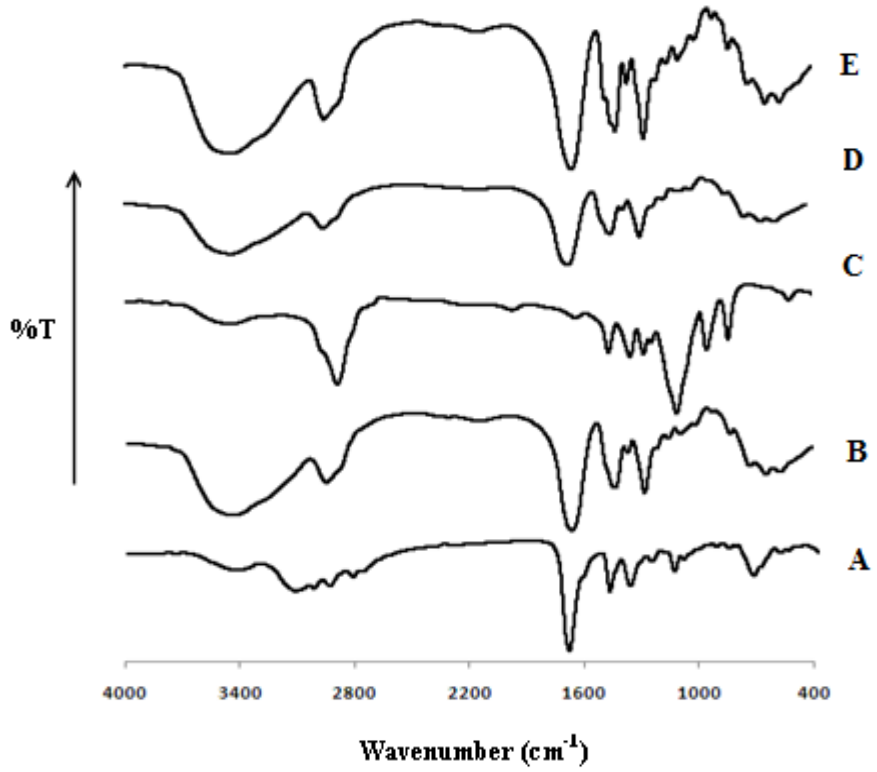

Figure 10. FTIR spectra of (A) pure domperidone (DMP), (B) polyvinyl pyrrolidone K30 (PVP K30), (C) pluronic F-127 (PL F-127), (D) physical mixture of 1:9:0.25 DMP/PVP K30/PL F-127, and (E) solid dispersion of 1:9:0.25 DMP/PVP K30/PL F-127.

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.

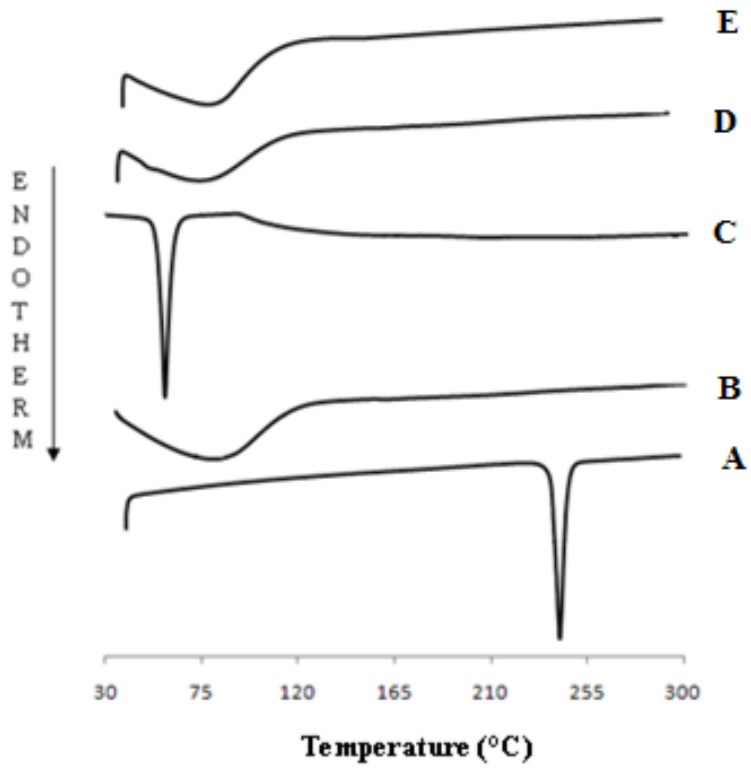

Figure 11. DSC thermograms of (A) pure domperidone (DMP), (B) polyvinyl pyrrolidone K30 (PVP K30), (C) pluronic F-127 (PL F-127), (D) physical mixture of 1:9:0.25 DMP/PVP K30/PL F-127 and (E) solid dispersion of 1:9:0.25 DMP/PVP K30/PL F-127.

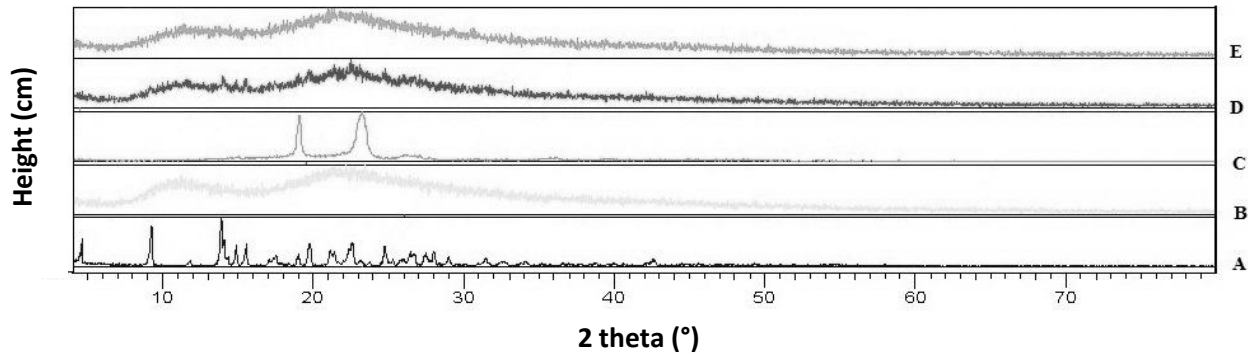

Figure 12. PXRD patterns of (A) pure domperidone (DMP), (B) polyvinyl pyrrolidone K30 (PVP K30), (C) pluronic F-127 (PL F-127), (D) physical mixture of 1:9:0.25 DMP/PVP K30/PL F-127, and (E) solid dispersion of 1:9:0.25 DMP/PVP K30/PL F-127.

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

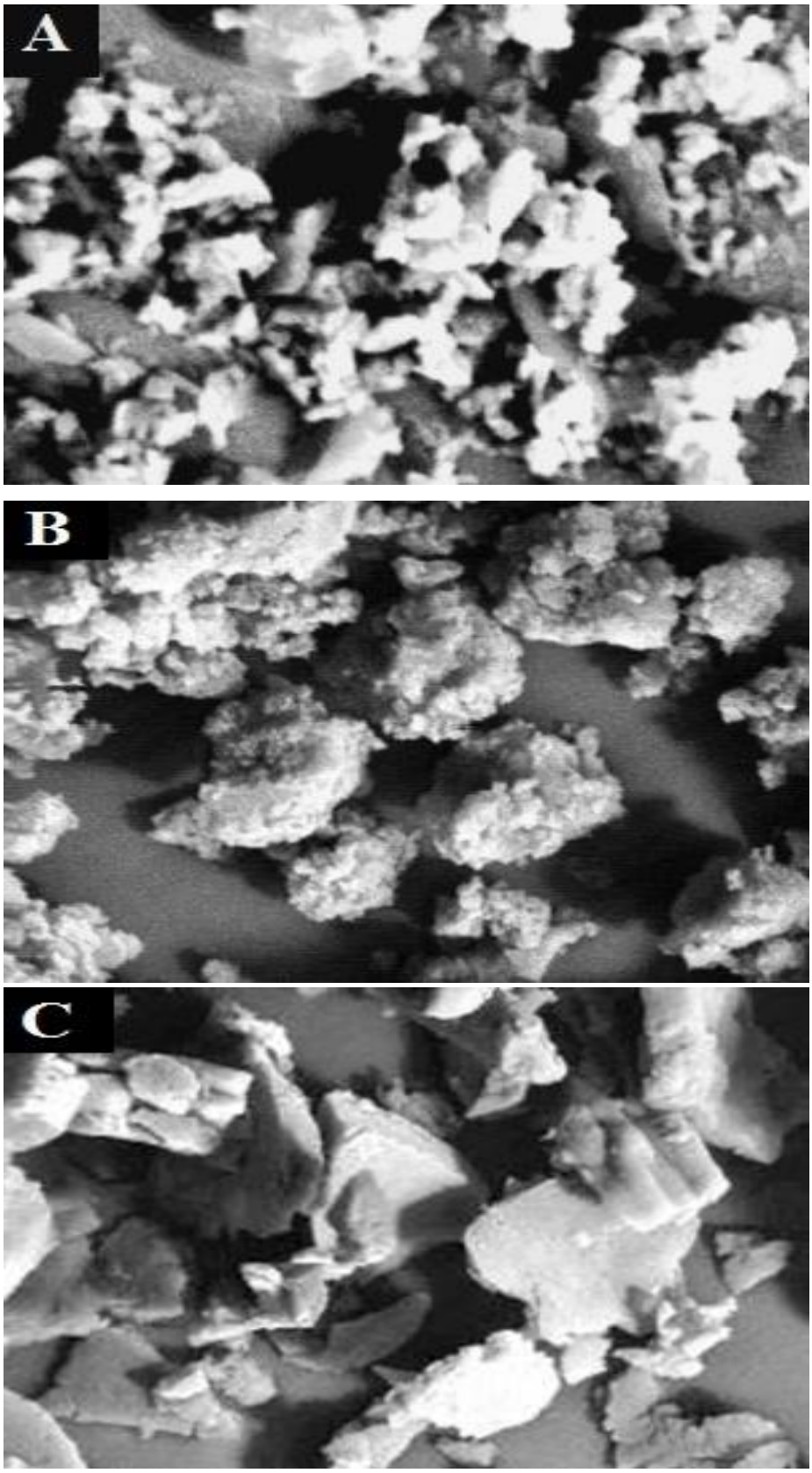

Figure 13. SEM microphotographs of (A) pure domperidone (DMP), (B) physical mixture of 1:9:0.25 DMP/Polyvinyl pyrrolidone K30/Pluronic F-127, and (C) solid dispersion of 1:9:0.25 DMP/Polyvinyl pyrrolidone K30/Pluronic F-127.

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

[a]Domperidone; [b]Polyethylene glycol 8000; [c]Polyvinylpyrrolidone K30; [d]Pluronic F-127; and [e]Hydroxypropyl 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

[a]Relative degree of crystallinity; [b]Domperidone; [c]Physical mixture; [d]Polyvinylpyrrolidone K30; [e]Pluronic F-127 and [f]Solid dispersion.

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

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Vol. 8(3), pp. 81-86, 22 January, 2014
DOI: 10.5897/AJPP2013.3777
ISSN 1996-0816 © 2014 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Improving effect of zinc supplementation on pituitary gonadotropins secretion in smokers

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Accepted 16 January, 2014

This study aimed to investigate the effect of zinc supplementation on testicular tissue as a feed-back mechanism of the pituitary secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) on cigarette smoker subjects. Total of twenty subjects participated in this study as per the national bioethics committee guidelines. Venous blood samples were collected for determination of serum level of LH and FSH before and after zinc supplementation. LH and FSH determination were done using enzyme-linked immunosorbent assay (ELISA). A t-test was used to compare the means of two groups, P value was considered significant if ≤ 0.05. Results showed that LH serum levels were elevated in smokers as compared to the control group (p 0.05). Similar observation was found in FSH levels (p<0.05). After one month of zinc supplementation, LH levels decreased in smokers group (after: 4.14 ± 2.38 mIU/ml, before: 7.43 ± 4.32 mIU/ml; p<0.05). FSH levels also decreased after zinc supplementation in smokers group (after: 1.50 ± 1.12 mIU/ml, before: 3.88 ± 1.57 mIU/ml; p<0.05). The improving effect of zinc supplementation on LH and FSH pituitary and testicular steroid secretions for smoker subjects is obviously observed. These findings can be implemented to contribute to the outcome of zinc treatment associated with smoking.

Key words: Zinc, biometals, smoking, gonadotropins, luteinizing hormone (LH), follicle-stimulating hormone (FSH), trace elements.

INTRODUCTION

Cigarette smoke contains a large number of oxidative compounds. Both zinc and antioxidants delay the progression of oxidative degeneration, possibly by preventing cellular damage. Zinc is involved in numerous aspects of cellular metabolism, for example, it is required for the catalytic activity of approximately 100 enzymes. Pituitary gonadotropins include luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are essential for reproduction. While LH stimulates synthesis and secretion of testosterone hormone, FSH is critical for sperm production. Excessive secretion of FSH and/or LH is the most common result of gonad failure. Zinc, an essential trace element necessary for sustaining all animal life, is thought to protect cells from drought and disease. There are numerous functions in which zinc plays an important role such as prostate gland function and reproductive organ growth, immune function, protein synthesis , wound healing, DNA synthesis and cell division (Heiserman, 1992; Solomons, 1998; Prasad, 1995; Heyneman, 1996). It also supports normal growth and development of fetus during pregnancy and after birth in childhood and adolescence (Fabris and Mocchegiani, 1995; Maret and Sandstead, 2006). Zinc plays key role in different sensory functions like proper sense of taste

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and functions like proper sense of taste and smell (Prasad et al., 1997). Tissues with high rate of new cell development such as bone marrow, immune system cells and the lining of the gut, especially required zinc (David, 2006). Zinc involves in numerous aspects of cellular metabolism, approximately 100 enzymes require zinc for their catalytic activity (Sandstead, 1994). There are over 200 enzymes which contain zinc as a cofactor, and about the same number of transcription factors, zinc-containing enzymes are used by the body to regulate growth and development, promote fertility, aid digestion, and synthesize nucleic acid. Severe zinc deficiency is known to depress immune function (Navarro et al., 1994). The body requires zinc to develop and activate T-lymphocytes (Wintergerst et al., 2007). Individuals with low zinc levels have shown reduced lymphocyte proliferation response to mitogens and other adverse alterations in immunity that can be corrected by zinc supplementation (Beck et al., 1997). Both zinc and antioxidants delay the progression of age-related macular degeneration (AMD) and vision loss, possibly by preventing cellular damage in the retina (Marshall, 2000; Evans, 2006). Cigarette smoke contains a large number of oxidative compounds. These compounds appear to be the most potent compounds which can exert oxidative stress. Oxidative stress includes the generation of reactive oxygen species (ROS) by different cell type such as neutrophils and macrophages which may be present in increased number or activated form.

Elevated serum FSH is a reliable indicator of germinal epithelial damage, and is usually associated with severe oligo-spermia or azoo-spermia of bad prognosis. In men with gonadotropins, insufficiency testosterone cannot maintain spermatogenesis contrary, therefore normal levels of both FSH and LH are required to achieve quantitatively normal sperm-production (Martin-du, 2012). In the present study, the effect of cigarette smoke on pituitary secretion of FSH and LH will be assayed and the effect of zinc supplementation on pituitary secretion of these hormones will be monitored in cigarette smoker subjects.

MATERIALS AND METHODS

Ten healthy male and ten smoker male subjects were invited to participate in the study. Each subject gave written informed consent for the study. The study has been designed ethically according to National Committee of BioEthics at King Abdulaziz City for Science and Teschnology (NCBE/KACST) and Al Jouf University (JU) bioethics committee guidelines. The study took place at Aljouf University facilities, Al Jouf province, KSA. The age of the subjects ranged between 19 and 26 years. The duration of smoking ranged between 4 and 7 years ago (20 cigarettes/day). Ten healthy male nonsmoker subjects were also included as control group who were matched to the smokers in age and socioeconomic status. For each subject of smoker and control groups, the following procedures were carried out: medical history collection, general clinical examination, venous blood sample collection for determination of serum levels of LH and FSH. Zinc supplementation was then started to smoker subjects for one month as one tablet daily of stress tablets with zinc, manufactured by Wyeth Canada. The oral tablet contains zinc sulfate (23.9 mg) and copper (3 mg), prescribed to be taken after meal with continuing the same smoking rate (20 cigarettes/day). High zinc intakes can inhibit copper absorption, sometimes producing copper deficiency and associated anemia (Whittaker, 1998; Broun et al., 1990). Considering the fact, dietary supplement formulations should contain high levels of zinc and copper (Broun et al., 1990). Venous blood samples were collected for determination of serum level of LH and FSH after zinc supplementation. Quantitative determination of LH and FSH serum levels were done using Accu-Bind enzyme-linked immunosorbent assay (ELISA) microwells (product code 625-300 for LH and product code 425-300 for FSH).

Estimation of serum zinc levels

They were measured by an atomic absorption spectrophotometry (mode 12380; Perkin Elmer). The monochromatic slit was adjusted to 0.7 and the wave length was set to the zinc resonance line at 213.9 nm (Pekarek et al., 1972).

Statistics

All the statistical analyses were processed using Statistical Program of Social Sciences (SPSS) for windows, version 21.0. Values of the measured parameters were expressed as mean value ± standard deviation (SD) and the difference between each of the two groups was determined using unpaired student's t-test, and the significance was considered at p values as less than 0.05. Analysis of variance (ANOVA) was used to compare the means of more than two groups.

RESULTS

The LH serum level was found to have a very high significant increase in smokers group before zinc supplementation compared to nonsmokers group as illustrated in Table 1 ($p<0.05$). LH serum level was found with very low significant decrease in smokers group after zinc supplementation as compared with smokers before zinc supplementation ($p<0.05$), while the LH serum levels after zinc supplementation shows nonsignificance difference as compared with nonsmokers as shown in Table 1 and Figure 1. Similarly, FSH serum level was found also with very high significant increase in smokers before zinc supplementation compared to nonsmokers group ($p<0.05$) as shown in Table 2. FSH serum level was also found with very high significant decrease in smokers group after zinc supplementation as compared with nonsmokers ($p<0.05$). And the FSH serum levels after zinc supplementation shows nonsignificance difference as compared with nonsmokers ($p>0.05$) as shown in Table 2 and Figure 2. Also, zinc serum level was also found with very high significant decrease in smokers before zinc supplementation compared to nonsmokers group ($p<0.05$) as shown in Table 3. Zinc serum level was also found with very high significant increase in smokers group after zinc supplementation as compared with smokers before zinc supplementation ($p<0.05$). The

Table 1. Comparison between serum level of LH in smokers (before and after Zn supplementation) and nonsmokers

Parameter	Smokers		Nonsmokers	ANOVA	
	Before Zn	After Zn		F ratio	P value
Serum level of LH (mIU/ml)	7.05 ± 0.45[a]	3.29±0.41[bc]	3.02 ± 0.25	345.0	<0.05***

(a) Very high significant differences between smokers before Zn supplementation and non smokers($p<0.05$); (b) Very high significant differences between smokers after Zn supplementation and smokers before Zn supplementation($p<0.05$); (c) Nonsignificant differences between smokers after Zn supplementation and nonsmokers ($p>0.05$).

Table 2. Comparison between serum level of FSH in smokers (before and after Zn supplementation) and nonsmokers.

Parameter	Smokers		Nonsmokers	ANOVA	
	Before Zn	After Zn		F ratio	P value
Serum level of FSH (mIU/ml)	3.45 ± 0.67[a]	2.01 ± 0.47[bc]	2.00 ± 0.18	31.89	<0.05***

(a) Very high significant differences between smokers before Zn supplementation and non smokers ($p<0.05$); (b) Very high significant differences between smokers after Zn supplementation and smokers before Zn supplementation ($p<0.05$); (c) Nonsignificant differences between smokers after Zn supplementation and nonsmokers ($p > 0.05$).

Table 3. Comparison between serum level of Zn in smokers (before and after Zn supplementation) and nonsmokers.

Parameter	Smokers		Nonsmokers	ANOVA	
	Before Zn	After Zn		F ratio	P value
Serum level of zinc (µg/dl)	84.21 ± 13.70[a]	107.35 ± 5.68[bd]	115.03 ± 8.7	26.03	<0.05***

(a) Very high significant differences between smokers before Zn supplementation and non smokers ($p<0.05$); (b) Very high significant differences between smokers after Zn supplementation and smokers before Zn supplementation ($p<0.05$); (d) Significant differences between smokers after Zn supplementation and nonsmokers ($p<0.05$).

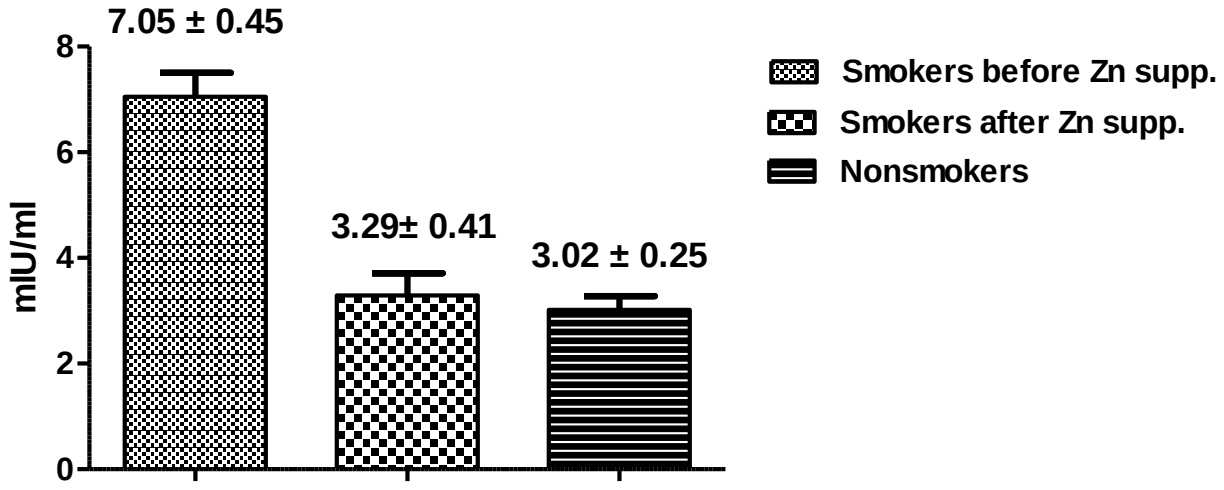

Figure 1. Comparison between serum level of LH (mIU/ml) in smoker (before and after Zn supplementation) and nonsmokers.

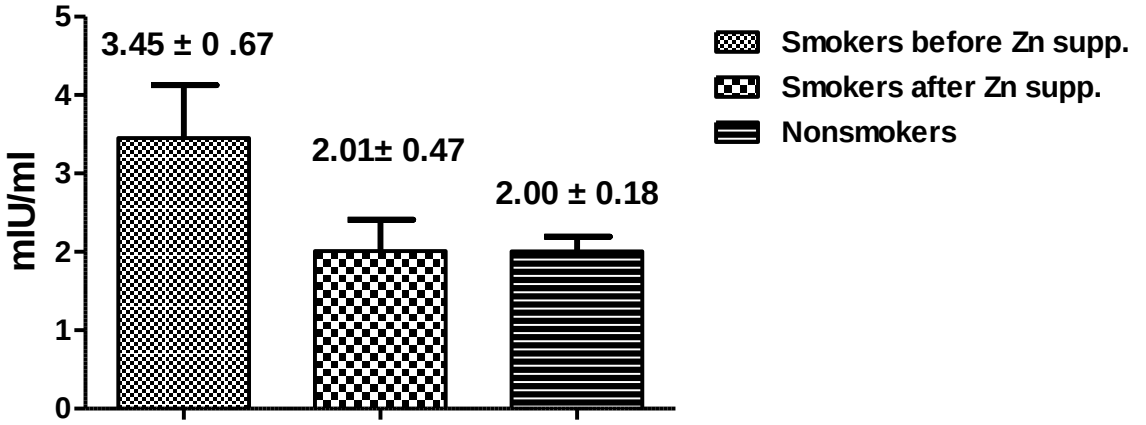

Figure 2. Comparison between serum level of FSH (mIU/ml) in smoker (before and after Zn supplementation) and nonsmokers.

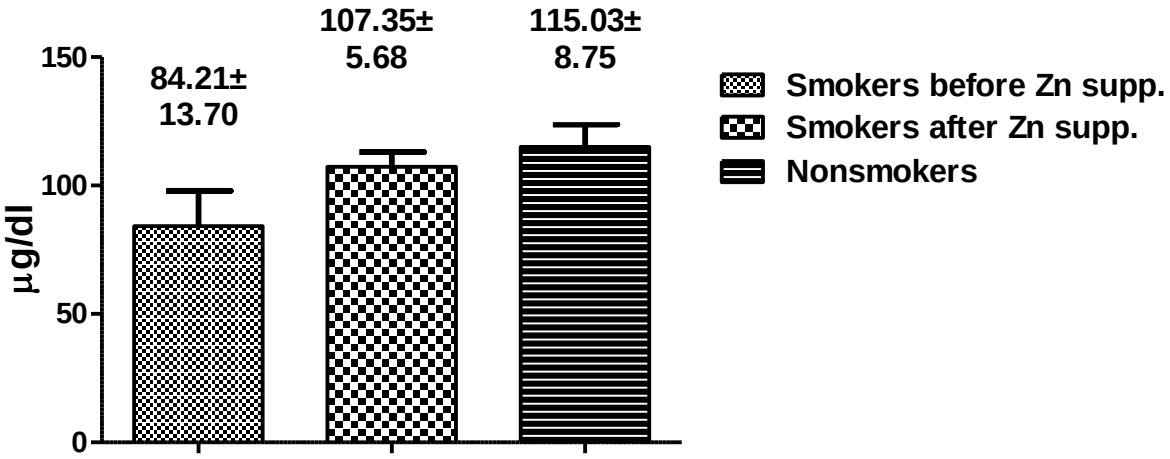

Figure 3. Comparison between serum level of Zinc (µg/dl) in smoker (before and after Zn supplementation) and nonsmokers.

zinc serum levels after zinc supplementation shows significance difference as compared with nonsmokers ($p<0.05$) as depicted in Table 3 and Figure 3.

DISCUSSION

The improving effect of zinc supplementation on LH and FSH pituitary and testicular steroid secretions for smoker subjects is obviously observed in this study. These findings can contribute to the outcome of zinc treatment associated with smoking and zinc involvement in relevant biological processes. Zinc has potential to influence many metabolic functions and to impact a range of diseases (Hughes and Samman, 2006). Testicular toxicants such as polycyclic aromatic hydrocarbons exert their effects at least in part by free radical-dependent mechanisms. Although, the role of trace elements and the mechanism of their kinetics in relation to these diseases remain controversial, the serum copper/zinc ratio (CU/Zn) has been claimed to be highly sensitive in the diagnosis of the diseases in question (Hiura et al., 2003). The decrease in the zinc levels may be due to the decreased intake of zinc, disturbed intestinal absorptionof zinc, decreased serum albumin as a carrier of zinc, decreased storage of zinc in liver and increased urinary excretion of zinc (Lin et al., 2006). Pro-oxidant states often result in impairment of this defense. The testis tissue and sperm function are particularly vulnerable to per-oxidative injury produced by reactive oxygen species. Defective sperm function of infertile men is associated with increased lipid per-oxidation and impaired function of antioxidant enzymes in

spermatozoa (Fridovich and Freeman, 1986; Aitken et al 1989). Smoking is associated with a high significant reduction in sperm output and motility (Handelsman et al., 1984). Superoxide anions are potent oxidants (Halliwell and Gutteridge, 1999) moderated by the activities of various forms of superoxide dismutase (SOD) (Zou et al., 2002). Zinc is postulated to influence oxidants via its role as a cofactor for Cu, Zn-SOD (O'Dell, 2000; Powell, 2000). Both low and high zinc intakes impact Cu, Zn-SOD activity (Ruz et al., 1992). Increase in zinc intake raises plasma and erythrocyte SOD (Davis et al., 2000). Zinc supplementation decreased plasma lipid peroxides; therefore, adequate zinc intake could play an important role in the prevention and/or modulation of diseases. Hypozincemia and marked hypercupremia have been reported in patients with digestive, hepatic, breast, and lung cancers (Maeda et al., 2005).

The evaluated levels of the LH and FSH pituitary secretions in the male subjects showed a significant increase in serum level of LH in smokers group compared to control group ($p<0.05$) and a significant increase in serum level of FSH in smokers group as compared to control group ($p<0.05$). These results are in accordance with literature reviews. Such trend in LH and FSH secretions is an indicator of the testicular dysfunction in smoker subjects.

After one month zinc supplementation, the serum levels of LH and FSH were measured in smokers group. LH serum level significantly decreased in smokers group after zinc supplementation in comparison to the level before supplementation ($p<0.05$). FSH serum level also significantly decreased in smokers group after zinc supplementation compared to the level before supplementation ($p<0.05$). This is an indicator about the improvement of testicular function of smoker subjects as a feedback regulation. Decreased blood levels of gonadotropins usually reflect elevation of steroid feedback. The improvement of LH pituitary secretion in smokers group after zinc supplementation is statistically nonsignificant in comparison to control group ($p>0.05$). Similarly, the improvement of FSH pituitary secretion of smoker group in comparison to control group is not significant ($p>0.05$).

Zinc supplementation is associated with decreased oxidative stress and improved immune function among possible mechanism for its disease prevention (Prasad and Kucuk, 2002). Baltaci et al. (2006) reported that LH serum levels in rats having zinc supplementation (3 mg/kg/day) were lower than castrated rats. Free and total testosterone serum levels in zinc supplemented rats were higher than in control group. Nakayama et aI. (2002) reported a progressive decrease in serum zinc levels in patients with liver diseases from chronic hepatitis, alkaline phosphatase activity in zinc supplemented adult male group. Sunar et al. (2009) studied 40 adult female rats, which were allocated to 4 groups; control (group 1), ovariectomized control (OVX) (group 2), OVX-zinc-supplementation (group 3), OVX-zinc deficient (group 4). Estrogen and progesterone levels in group 3 were higher than those in groups 2 and 4. Group 3 had the highest serum zinc level. The findings by Sunar et al. (2009) also reflect the improving effect of zinc on LH and FSH pituitary secretion. Testicular dysfunction existed in smoker subjects before zinc supplementation which is supported by the elevation in serum levels of LH and FSH with zinc supplementation. The improving in testicular steroid secretion in smoker subjects as a feedback regulation appeared by a decrease in serum levels of LH and FSH. The decrease in secretion of LH and FSH is the most common result of gonad improvement. This reflects elevation of steroid feedback. Moreover, this may significantly contributes to the outcome of zinc therapeutic intervention in gonad dysfunction and oxidative stress conditions.

In conclusion and as a preventive measure, an implementation of short term plan to reduce the present health hazards of high levels of LH and FSH were recommended due to smoking. Further studies with higher sample size are warrantied to confirm these results for improving benefits of zinc supplementation to prevent certain health disorders may be necessary.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Clinical Laboratory Sciences at the College of Applied Medical Sciences, Aljouf University for support provided to complete this work.

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Vol. 8(3), pp. 87-92, 22 January, 2014
DOI: 10.5897/AJPP2013.3823
ISSN 1996-0816 © 2014 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

The suspending properties of *Terminalia randii* gum in magnesium carbonate suspension

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Accepted 20 December, 2013

Terminalia randii **tree is widely planted in Nigeria as an exotic plant, and gum can be obtained from the incised trunk. The natural gum from *T. randii* is a hydrophilic carbohydrate polymer. The purpose of this work was to develop cheap and effective natural excipient that can be used as an alternative for the formulation of pharmaceutical suspensions. The suspending properties of *T. randii* gum (Combretaceae) was studied in magnesium carbonate suspension and compared with acacia, compound tragacanth and gelatin at a concentration range of 1 to 4% $^{w}/_{v}$. Sedimentation volume, rheology, redispersibility and flow rate of suspensions were used as evaluation parameters. The ranking of sedimentation volume, viscosity and ease of redispersibility of the suspensions were in the order of *T. randii* gum > compound tragacanth > gelatin > acacia with non-Newtonian flow. The flow rates of the suspensions were in the order of acacia > gelatin > compound tragacanth > *T. randii.* At concentrations above 2.5% $^{w}/_{v}$, the viscosity of suspensions containing *T. randii* reduced the ease of redispersibility. It can be concluded that the mucilage of *T. randii* gum can be used as an alternative suspending agent in suspension formulations. It could be exploited for use as a stabilizer and thickening agent in the food industry due to its high viscosity.**

Key words: *Terminalia randii* gum, suspension, viscosity, sedimentation volume, ease of redispersibility.

INTRODUCTION

Pharmaceutical suspensions are coarse disperse systems that are thermodynamically unstable and require the addition of thickeners or suspending agents to make them stable by reducing the settling rate of the particles (Martin, 2001). Suspensions offer several advantages over other dosage forms. Some of the benefits include: improved chemical stability of certain drugs, higher rate of bioavailability than other dosage forms, onset and duration of drug action can be controlled, suspensions are usually applicable for drugs which are insoluble or poorly soluble and it can also be used to mask the unpleasant or bitter taste of some drug substances (Fahr and Liu, 2007). Suspension drug delivery dosage forms are also extensively used in the pharmaceutical industry for different routes of administration such as oral, inhalation, topical and parenteral (Edman, 1994). Suspensions may be dilute (2 to 10% $^{w}/_{v}$ solid), concentrated (50% $^{w}/_{v}$ solid), flocculated or deflocculated (Aulton, 2002).

Synthetic materials seem to be taking over the place of naturally available excipients in the design of drug delivery systems (Kumar and Gupta, 2012). However, the use of natural polymers such as gums and starches as pharmaceutical excipients presents several advantages

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such as non-toxicity, low cost, high availability and biological compatibility (Bhardwaj et al., 2000). Natural gums are widely used in the pharmaceutical industries as suspending agents (Femi-Oyewo et al., 2004; Verma and Razda, 2003; Kumar et al., 2009), emulsifying agents (Odeku et al., 1997; Nasipuri et al., 1999), binders (Panda et al., 2008; Gangurde et al., 2012), disintegrants (Patel and Patel, 2011) and in the food industries as thickeners. *Terminali randii* tree is widely grown in Nigeria as an exotic plant and wind breaker. The gum is obtained from the incised trunk of this tree and has been used as a binder (Bamiro et al., 2010) and as a controlled release agent in carvedilol formulations (Bamiro et al., 2012). In the present study, the suspending property of the gum is being investigated in magnesium carbonate formulation.

MATERIALS AND METHODS

The materials used are light magnesium carbonate, gelatin (Merck, Germany), acacia gum (Mytonjaundrs and Co. Ltd, Liverpool), compound tragacanth powder, benzoic acid, chloroform water and distilled water. *Terminalia* gum was collected from the incised trunk of *T. randii* tree at the premises of Olabisi Onabanjo University, Ogun State in South Western part of Nigeria. The extraction of the gum has been described elsewhere (Bamiro et al., 2010).

Suspension preparation

Light magnessium carbonate suspension (10%$^{w}/_{v}$) was prepared by trituration method. *T. randii* gum (1 to 4% $^{w}/_{v}$) served as the suspending agent and was compared with standard agents (acacia gum, compound tragacanth gum and gelatin). Benzoic acid (0.1% $^{v}/_{v}$) and chloroform water (50% $^{v}/_{v}$) served as preservatives and the dispersing vehicle (distilled water) was used to make up to volume.

Determination of suspension properties

The properties of the suspension were evaluated using the methods described:

Sedimentation volume

Triplicate samples of fifty (50) ml of suspension was stored in a 50 ml measuring cylinder and left undisturbed for one week at room temperature (33°C). The sedimentation volume of each suspension was taken at 1 h intervals for 7 h and every 24 h for 7 days. Readings were done in triplicates. Sedimentation volume was calculated using the formula:

$$F = V_u/V_o \quad (1)$$

Where: V_u is the ultimate volume of the sediment and V_o is the original volume of the suspension.

Flow rate

The time required for 10 mls of each suspension sample to flow through a 10 ml pipette (from a fixed height) was determined and determinations were done in triplicate. The flow rate was calculated using the formula:

$$\text{Flow rate } (\eta\alpha) = \frac{\text{Volume of pipette (ml)}}{\text{Flow time (s)}} \quad (2)$$

Viscosity

The Brookfield Synchro-lectric viscometer, model LVF (Brookfield Laboratories, Massachusetts) was used to carry out viscosity test on the suspensions using spindle size 3 at 25 ± 2°C at 30 revolutions per minute. All determinations were done in triplicate.

Particle size analysis

The method of Patel et al. (1986) was used to analyze the particle size.Ten (10) ml of each sample was transferred into 200 ml cylinder after shaking the suspension for 2 min. Distilled water (150 ml) was then added, mixed, and 10 ml aliquot was removed at a distance of 10 cm below the surface of the mixture at 1 to 30 min using 5 min interval. Each aliquot was transferred into an evaporating dish and dried in an oven at 105°C and the residue weighed. The particle diameter (d in cm) was then calculated using the Stokes equation.

$$d = \frac{18\eta h}{(\rho_s - \rho_o)\, gt} \quad (3)$$

Where h is the distance of fall of the particle (cm), t is the time (s), η is the viscosity of the dispersion medium (poise), ρ_s - ρ_o is the density gradient between the dispersed particles and the liquid (g cm^{-3})

Redispersibility test

This was carried out by rotating the bottle containing the suspension at an angle 180° and the ease of re-dispersibilty was noted (Saeedi et al., 2003).

Statistical analysis

All tests were conducted in triplicates and analyzed using Graph Pad InStat (Graphpad Software Inc., San Diego, USA). Unpaired student's t-test, analysis of variance and linear regression tests were utilized. The null hypothesis in each test was that there were no significant differences between or within the treatments. P values of < 0.05 (that is, 95% confidence interval) were considered significant.

RESULTS AND DISCUSSION

Sedimentation volume

The sedimentation volume (F) of the suspensions containing the different suspending agents is presented in Table 1. It was observed that the sedimentation volume of the suspensions increased with increase in concentration of suspending agent. This could be due to an increase in viscosity of the suspensions with increase

Table 1. Values of sedimentation volume of magnesium carbonate suspensions using different concentrations of suspending.

Suspending agent	Concentration (% $^{w}/_{v}$)	Sedimentation volume time (h)														
		0	1	2	3	4	5	6	7	24	48	72	96	120	144	168
Terminalia randii gum	0	1.00	0.60	0.55	0.54	0.52	0.50	0.50	0.50	0.48	0.46	0.46	0.46	0.46	0.46	0.46
	1	1.00	0.72	0.69	0.68	0.67	0.67	0.65	0.64	0.61	0.60	0.60	0.60	0.60	0.60	0.60
	2	1.00	0.80	0.78	0.76	0.76	0.75	0.74	0.73	0.70	0.69	0.68	0.68	0.68	0.68	0.68
	2.5	1.00	0.90	0.90	0.88	0.85	0.84	0.83	080	0.79	0.78	0.78	0.78	0.78	0.78	0.78
	3	1.00	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
	3.5-4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tragacanth gum	1	1.00	0.70	0.68	0.64	0.62	0.61	0.59	0.58	0.56	0.55	0.55	0.55	0.55	0.55	0.55
	2	1.00	0.78	0.77	0.76	0.75	0.73	0.72	0.71	0.68	0.67	0.67	0.67	0.67	0.67	0.67
	2.5	1.00	0.81	0.80	0.79	0.78	0.76	0.75	0.73	0.71	0.70	0.70	0.70	0.70	0.70	0.70
	3	1.00	0.84	0.82	0.81	0.80	0.78	0.77	0.76	0.73	0.72	0.72	0.72	0.72	0.72	0.72
	3.5	1.00	0.86	0.85	0.83	0.82	0.80	0.80	0.78	0.75	0.74	0.74	0.74	0.74	0.74	0.74
	4	1.00	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Gelatin	1	1.00	0.63	0.60	0.59	0.57	0.56	0.54	0.52	0.51	0.51	0.50	0.50	0.50	0.50	0.50
	2	1.00	0.72	0.70	0.68	0.66	0.64	0.62	0.60	0.57	0.56	0.56	0.56	0.56	0.56	0.56
	2.5	1.00	0.78	0.76	0.74	0.73	0.71	0.69	0.65	0.63	0.63	0.63	0.63	0.63	0.63	0.63
	3	1.00	0.80	0.78	0.77	0.75	0.74	0.73	0.70	0.68	0.67	0.67	0.67	0.67	0.67	0.67
	3.5	1.00	0.84	0.83	0.81	0.80	0.78	0.77	0.75	0.73	0.72	0.72	0.72	0.72	0.72	0.72
	4	1.00	0.85	0.83	0.82	0.81	0.80	0.79	0.77	0.75	0.74	0.74	0.74	0.74	0.74	0.74
Acacia	1	1.00	0.62	0.60	0.58	0.55	0.54	0.53	0.51	0.50	0.49	0.48	0.48	0.48	0.48	0.48
	2	1.00	0.70	0.68	0.64	0.62	0.59	0.57	0.56	0.53	0.51	0.49	0.49	0.49	0.49	0.49
	2.5	1.00	0.75	0.73	0.72	0.69	0.67	0.66	0.64	0.61	0.59	0.59	0.59	0.59	0.59	0.59
	3	1.00	0.78	0.77	0.74	0.70	0.68	0.67	0.65	0.61	0.60	0.60	0.60	0.60	0.60	0.60
	3.5	1.00	0.79	0.78	0.76	0.74	0.72	0.70	0.68	0.65	0.64	0.63	0.63	0.63	0.63	0.63
	4	1.00	0.80	0.79	0.77	0.75	0.73	0.71	0.69	0.67	0.67	0.67	0.67	0.67	0.67	0.67

n=3, P<0.05

in the concentrations of suspending agent which led to a decrease in the rate of settling of the particles. The dispersed particles sediment at a faster rate in suspensions containing 0.1 and 2% $^{w}/_{v}$ of suspending agent and the initial sedimentation sedimentation during the first 24 h are much faster than afterwards. All suspension samples prepared maintained steady sedimentation volume after 72 h with the different concentrations of suspending agent. Formulations containing *Terminalia* gum at 3.5 to 4% $^{w}/_{v}$ did not show a reduction in sedimentation volume throughout the period of observation. This was due to the high viscosity and the suspension was difficult to pour from the container. At 2.5% $^{w}/_{v}$, there was an initial

Table 2. The flow rate and viscosity of magnesium carbonate suspensions prepared using different types of suspending agents.

Suspending agents	Concentration of suspending agents (% $^{w}/_{v}$)	Flow rate (ml/s)	Viscosity at 25°C* (CentiPoise)
Terminalia randii gum*	0.00	0.51	50.00
	1.00	0.85	415.00
	2.00	0.69	600.00
	2.50	0.43	800.00
	3.00	0.29	920.00
	3.50-4.00	**	**
Tragacanth gum	1.00	1.01	40.00
	2.00	0.82	70.00
	2.50	0.70	74.00
	3.00	0.42	100.00
	3.50	0.35	110.00
	4.00	0.32	160.00
Gelatin	1.00	1.08	20.00
	2.00	1.05	40.00
	2.50	1.00	60.00
	3.00	0.93	80.00
	3.50	0.67	100.00
	4.00	0.65	120.00
Acacia gum	1.00	1.56	14.00
	2.00	1.51	30.00
	2.50	1.44	45.00
	3.00	1.40	50.00
	3.50	1.33	55.00
	4.00	1.26	65.00

**Too viscous to be determined, n = 3, *p < 0.001.

steady decline in sedimentation volume for all the suspending agents which gradually became constant with *Terminalia randii* gum having the highest (0.78). The trend of sedimentation volume for all the suspending agents at 2.5% $^{w}/_{v}$ was: *Terminalia* > compound tragacanth > gelatin > acacia.

Flow rate

The results of flow rate are presented in Table 2. The flow rate of the suspensions was observed to decrease with increase in concentration of suspending agent. Formulation containing *Terminalia* gum at 3.5 to 4.0 % $^{w}/_{v}$ concentration was too viscous and could not flow through the pipette. A good suspension is one that can easily flow through a container. This also indicates that the dosing of the suspension would not be uniform. The flow rates of the suspensions were in the order of acacia > gelatin > compound tragacanth > *T. randii.*

Viscosity

The viscosities (Table 2) of the suspensions containing the different suspending agents was observed to increase with increase in concentration of suspending agents (Figure 1) and the ranking of viscosity was in the order of *T. randii* gum > compound tragacanth > gelatin > acacia. Table 3 also shows the result of viscosity data analyzed using linear regressions and good correlations (r^2 = 0.910 to 0.996) between viscosity and concentrations were obtained from the suspending agents with a trend of *Terminalia* > compound tragacanth> gelatin > acacia. *Terminalia randii* gum also gave significantly higher ($p < 0.001$) viscosities than the other agents at all concentrations used. This shows that whenever a material with high viscosity is required, the gum will be readily

Table 3. The correlation of viscosities of suspending agents with concentration in magnesium carbonate suspension

Suspending agent	Correlation coefficients (r^2)
Terminalia randii gum	0.9961
Tragacanth gum	0.9096
Gelatin	0.9796
Acacia	0.9804

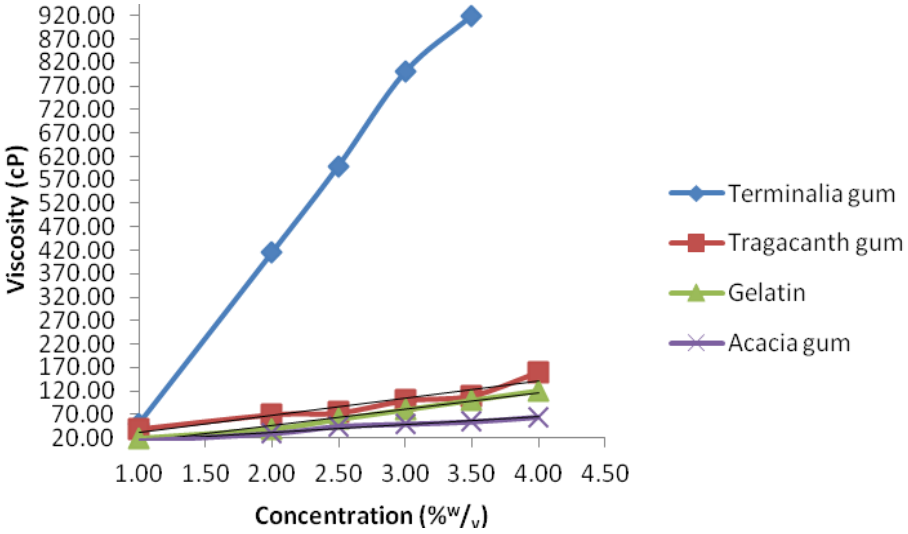

Figure 1. Effect of concentration of suspending agents on the viscosity of Magnesium carbonate suspensions.

gum will be readily useful and quantity needed might be low and this is of economic importance.

Particle size analysis

All the formulations obeyed Stoke's equation when subjected to particle size analysis (Data not shown). The ranking of particle size was terminalia < compound tragacanth < gelatin < acacia. This implies that the particle diameter of dispersed phase was directly proportional to the viscosity of the medium as imparted by the suspending agent. The proportion of smaller particle sizes were also inversely proportional to the sampling time implying that the longer the sampling time, the higher the proportion of smaller particles obtained. This is consistent with the expected behavior of dispersed solids in which the bigger particles fall faster than the smaller ones.

Redispersibility

Generally, the formulated suspensions have a non-Newtonian flow and a ranking of *T. randii* gum>compound tragacanth > gelatin > acacia with respect to sedimentation volume, viscosity and ease of re-dispersibility. Optimum stability and ease of redispersion of magnesium carbonate suspension was obtained at a moderate concentration of 2.5% $^{w}/_{v}$ for terminalia gum.

Conclusion

In view of these results, *T. randii* gum exhibited the best suspending properties when compared to all the other agents employed in the formulations and can be used as an alternative suspending agent in the formulation of pharmaceutical suspensions. Moreover, its high viscosity could be exploited for use as a stabilizer and thickening agent in the food industry.

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