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Research Article

Improvement of Dissolution and Hypoglycemic Efficacy of Glimepiride by Different Carriers

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Abstract. Effects of tromethamine (Tris), polyvinylpyrrolidone (PVP-K25), and low molecular weight chitosan (LM-CH) on dissolution and therapeutic efficacy of glimepiride (Gmp) were investigated using physical mixtures (PMs), coground mixtures, coprecipitates (Coppts) or kneaded mixtures (KMs), and compared with drug alone. Fourier transform infrared spectroscopy, differential scanning colorimetry, and X-ray diffractometry were performed to identify any physicochemical interaction with Gmp. Surface morphology was examined via scanning electron microscopy. The results of Gmp *in vitro* dissolution revealed that it was greatly enhanced by Coppt with Tris or PVP-K25 and KM with LM-CH at a drug to carrier ratio of 1:8. Gmp amorphization by PVP-K25 and LM-CH was a major factor in increasing Gmp dissolution. Being basic, Tris might increase the pH of the microdiffusion layer around Gmp particles improving its dissolution. Formation of water-soluble complexes suggested by solubility study may also explain the enhanced dissolution. Capsules were prepared from Coppts and KM 1:8 drug to carrier binary systems and also with Tris PMs. *In vivo*, the hypoglycemic efficacy of Gmp capsules in rabbits increased by 1.63-, 1.50-, and 1.46-fold for 1:8 Coppts with Tris or PVP-K25 and KM with LM-CH respectively, compared with Gmp alone. Surprisingly, the response to Tris PM 1:20 capsules was 1.52-fold revealing statistically insignificant difference to that of Tris Coppt 1:8 (1.63 fold). As a conclusion, dissolution enhancement and hypoglycemic potentiation by 1:20 PM of Gmp/Tris, being simple and easy to prepare, may enable development of a reduced-dose and fast-release oral dosage form of Gmp.

KEY WORDS: dissolution enhancement; glimepiride; low molecular weight chitosan; polyvinylpyrrolidone; tromethamine.

INTRODUCTION

On therapeutic application, poorly water-soluble agents encounter a number of serious proplems such as, poor absorption and bioavailability upon oral administration and formation of aggregates following intravenous administration that can cause embolization of blood vessels resulting in serious respiratory system failure, local toxicity, and/or lowered systemic bioavailability (1). Hence, about 40% of potentially valuable drug candidates identified by high throughput screening are rejected and never enter a formulation development stage due to their poor water solubility (2). Consequently, various techniques such as micronization, solubilization, salt formation, complexation, change in physical form, use of prodrugs, drug derivitization, alteration in pH, addition of surfactants, and others have been employed in order to improve the dissolution and bioavailability of sparingly soluble drugs (3,4). Hydrophilic carriers, such as Tris, LM-CH, and PVP (Fig. 1b, c, and d respectively) have been widely used for this purpose. For example, different solid binary systems as physical mixtures (PMs) and coprecipitates (Coppts) with Tris and PVP were successful in increasing the dissolution and the analgesic effect of nimesulide (5). Dissolution rates of benzoic acid (6), hydrochlorothiazide (7), and furosemide (8) were markedly enhanced by use of Tris as a carrier. Enhancement of naproxen dissolution by PMs, coground mixtures (GMs), and kneaded mixtures (KMs) with chitosan has been reported (9). The ability of chitosan to improve the solubility of some drugs as aceclofenac has been illustrated (10). Dissolution improvement of poorly water-soluble sulfonylureas was reported in purpose of increasing their therapeutic efficacy. For example, glibenclamide solubility was enhanced by PVP (11) and Tris (12). That of repaglinide was increased by ultra-rapid freezing with Tris (13). The effects of cyclodextrin and chitosan on the solubility of glyburide have been investigated (14).

Gmp, Fig. 1a, is one of the third generation sulfonylurea drugs used for treatment of diabetis mellitus type 2 (15). Several investigations reported that this hypoglycemic drug showed number of advantages over the other sulfonylureas including lower dosage, rapid onset, and longer duration of action (16,17). However, its poor water solubility and slow dissolution may result in irreproducible clinical response or therapeutic failure because of subtherapeutic plasma levels (18). Recently, dissolution improvement of the widely used

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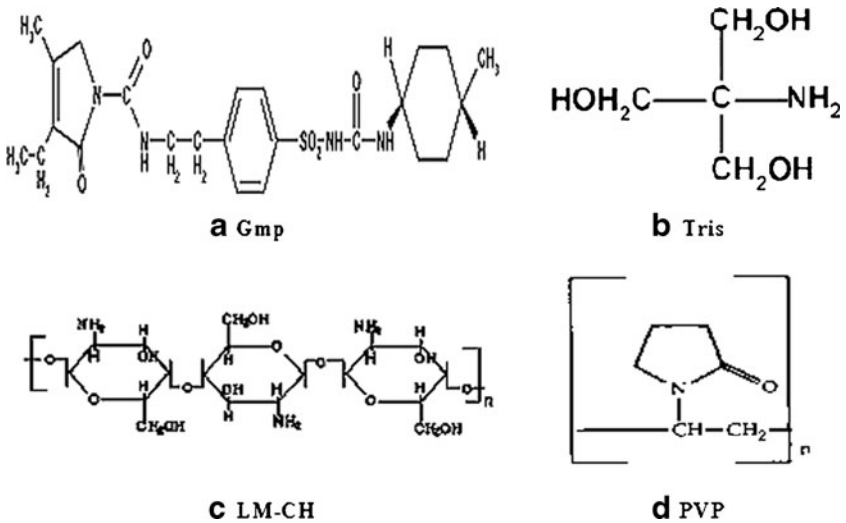

Fig. 1. Chemical structures of the drug and the tested carriers (*Gmp* glimepiride (21), *Tris* tromethamine (5), *LM-CH* low molecular weight chitosan (24), *PVP* polyvinylpyrrolidone (30))

sulfonylurea, Gmp, was obtained utilizing complexes with cyclodextrin and its derivatives (19), solid dispersion with polyethylene glycol 6000 (20) or hyperbranched poly(esteramides) (21), cosolvents (22), and microencapsulation by spray congealing technology using hydrophilic meltable carriers (23). Therefore, it was worthy of trying to prepare different Gmp binary systems with each of Tris, LM-CH, and PVP-K25, investigate and compare the effects of these carriers in the prepared systems on dissolution and hypoglycemic efficacy of Gmp.

MATERIAL AND METHODS

Materials

Gmp (Batch # RM0700075) was kindly supplied by Delta Pharma Co., The 10th of Ramadan City, Egypt. Tris and PVP-K25 were obtained from Merck Co., Darmstadt, Germany. LM-CH was purchased from Sigma Chem. Co., St Louis, MO, USA. Dicalcium phosphate was obtained from Mendell, UK. Glucose oxidase peroxidase (GOD/POD) kit was

Table I. Dissolution Efficiency (DE) and Relative Dissolution Rate (RDR), from Different Binary Systems and Capsules

	Binary system[a]		Capsules[b]		Fold increase[c]	
Drug:carrier	DE_{30}[d]	RDR_{30}[e]	DE_{60}[f]	RDR_{60}[g]	DE_{30}[d]	DE_{60}[f]
Gmp alone	6.19	_	6.01	_	_	_
Tris PM 1:8	13.06	2.10	_		2.11	_
Tris PM 1:10	15.58	2.89	21.70	3.19	2.52	3.61
Tris PM 1:15	19.72	3.75	26.72	4.13	3.19	4.44
Tris PM 1:20	26.99	3.99	33.48	5.21	4.36	5.57
Tris GM 1:8	38.43	5.85	_		6.21	_
Tris GM 1:20	46.77	6.95	_		7.56	_
Tris Coppt 1:8	65.17	9.24	49.45	8.65	10.53	8.23
LM-CH PM 1:8	13.76	1.87	_		2.22	
LM-CH PM 1:20	16.52	2.45	_		2.67	
LM-CH GM 1:8	38.37	5.70	_		6.2	
LM-CH GM 1:20	46.10	7.04	_		7.45	
LM-CH KM 1:8	59.80	9.28	44.45	8.29	9.66	7.39
PVP-K25 PM 1:8	18.93	2.79	_		3.06	
PVP-K25 PM 1:20	9.73	1.52	_		1.57	
PVP-K25 GM 1:8	37.70	5.80	_		6.09	
PVP-K25 GM 1:20 11:20	32.05	5.02	_		5.18	
PVP-K25 Coppt 1:8	67.33	9.81	54.78	9.11	10.88	9.11

[a] *PM* indicates physical mixture; *GM* ground mixture; *Coppt* coprecipitate; and *KM* kneaded mixture
[b] Each capsule contains 3 mg Gmp and amount of carrier specified by its ratio. The final weight was 50 mg by dilution with dicalcium phosphate excipient when needed
[c] Compared with Gmp alone
[d] The dissolution efficiencies at 30 min for the powders and capsules
[e] The relative dissolution rate at 30 min for the powders and capsules
[f] The dissolution efficiencies at 60 min for the powders and capsules
[g] The relative dissolution rate at 60 min for the powders and capsules

Table II. Solubility Parameters of Gmp with The Tested Carriers at 37°C

	In buffer pH 6.8		In water	
Carrier	Slope	Stability constant (K_s; mg^{-1} ml)	Slope	Stability constant (K_s; mg^{-1} ml)
Tris	0.0026	0.965	0.0024	1.002
LM-CH	0.0009	0.334	0.0008	0.334
PVP-K25	0.0019	0.705	0.0018	0.751

purchased from Spinreact, S. A. U., Spain. Amaryl 3 mg tablets manufactured by Aventis, Cairo, Egypt, were used as a commercial product for comparison. All other chemicals were of reagent grade.

Methods

Preparation of the Different Solid Binary Systems and Capsules

Gmp with each of Tris, LM-CH, and PVP-K25 as PMs, GMs, and Coppts in different drug/carrier ratios were prepared (Table I). With LM-CH, however, the Coppts were replaced with KMs.

PMs were prepared by geometric proportion technique. GMs were prepared by grinding the corresponding PM in vibrational mill for 1 h (Microne Micronising Mill, England). The Coppts were prepared by the solvent evaporation technique (5,20). The solvent was methanol and evaporation was under vacuum at 40°C. To prepare KMs of LM-CH, the corresponding PM was kneaded for 0.5 h with distilled water in an amount equal to 1.2 times of total weight of the PM using mortar and pestle (24). The resulting KM was dried at room temperature in a desiccator containing anhydrous calcium chloride for 48 h. The resulting binary systems were ground if necessary and passed through a 250-μm sieve.

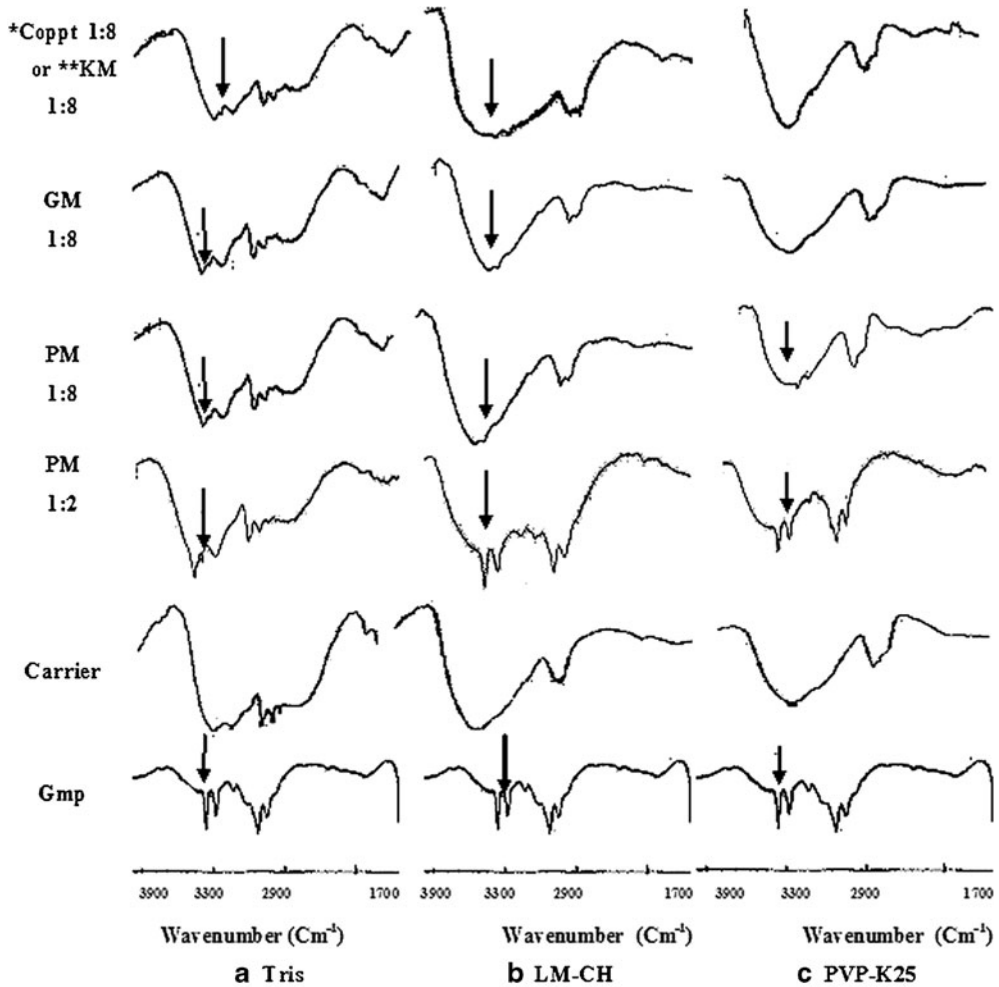

Fig. 2. FTIR spectra of some prepared binary systems compared with Gmp and carriers, each alone. The *arrows* indicate Gmp N–H stretching peaks (*PM* physical mixture, *GM* coground mixture; *Coppt* coprecipitate, *KM* kneaded mixture, **Coppt* with Tris or PVP-K25, ***KM* with LM-CH)

Weights of the prepared systems, each equivalent to 3 mg Gmp, was diluted with dicalcium phosphate when needed, as excipient, to 50 mg total weight and dispensed in hard gelatin capsules (Table I).

Estimation of Drug Content

An accurately weighed amount of each of the binary systems equivalent to 3 mg of Gmp was dissolved in minimum amount of methanol with sonication. The volume was adjusted with distilled water to 100 ml in a volumetric flask filtered to remove undissolved material if any. The solution was assayed for drug content spectrophotometrically at 226 nm using blank containing an equivalent amount of the corresponding carrier. Three replicates were prepared and the average drug contents were estimated.

Characterization of the Prepared Solid Binary Systems

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectra of all carriers and binary systems were obtained using Mattson 5000 FTIR Spectrophotometer (Madison Instruments, Middleton, Wisconsin, USA). KBr discs were prepared by means of hydrostatic press. The scanning range was 500 to 3900 cm^{-1}

Differential Scanning Colorimetry (DSC)

Differential scanning colorimetry (DSC) data were performed using a Perkin-Elmer differential scanning colorimeter model DSC-4 (New York, USA). It was calibrated with indium (99.99% purity, m.p. 156.6°C), at heating rates of 10°C/min. One- to 5-mg samples were heated in aluminum crimped pans under nitrogen gas flow, in the 30–450°C range.

X-Ray Diffractometry

X-ray diffractometry (XRD) patterns were obtained using a Diano X-ray diffractometer equipped with Co Kα (USA). The tube operated at 45 kv, 9 mA.

Scanning Electron Microscopy

The surface morphology of Gmp 1:8 Coppt with each of Tris and PVP-K25 were examined by means of a scanning electron microscope (JEOL, Japan), compared with Gmp alone and both the 1:8 and 1:20 PMs. The powders

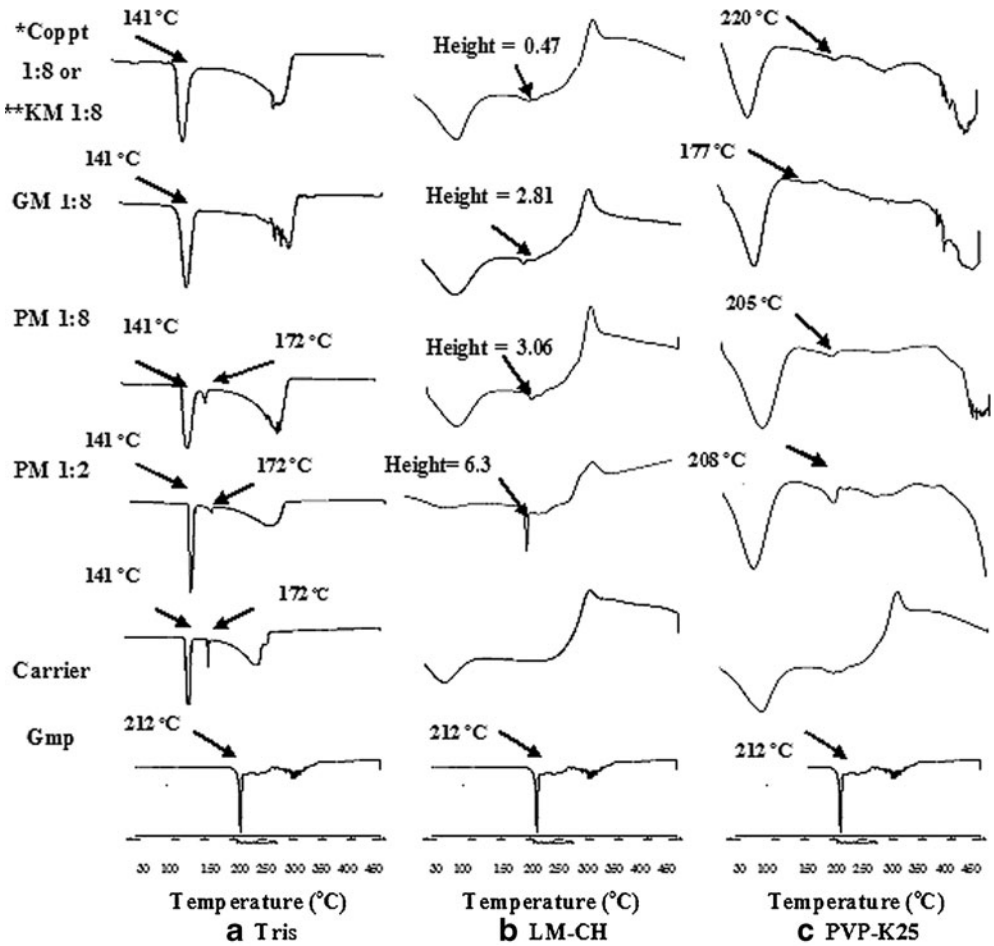

Fig. 3. DSC curves of some prepared binary systems with the different carriers compared with Gmp and carriers, each alone (*PM* physical mixture, *GM* coground mixture, *Coppt* coprecipitate, *KM* kneaded mixture, **Coppt* with Tris or PVP-K25, ***KM* with LM-CH)

were fixed on a brass stub using double-sided adhesive tape and then made electrically conductive by coating, in a vacuum, with a thin layer of gold (approximately 150 Å) for 30 s. The pictures were taken at an excitation voltage of 25 kV.

Solubility Studies

Solubility measurements were carried out according to the method of (25). An excess amount of Gmp in either aqueous or phosphate buffer (pH=6.8) solutions containing different concentrations of each carrier equivalent to drug to carrier ratios of 1:1, 1:2, 1:4, 1:8, 1:10, 1:20, 1:40, and 1:60. The suspensions were shaken at 37 ±0.5°C for 72 h and then filtered through a millipore filter (0.45 μ). An aliquot portion of the filtrate was properly diluted when necessary and analyzed for its drug content by measuring its ultraviolet absorbance at 226 nm against blank solution containing the same concentration of the studied carrier. The solubility phase diagrams were constructed and the apparent stability constants (K_s) were calculated according to the following equation (26):

$$K = \text{slope}/\text{intercept}(1 - \text{slope})$$

Where, the intercept is the solubility of Gmp alone in the respective medium.

In Vitro Dissolution Study

Dissolution of Gmp from all prepared binary systems was assessed by USP apparatus II (paddle method) at 37°C using 500 ml of phosphate buffer (pH 6.8) (19) and stirred at 100 rpm. With respect to capsules, the same procedure was employed except that USP apparatus I (basket method) was used. Aliquots from the dissolution medium were withdrawn at time intervals of 15, 30, 60, 90, 120 and 180 min and the medium was replenished by equal volumes of fresh dissolution medium. The samples were filtered through a millipore filter (pore size 45 μm) and analyzed for Gmp content by measuring its absorbance at 226 nm. Buffer containing an equivalent amount of the tested carrier (Tris, LM-CH, or PVP-K25) and/or excipient was

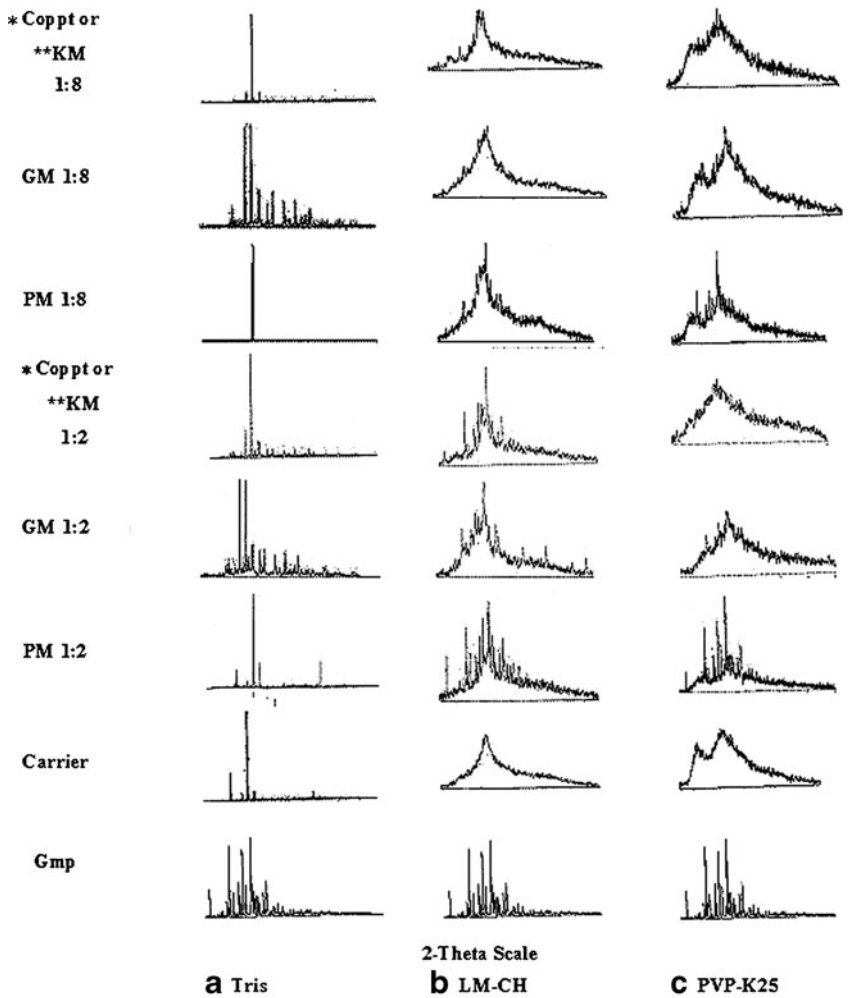

Fig. 4. XRD spectra of some prepared binary systems with the different carriers compared with Gmp and carriers, each alone (*PM* physical mixture, *GM* coground mixture, *Coppt* coprecipitate, *KM* kneaded mixture, **Coppt* with Tris or PVP-K25, ***KM* with LM-CH)

used as a blank after filtration. Each experiment was done in triplicate and the average % dissolved was calculated at each time interval. Values of dissolution efficiency (DE) were calculated as the percent ratio of area under the dissolution curve up to the time, *t*, to that of the area of the rectangle described by 100% dissolution at the same time (26,27). The value of relative dissolution rate (RDR) was estimated as the ratio of the percentage dissolved Gmp from each binary system or capsule to that of Gmp alone at the same time (5).

Therapeutic Efficacy Assessment

Male rabbits weighing 1.75–2 kg were kept on a standard diet. The animals were fasted overnight prior to the experiment with water *ad libitum*. Food was withheld during the experiment. The rabbits were randomized into eight groups each of six animals. Animal use protocol was approved by the research ethical committee at Mansoura University in accordance with "principles of laboratory animal care" (NIH publication No. 85-23, revised 1985). One group of animals served for the control capsule and another for the marketed one (amaryl 3 mg). The other groups were for capsules of the selected binary systems as shown in Table II. Blood samples were collected from the marginal ear vein of the animal at time intervals 1, 2, 3, 4, 5, 6, 8, 10, and 24 h after drug administration (BGa). Each animal served as its own control by taking a sample of blood before drug administration (BGb). Blood glucose level was assessed by glucose oxidase peroxidase method using GOD/POD kit (28). The hypoglycemic response was evaluated as percentage reduction in blood glucose level (%RBGL) and the data were plotted as mean values *versus* time. The area under these response curves ($AUC_{0-24\ h}$) were calculated using Prism 4 software (19,29). The values of %RBGL were calculated as follows:

$$\%\mathrm{RBGL} = \frac{\mathrm{BGb} - \mathrm{BGa}}{\mathrm{BGb}} \times 100$$

Statistical Analysis

In vitro, the dissolution data were represented as average of triplicate and standard deviation. The values of the maximum %RBGL and $AUC_{0-24\ h}$ of the different groups were statistically analyzed based on one-way analysis of variance test followed by Tukey–Kramer test to compare between

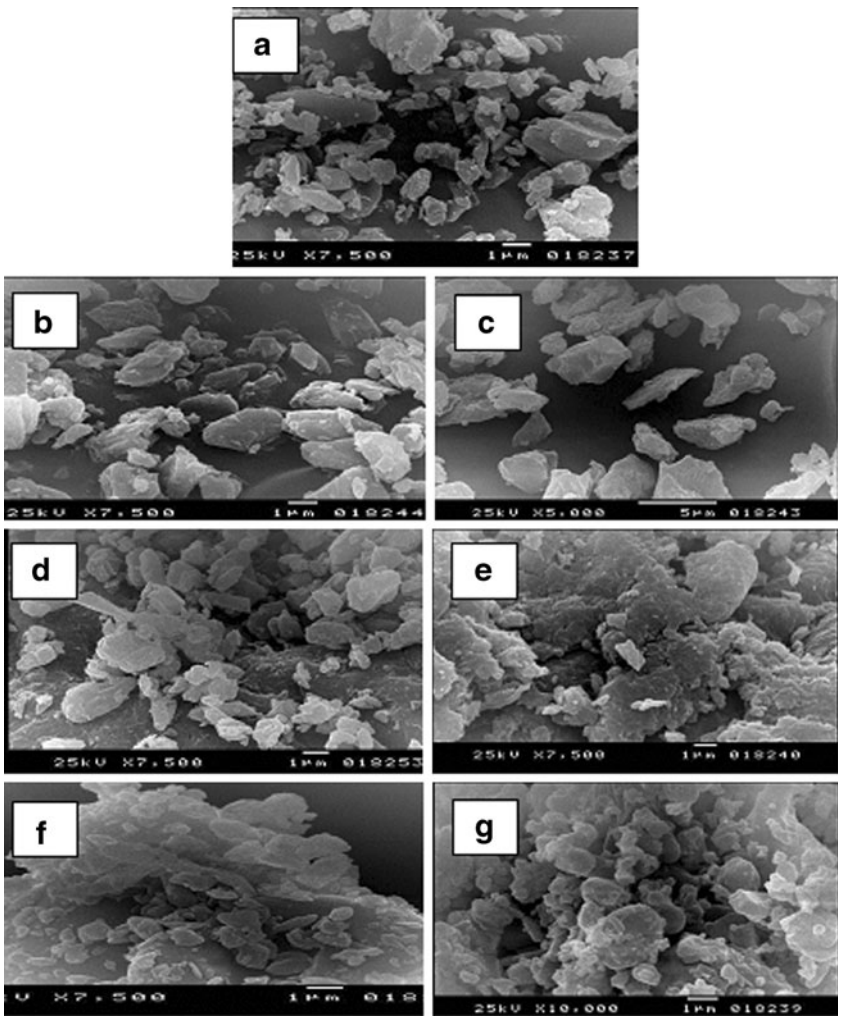

Fig. 5. SEM of Gmp alone (**a**), Gmp/Tris PM 1:20 (**b**), Gmp/Tris Coppt 1:8 (**c**), Gmp/LM-CH PM 1:8 (**d**), Gmp/LM-CH KM 1:8 (**e**), Gmp/PVP-K25 PM 1:8 (**f**), and Gmp/PVP-K25 Coppt 1:8 (**g**)

pairs. This statistical analysis was computed with GraphPad InStat version 3.0 (GraphPad Software, Inc., CA, USA) at different levels of *P* values.

RESULTS AND DISCUSSION

Drug Content of the Prepared Solid Binary Systems

Gmp content in different binary systems ranged from 92.07% to 99.3%. PMs with the three carriers showed higher average drug contents than the corresponding GMs, Coppts and KMs may be due to the simpler method of preparation that minimized drug loss.

Characterization of the Prepared Solid Binary Systems

FTIR

FTIR spectra of the 1:8 drug/carrier ratio are shown in Fig. 2. The 1:2 ratio was added for comparison. The spectrum of Gmp reveals its characteristic peaks of N–H stretch at wavenumbers of 3369 and 3288 cm^{-1} (19). Spectra of Gmp with each of 8 Tris and LM-CH either as PM, GM and Coppt or KM appeared as combined spectra of both drug and the corresponding carrier (Fig. 2a and b, respectively). These results may indicate absence of identified interaction between Gmp and either of Tris or LM-CH. On the other hand, Gmp with PVP-K25 1:8 either as GM or Coppt showed a broadening or complete disappearance of Gmp N–H stretching peaks contrary to the corresponding PM. This may be due to an interaction between Gmp N–H groups and PVP-K25 carbonyl groups through hydrogen bonding on cogrinding and coprecipitation. A similar reaction of PVP carbonyl groups with hydroxyl groups of anionic indomethacin through hydrogen bonding has been reported (30).

DSC

Figure 3 illustrates DSC data of the prepared systems with the tested carriers at drug to carrier ratio of 1:8 compared with those of Gmp and carriers each alone. DSC curves of PMs of the three carriers with the drug at a ratio of 1:2 was included in this figure to demonstrate the effect of dilution on the height of Gmp peak. The crystalline anhydrous nature of untreated Gmp was expressed by a sharp endothermic peak (T_{peak}=212°C) corresponding to its melting point (20). Pure Tris experienced two sharp endothermic peaks: at 172.8°C close to its reported melting point range (168–172°C) (31) and at 141°C representing its solid–solid transition (8) (Fig. 3a). The disappearance of the melting point peak of Gmp (T_{peak}=212°C) in the PMs (1:2 and 1:8) with Tris might be due to the thermal energy supplied during DSC scan which brought both Gmp and Tris to a highly dispersed state. Thus, more contact may be allowed resulting in an interaction that could not occur in their solid PMs and was not seen in their FTIR results. The possibilty of conversion of some crystalline compounds into highly dispersed state due to the thermal energy supplied during DSC scan has been reported in other studies (5,32). Excess Tris without interaction during DSC scan may account for the presence of the two peaks (141°C

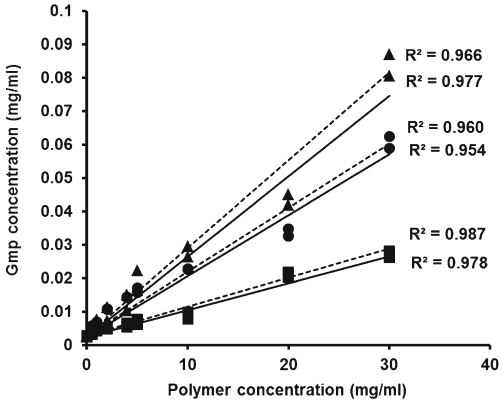

Fig. 6. Phase-solubility diagrams of Gmp in phosphate buffer pH 6.8 (*dotted lines*) and distilled water (*solid lines*) in the presence of increasing concentrations of tromethamine (Tris, *triangles*), low molecular weight chitosan (LM-CH, *squares*), and polyvinylpyrrolidone-K25 (PVP-K25, *circles*)

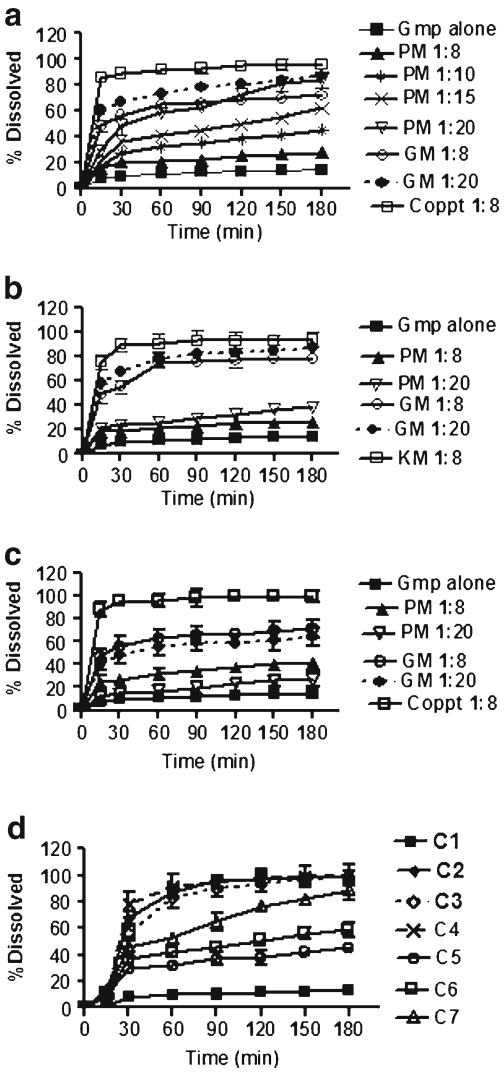

Fig. 7. Dissolution of Gmp in phosphate buffer pH 6.8 from some binary systems with **a** Tris, **b** LM-CH, **c** PVP-K25 and **d** the prepared capsules; all compared with Gmp alone; (see Table III for capsules composition; *PM* physical mixture, *GM* coground mixture, *Coppt* coprecipitate, *KM* kneaded mixture)

and 172°C). Both GM and Coppt with Tris experienced only one peak for Tris (140°C) and absence of the peak at 171°C. This could be explained by change of the carrier crystals with these harsh treatments as has been suggested by others (33). The broad endotherm of LM-CH confirmed its amorphous hydrated nature (Fig. 3b; 9). This figure shows that Gmp peaks existed in all LM-CH preparations (PM1:2, PM 1:8, GM 1:8 and KM 1:8) negating interaction of this carrier with Gmp as evidenced by FTIR data. The peak heights were 3.06, 2.81, and 0.47 mv for PM, GM, and KM in 1:8 drug carrier ratio, respectively, indicating variation in crystalinity of Gmp in the different systems with LM-CH. More amorphization of the drug by kneading took place than by cogrinding with LM-CH. The broad endotherm of PVP-K25 indicated its amorphous nature (Fig. 3c). Coppt in 1:8 of Gmp/PVP-K25 showed a broadening and shift in Gmp endothermic peak. The shift in Gmp peak was small in case of PM compared with Coppt. In accordance with FTIR data, these findings indicate interaction of Gmp and its amorphization on cogrinding and coprecipitation with PVP-K25.

XRD

XRD spectra at a diffraction angle of 2-theta for different binary systems with each of Tris, LM-CH, and PVP-K25 are depicted in Fig. 4a, b, and c, respectively. Drug and carriers, each alone, were included for comparison. XRD patterns at a ratio of 1:2 drug/carrier were added to the spectra to show clearly the impact of the carrier ratio and the method of preparation on Gmp crystallinity compared with 1:8.

Numerous sharp diffraction peaks of pure Gmp were recorded indicating its crystalline nature (20). Tris alone exhibited a number of short diffraction peaks and one long distinct peak that confirmed its highly crystalline nature (Fig. 4a). The peaks of crystalline Gmp and Tris exist in their binary systems. In case of Tris GMs and Coppts with Gmp, possible change of the crystalline structure of the carrier might account for their different diffraction patterns from those of the corrsponding PMs (33).

LM-CH (9) and PVP-K25 (5,32) amorphous natures were represented by halo diffraction patterns characterized by absence of sharp peaks (Fig. 4b and c, respectively). The ratio of 1:2, GM and KM with LM-CH showed less crystallinity of Gmp than that noticed with corresponding PM. These findings were more clear at a ratio of 1:8 may be due to dilution with higher LM-CH content. GMs and Coppts with PVP-K25, at both ratios 1:2 and 1:8, resulted in a pronounced reduction in Gmp crystallinity compared with the corresponding PMs. The results obtained with scanning electron microscopy (SEM) hereafter together with that seen with XRD would augment each other.

SEM

The photomicrographs of the different drug/carriers ratios are shown in Fig. 5. The morphology of the PM 1:20 and Coppt 1:8 with Tris appeared as broken crystals with irregular shape and size similar to that of drug alone. Factors other than amorphization helped in increasing the dissolution rate of the drug in this case, possibly the pH of the microdiffusion layer around the drug particles. With LM-CH and PVP-K25, however, the PMs showed crystals of the drug dispersed in amorphous matrix of either polymer. On the other hand, the photomicrographs of the KM 1:8 with LM-CH or Coppt 1:8 with PVP-K25 reveal amorphous-looking lumps. The closer contact between the hydrophilic carriers and drug in addition to the amorphization were influential in increasing the dissolution rate of Gmp.

Table III. Statistical Analysis of The Hypoglycemic Response Parameters

Capsules[a]	T_{max} (h)	Mean max. %RBGL±S.E.	$AUC_{0-24\ h}$±S.E.	*P* value	
				Mean Max. %RBGL	$AUC_{0-24\ h}$
C1 Control Gmp alone	4	33.3±1.5	311.7±15.2	–	–
Marketed tablet	4	34.0±0.6	342.9±13.8	>0.05[b]	>0.05[b]
C2 Coppt Gmp/Tris 1:8	4	44.7±2.6	509.3±27.6	<0.01[b,c] >0.05[d,e,f]	<0.001[b] <0.01[c] >0.05[d,e,f]
C3 KM Gmp/LM-CH 1:8	4	41.7±2.2	456.3±16.7	<0.05[b] >0.05[c]	<0.01[b] <0.05[c]
C4 Coppt Gmp/PVP-K25 1:8	4	42.2±1.8	466.5±45.2	<0.05[b,c]	<0.01[b] <0.05[c]
C5 PM Gmp/Tris 1:10	4	35.2±1.5	354.2±14.9	>0.05[b,c]	>0.05[b,c]
C6 PM Gmp/Tris 1:15	4	36.5±1.3	404.4±33.6	>0.05[b,c]	>0.05[b,c]
C7 PM Gmp/Tris 1:20	4	41.2±1.3	474.8±41.1	<0.01[b,c] >0.05[d,e,f]	<0.01[b] <0.05[c] >0.05[d,e,f]

[a] Each capsule contains 3 mg Gmp
[b] Compared with C1
[c] Compared with marketed tablet
[d] Compared with C3
[e] Compared with C4
[f] Compared with C7

Solubility Studies

The solubilities of Gmp in phosphate buffer 6.8 and water were 2.66 and 2.41 μg/ml, respectively, indicating its poor solubility in both media. A linear increase in Gmp solubility in the following order: Tris>PVP-K25>LM-CH has been recorded with increased carrier levels (Fig. 6). Formation of water-soluble complexes between Gmp and these carriers can be suggested. Water-soluble complexes of Tris with nimesulide (5), PVP with acetaminophen (34), and chitosan with naproxen (9) have been reported. Some authors used the slopes of the linear solubility curves (A_L) and the apparent stability constants (K_s) as indicatives of the solubilizing efficiency and the binding affinity of the carriers towards examined drugs, respectively (26). Tris showed highest solubilizing efficiency and binding affinity followed by PVP-K25 then LM-CH as clarified by the slope and K_s values, respectively, of the corresponding linear curves (Table II). Being compratively strong basic, Tris may be expected to form more stabilized complexes with Gmp, hence higher solubility of this drug was observed (5,7,13). The hydrogen bonding of PVP with Gmp as suggested by FTIR results might stabilize the complex with this drug (Fig. 2c).

In Vitro Dissolution

The tested binary systems were prepared at different drug to carrier ratios, however, a noticeable enhancement in Gmp dissolution was observed at a drug to 12 carrier ratio of 1:8. PMs and GMs at a drug to carrier ratio of 1:20 were formed to investigate the possibility of optimal dissolution using these techniques that are simpler than kneading and coprecipitation. Hence, the dissolution results in phosphate buffer pH 6.8 at only these two ratios were illustrated for binary systems with Tris, LM-CH, and PVP-K25 in Fig. 7a, b, and c respectively. Table I represents the values of DE and RDR of Gmp and its binary systems with the three carriers, as well as those of the prepared capsules. Generally the ratio of 1:8, Coppt with Tris or PVP-K25 and KM with LM-CH showed almost complete dissolution after 30 min (burst effect) with 10.53-, 10.88-, and 9.66-fold increase in DE_{30} respectively, compared with drug alone (Table I). In accordance, the values of RDR_{30} of the above mentioned binary systems were 9.24, 9.81, and 9.28, respectively. With the three carriers, the increase in Gmp dissolution at a drug to carrier ratio 1:8 was in the following order: Coppt or KM>GM>PM (Fig. 7). The DE_{30} and RDR_{30} values for these systems, illustrated in Table I, confirm these orders.

The superiority of dissolution in case of coprecipitation with PVP-K25 and kneading with LM-CH can be attributed to amorphization of Gmp as confirmed with XRD and SEM results. An amorphous drug would be expected to dissolve faster than a crystalline material because of its high energy state (5). The increased dissolution of some drugs by cogrinding and kneading with chitosan was represented in the literature (9,35). Coppts with each of Tris and PVP potentiated the dissolution and the analgesic effect of nimesulide (5).

With LM-CH and PVP-K25, the less crystalline structure of Gmp confirmed by XRD data on cogrinding may explain the

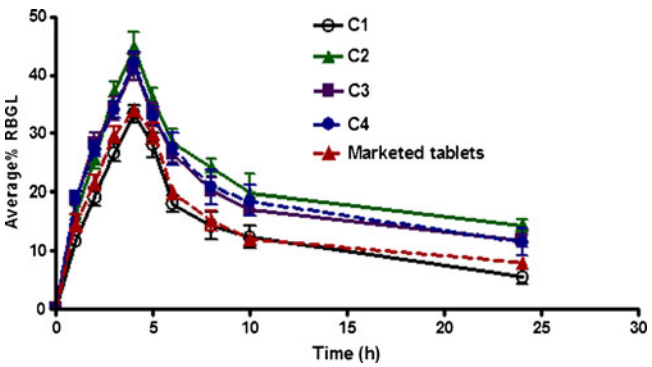

Fig. 8. Hypoglycemic efficacy of Gmp from capsules with different systems compared with the drug alone and marketed tablet (*%RBGL* percentage reduction in blood glucose level, see Table III for capsules composition)

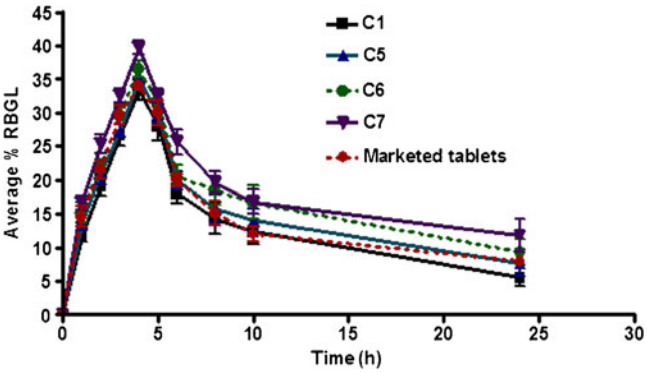

Fig. 9. Effect of capsules containing different ratios of physically mixed Tris on the hypoglycemic efficacy of Gmp compared with that containing Gmp alone and marketed tablet (*%RBGL* percentage reduction in blood glucose level, see Table III for capsules composition)

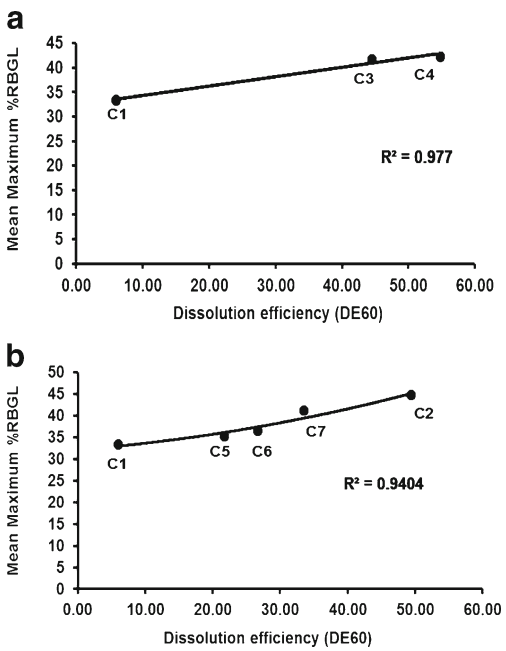

Fig. 10. **a** *In vitro–in vivo* correlation of capsules containing **a** KM 1:8 with LM-CH (*C3*), Coppt 1:8 with PVP-K25 (*C4*) and that of Gmp alone (*C1*). **b** Coppt 1:8 (*C2*), PM 1:10, PM 1:15, and PM 1:20 with Tris (*C5*, *C6*, and *C7*, respectively) and that of Gmp alone (*C1*; see Table III for capsules composition and Table I for DE_{60})

higher dissolution observed with GMs than the corresponding PMs (Fig. 4b and c, respectively). A pronounced increase in the viscosity of the diffusion layer due to higher PVP concentration may account for the lower Gmp dissolution from PMs and GMs with this carrier at a drug to carrier ratio of 1:20 than the corresponding 1:8 binary systems. Similar behavior with PVP was documented in the literature (5). A unique factor pertinent to Tris; being basic could increase the pH of the microenvironment around Gmp particles augmenting dissolution. To ascertain this, PM of Gmp/Tris in 1:10, 1:15 and 1:20 were prepared and dissolution rates were assessed. A gradual increase in dissolution rate of Gmp was noticed with the increase in Tris ratio. The increase in DE_{30} for Gmp/Tris PMs 1:10, 1:15 and 1:20 was 2.52-, 3.19- and 4.36- fold of Gmp alone, respectively (Table I). Nevertheless, these values were lower than that of Gmp/Tris 1:8 Coppt (10.53-fold, Table I). Higher dissolution of Gmp from GMs and Coppts with Tris than the corresponding PMs was noted probably due to more intimate contact between Gmp and this carrier.

The Coppts and KM in 1:8 ratio were selected to prepare capsules of Gmp (Table I). Moreover, capsules of Gmp physically mixed with different Tris ratios (1:10, 1:15, and 1:20) were also included for comparison (Table I). Gmp dissolution from the prepared capsules is described in Fig. 7d together with that of the drug alone. Generally, *in vitro* dissolution of Gmp from capsules of the different systems was higher than that observed with the drug alone and parallel to that of the corresponding powders. Complete dissolution was within 60 min with lag time of 30 min, for the gelatin shell to swell and dissolve. The highest Gmp dissolution observed with capsules of Tris PMs was that of 1:20 ratio (DE_{60} 33.48). However, it was lower than that experienced with capsules containing the Coppt of Gmp/Tris in 1:8 binary system (DE_{60} 49.45; Table I).

Therapeutic Efficacy Assessment

Capsules containing Coppts or KM in 1:8 Gmp/carrier ratio as well as PMs with different ratios of Tris were selected for *in vivo* experiment in rabbits (Table III). Figures 8 and 9 elucidate the average %RBGL in normal rabbits after treatment with capsules of the selected binary systems as in Table III. The area under the curve of %RBGL-time was used as a reflection of the insulin level by some authors (29). The results as %RBGL and $AUC_{0-24\ h}$ were statistically compared with those capsules of Gmp alone and marketed tablet (amaryl 3 mg; Table III) (19). The data of the two Coppts and KM, were statistically insignificant between themselves, $P > 0.05$, while each was significantly different from capsules of Gmp alone and the marketed tablet, $P < 0.05$ or < 0.01 (Table III). The hypoglycemic efficacy ($AUC_{0-24\ h}$) of Tris and PVP-K25 Coppts and LM-CH KM were higher by 1.63-, 1.50-, and 1.46-fold respectively, compared with that of Gmp alone (Table III).

Conversely from dissolution studies, the 1:20 PM with Tris showed a statistically similar response ($P > 0.05$) to Coppt Gmp/Tris and to that with PVP-K25 (Table III) despite the amorphizing power of the later revealed by solid state analysis. This might be because of the basic effect of Tris on the microenvironmental pH around Gmp particles in the GIT resulting in higher dissolution rate. This result makes the PM of Gmp/Tris in 1:20 ratio a promising system of high Gmp bioavailability. Repaglinide an antidiabetic drug with slight solubility has been ultra-rapid freezed with Tris (13). The 1:10 and 1:15 PMs with Tris, C5 and C6, respectively, exhibited statistically insignificant difference from the control (C1) and marketed tablet (Table III).

In Vitro–In Vivo Correlation

In vitro and *in vivo* correlation was examined using DE_{60} of the tested capsules as the *in vitro* parameter and mean maximum %RBGL as the *in vivo* one.

After some trials to get the highest correlation coefficient squared (r^2) value possible, it was found that with LM-CH as KM 1:8 (C3), PVP-K25 as Coppt 1:8 (C4) and Gmp alone (C1; Table III), the value of r^2 was 0.9770 (Fig. 10a). A slightly lower r^2 value, 0.9404, was obtained with Tris PMs 1:10, 1:15 and 1:20 (C5, C6 and C7, respectively), and Coppt 1:8 (C2) together with Gmp alone (C1; Fig. 10b). Surprisingly, these correlations came in accordance with the suggestion of the mechanisms involved in increasing the dissolution rate of Gmp. In the first case (Fig. 10a), amorphization of Gmp with the carriers whether LM-CH or PVP-K25 was the common mechanism. In the second case (Fig. 10b) however, alkalization of the diffusion layer around Gmp particles by Tris could play a major role.

CONCLUSIONS

Gmp/carrier at a ratio of 1:8, Coppt with each of Tris and PVP-K25 and KM with LM-CH showed highly enhanced dissolution of the drug by 9.24-, 9.81-, and 9.28-fold respectively, compared with the drug alone. Gmp amorphization by PVP-K25 and LM-CH was a major factor in increasing Gmp dissolution rate. Being basic, Tris possibly increased the pH of the microdiffusion layer around Gmp particles improving its dissolution. As well, formation of water-soluble complexes may account for the enhanced dissolution. Capsules of PM 1:20 with Tris resulted in an improvement in the therapeutic effect statistically similar to that obtained with Coppt 1:8 drug/Tris possibly due to the increase in the microenvironmental pH of the drug particles in the GIT. Being simple and easy to prepare, the PM 1:20 Gmp/Tris might be promising as a soluble form with a reduced dose of the drug.

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