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Dissolution Enhancement of Gliclazide Using pH Change Approach in Presence of Twelve Stabilizers with Various Physico-Chemical Properties

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Abstract – Purpose. The micronization using milling process to enhance dissolution rate is extremely inefficient due to a high energy input, and disruptions in the crystal lattice which can cause physical or chemical instability. Therefore, the aim of the present study is to use *in situ* micronization process through pH change method to produce micron-size gliclazide particles for fast dissolution hence better bioavailability. **Methods.** Gliclazide was recrystallized in presence of 12 different stabilizers and the effects of each stabilizer on micromeritic behaviors, morphology of microcrystals, dissolution rate and solid state of recrystallized drug particles were investigated. **Results.** The results showed that recrystallized samples showed faster dissolution rate than untreated gliclazide particles and the fastest dissolution rate was observed for the samples recrystallized in presence of PEG 1500. Some of the recrystallized drug samples in presence of stabilizers dissolved 100% within the first 5 min showing at least 10 times greater dissolution rate than the dissolution rate of untreated gliclazide powders. Micromeritic studies showed that *in situ* micronization technique via pH change method is able to produce smaller particle size with a high surface area. The results also showed that the type of stabilizer had significant impact on morphology of recrystallized drug particles. The untreated gliclazide is rod or rectangular shape, whereas the crystals produced in presence of stabilizers, depending on the type of stabilizer, were very fine particles with irregular, cubic, rectangular, granular and spherical/modular shape. The results showed that crystallization of gliclazide in presence of stabilizers reduced the crystallinity of the samples as confirmed by XRPD and DSC results. **Conclusion.** In situ micronization of gliclazide through pH change method can successfully be used to produce micron-sized drug particles to enhance dissolution rate.

INTRODUCTION

It is well established that the active ingredient in a solid dosage form must undergo dissolution before it is available for absorption from the gastrointestinal tract. Many potential drug candidates are characterized by a low oral bioavailability. Often, poor drug dissolution and solubility rather than limited permeation through the epithelia of the gastrointestinal tract are responsible for low bioavailability of orally taken drugs. Therefore, the solubility of a drug is an important factor in determining the rate and extent of its absorption (1); and an enhancement in dissolution rate is important to attain suitable blood-levels of class II drugs (low solubility and high permeability).

There are several methods for enhancing dissolution rate of poorly water-soluble drugs including producing solid dispersion (2-6), making liquisolid formulations (7-11) and milling

or grinding of particles (12). The main mechanism behind the dissolution enhancement using the mentioned approaches is an increase in specific surface area of particle available for dissolution. One of the most common techniques to increase surface area of drug particles hence dissolution rate is milling the particles (13). The milled particles have the tendency to agglomerate as a result of their hydrophobicity, thus reducing their available surface area (14). Milling process which is usually used to obtain small particles is the disruption of large crystals. The micronization process using mills is extremely inefficient (15) due to a high energy input, and disruptions in the crystal lattice that can cause physical or chemical instability (16, 17).

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To overcome these problems, hydrophobic drugs can be microcrystallized using *in situ* micronization techniques (18). Micronized powders with a higher energetic surface show poorer flow properties (19). As the drug powders are prepared directly in micronized state during particle formation without any size reduction, this technique can be described as an *in situ* micronization technique (20). *In situ* micronization is a suitable method for the production of micron-sized drugs. Compared to milled products drug properties are optimized as all particle surfaces are naturally grown, the particle size is more uniformly distributed and the powder is less cohesive (21).

One of the major challenges to drug development today is poor solubility, as an estimated 40% of all newly developed drugs are poorly soluble or insoluble in water (22). In addition, up to 50% of orally administered drug compounds suffer from formulation problems related to their high lipophilicity (23). Therefore, the aim of this study was to explore pH change method as an efficient approach to produce micron-sized particles in presence of twelve different stabilizers to enhance the dissolution and consequently the absorption of poorly water soluble drug, gliclazide. In the present study, solid state of recrystallized microparticles was also investigated using DSC, FT-IR and XRPD.

MATERIALS AND METHODS

Materials

Gliclazide was purchased from CFM Co., Italy. All Brij's and PEGs were obtained from Fluka, Germany. Pluronic F68 (Sigma, UK), hydroxyethylcellulose (Aldrich, UK), PVP K30 (BASF, Germany) and Carboxymethylcellulose (Aldrich, UK) were used. All the other reagents were of analytical grade.

In Situ Micronization Technique

Preliminary investigation

In order to find ideal conditions for preparation of microcrystals (smaller particle size with less aggregation) using *in situ* microcrystallization through pH change technique, the preliminary studies were carried out. The aim of the preliminary study was to select the parameters such as the speed of homogenization, concentration of stabilizers and gliclazide and the type of stabilizers. In brief, the results showed

that reducing pH from 11 to 5 by adding HCl within 5 min, while stirring with high sheer homogenizer at 26000 rpm, produced microcrystals without aggregation. The best concentration for any type of stabilizer and gliclazide concentration using the above condition were 0.05% and 0.3% (w/v) respectively. The results showed that type of stabilizer did not change the preliminary results. All experiments were carried out in 100 ml volume and ice bath temperature.

Main crystallization procedure

After the preliminary study as described above, the concentration of drug and stabilizers, speed of homogenization, range of pH, and temperature of the experiment to produce gliclazide microparticles with least aggregation were determined. So, the above parameters were kept constant with the exception of the type of stabilizer. On the basis of preliminary experiments, 0.3 g gliclazide was dissolved in 100 ml of distilled water at pH 11 (pH was adjusted using 0.1N NaOH) containing 0.05 g stabilizer. After complete dissolution of the drug under homogenization using a homogenizer with a speed of 26000 rpm (Heidolph, Silent crusher M, Germany) pH of the solution was reduced to 5 within 5 min by the addition of HCl 0.1 N when the temperature of the solution was reached around 5 °C. By reducing the pH of the solution a micron-size dispersion of drug was formed spontaneously due to low solubility of gliclazide at lower pH value. The mixture was allowed to be mixed in ice bath for 15 min and then it was transferred to the vacuum oven while stirring for 3 hr. After that the aqueous suspension was frozen at -20°C and then it was freeze-dried (Christ-Alpha 1-4, Braun Biotech-International-Germany) for 36 h. Particle size distribution of microcrystals was determined before and after freeze drying and there were no significant changes. In addition previous study showed no effect of freeze drying on particle size distribution (24).

Preliminary results showed that a simple filtration technique cannot be used for the separation of particles as it was very slow process due to the high viscosity of the solution. Moreover, during the filtration and drying all particles stick firmly together which make particles difficult to disperse in dissolution medium. In the present study various grades of PEG (PEG 300, 600, 1500, 4000, 6000), hydroxyethylcellulose (HEC), carboxymethylcellulose (CMC), different grades of Brij

(Brij 35, 76, 97), pluronic F68 and PVP K30 were used as stabilizers.

Scanning Electron Microscopy (SEM)

Electron micrographs of crystals were obtained using a scanning electron microscope (LEO 440i, UK) operating at 15 kV. The specimens were mounted on a metal stub with double-sided adhesive tape and coated under vacuum with gold in an argon atmosphere prior to observation.

Particle Size Analysis

The size distribution of ground and unground powder was measured with a laser diffraction particle size analyzer (SALD-2101 Shimadzu, Japan). The particle size distribution and mean particle size diameter were automatically calculated using the software provided. The size distribution was evaluated with the span value defined as follows.

Span = $(D_{90\%} - D_{10\%}) / D_{50\%}$ Where, DN% (N= 10, 50, 90) means the volume percentage of microparticles with diameters up to DN%. The smaller span value indicates the narrower particle size distribution.

X-Ray Powder Diffraction (XRPD)

X-ray diffractometry of drug, excipient and formulations were performed using Siemens diffractometer (Siemens, D5000-Germany). The cross section of samples was exposed to x-ray radiation (Cu K α) with wavelength of 1.5406 Å. The rate of the scanning was 0.6° /min. Samples, ground into powders with an agate mortar and pestle, were measured on a low background quartz plate in an aluminium holder.

Fourier Transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy was obtained on a Bomem 2000 T-IR system (Bomem Quebec, Canada) using the KBr disk method. Samples were mixed with KBr powder and compressed to 10-mm discs by hydraulic press at pressure of 150 bars for 30 s. The scanning range was 450–4000 cm⁻¹ and resolution was 4 cm⁻¹.

Thermal Analysis

The DSC was calibrated with indium and lead standards, then samples of gliclazide crystals (3-6

mg) were heated (range 30-200°C) at a scanning rate of 10°C/min in crimped aluminium pans using DSC instrument (Shimadzu DSC 60, Japan) under nitrogen gas. The thermograms were analysed in terms of melting point and enthalpy.

Compressibility Index

About 1 ml powder was carefully filled into a mounted measuring cylinder with known tare. The powder bed was leveled with a spatula, and the maximum bulk volume was read. A single tap was employed, and the volume was read again. This procedure was repeated, thereby gradually increasing the number of taps between individual readings, until three consecutive replicates of 100 taps did not reduce the powder volume further. Hence the minimum powder volume (to give the maximum bulk density) had been reached. The measuring cylinder was then weighed to determine the powder mass. Compressibility index (25) was calculated using eq. 1:

$$\text{Compressibility percent} = 100 \times (\rho_t - \rho_a) / \rho_t \quad \text{eq. 1}$$

Where ρ_t is the tapped density, ρ_a is the bulk density. All experiments were performed in triplicate.

Dissolution Studies

The USP paddle method (Erweka, DPT6R, Germany) was used for all the in vitro dissolution studies. In this method, the dissolution rate of gliclazide powders was measured at pH value of 1.2. The rate of stirring was 100 ± 2 rpm and the temperature of dissolution media was set at 37 °C. The powders were placed in 250 ml of HCl 0.1 N containing 0.25% Tween 80 and at appropriate intervals (5, 10, 15, 20, 30, 45, 60 and 90 min), 5 ml of the samples were taken and filtered through a 0.45 μm Millipore filter. The dissolution media was then replaced by 5 ml of fresh dissolution fluid to maintain a constant volume. After proper dilution, the concentration of dissolved drug in the medium was determined spectrophotometrically (Shimadzu, Japan) at 230. nm. In order to maintain sink conditions, Tween 80 was included to the dissolution medium and powders equivalent to 10 mg gliclazide was used in dissolution test. Gliclazide is weak acid and its solubility in acidic medium is less than its solubility in alkaline medium. Therefore, the aim of the investigation was to increase solubility/dissolution rate of gliclazide in acidic

conditions (simulating gastric fluid). It is obvious that as gliclazide belongs to class II drugs (low solubility, high permeability), an increase in its acidic solubility increases the dissolution rate and hence its bioavailability. This is why acidic condition was used to assess dissolution rate of gliclazide samples.

For comparison purposes, physical mixtures were prepared by mixing of drug with different types of stabilizers using a spatula on a glass plate for 2 min. The mixture was then transferred to a glass bottle for further mixing (5 min). The mixtures were kept in glass bottle until use.

Dissolution efficiency (%) up to 90 min (DE_{90min}) was calculated according to eq. 2 (26):

$$DE\% = \frac{\int_0^t y dt}{y_{100} t} \times 100 \quad \text{eq. 2}$$

Solubility Studies

Solubility measurements were performed according to the method of Higuchi and Connors (27). In brief, solubility studies of different samples of gliclazide were carried out at pH 1.2. Saturated solutions were prepared by adding excess drug to the medium and shaking on the shaker (Velp, Italy) for 48h at $25 \pm 0.5^\circ\text{C}$ under constant vibration. After this period the solutions were filtered, diluted and analysed by UV-spectrophotometer (Shimadzu, Japan). Three determinations were carried out for each sample to calculate the solubility of gliclazide.

RESULTS

Micronization of Gliclazide

Preliminary studies showed that in absence of the stabilizer the particle size was larger than in the presence of a stabilizer. When the drug was recrystallized without any stabilizer the particle size was 50-100 μm . The results also showed that in absence of a stabilizer the tendency of particle to agglomerate is very high. Therefore, due to the presence of larger particles and aggregates no further studies were carried out on particles produced without a stabilizer. Furthermore, there are a few studies showed that the presence of stabilizer is necessary to produce microparticles of atrovastatin calcium (28) and disodium cromoglycatevital (29). On the basis of the preliminary studies under the following

conditions microparticles of gliclazide with the least aggregation can be produced. The condition was as follows: the amount of stabilizer, stirring rate, drug concentration, time to change pH from 11 to 5 were 0.05% (w/v), 26000 rpm, 0.3% (w/v) and 5 min respectively. Therefore, the above conditions were set up for all crystallization procedures in presence of various stabilizers. In the present study the effects of 12 different stabilizers namely; PEG with different molecular weights (PEG300, PEG 600, PEG 1500, PEG 4000 and PEG 6000), various types of Brij (Brij 35, Brij 76 and Brij 97), cellulose derivatives (HEC and CMC), PVP-K30 and pluronic F68 on micrometric, morphology and dissolution behavior of recrystallized gliclazide were investigated.

Micromeritic Properties

The micromeritic behaviors (mean particle size, span and Carr's Index) of untreated gliclazide and recrystallized gliclazide in presence of various stabilizers are shown in Table 1. It is obvious from Table 1 that the original gliclazide (untreated drug) has larger mean particle size than all other gliclazide samples obtained via recrystallization technique. The smallest mean particle size was obtained for the sample recrystallized in presence of Brij 76. Carr's index which is an indication of powder flow behaviour showed that (Table 1), original gliclazide particles have lower Carr's index values than treated samples.

Morphology of Gliclazide Crystals

As morphology of drug particles has an impact on micromeritic properties and dissolution behavior, so, the morphology of the recrystallized samples was investigated using scanning electron microscope (SEM). The results of this study are shown in Figure 1. As it is clear from Figure 1, the presence of stabilizer/additive in crystallization medium of gliclazide had significant effect on particle shape. The untreated gliclazide is rod or rectangular shape, whereas the crystals produced in the presence of stabilizers, depending on the type of stabilizer, were very fine particles (in some cases agglomeration of fine particles) with irregular, cubic, rectangular, granular and spherical/modular shapes. It can be concluded that the type of stabilizer had significant effect on drug particle morphology which is apparent from SEM results (Fig. 1).

Table 1. Micromeritic properties of gliclazide samples recrystallized in presence of various stabilizers

Formulation	Mean Particle size (μm)	Span	Compressibility Index (%)
Untreated drug	290.43 \pm 36.05	2.10 \pm 0.20	20.04 \pm 0.10
PEG 300	3.69 \pm 0.01	1.50 \pm 0.01	60.05 \pm 0.52
PEG 600	3.90 \pm 0.00	1.40 \pm 0.03	60.23 \pm 0.72
PEG 1500	4.83 \pm 0.11	1.64 \pm 0.02	60.01 \pm 0.22
PEG 4000	4.34 \pm 0.14	1.65 \pm 0.01	60.00 \pm 0.15
PEG 6000	3.62 \pm 0.12	1.35 \pm 0.01	50.05 \pm 0.17
Brij 35	4.76 \pm 0.15	1.52 \pm 0.02	60.08 \pm 0.36
Brij 97	4.56 \pm 0.23	1.32 \pm 0.01	60.16 \pm 0.25
Brij 76	3.09 \pm 0.00	2.25 \pm 0.03	60.04 \pm 0.53
HEC	5.03 \pm 0.11	3.41 \pm 0.02	50.06 \pm 0.18
CMC	3.73 \pm 0.11	1.80 \pm 0.02	50.04 \pm 0.16
PVP K30	4.53 \pm 0.15	2.62 \pm 0.01	50.02 \pm 0.35
Pluronic F68	3.59 \pm 0.01	1.53 \pm 0.02	60.06 \pm 0.10

Dissolution studies

Dissolution profiles of untreated gliclazide and the crystals obtained in presence of various stabilizers are shown in Figures 2 to 5. For better comparison of dissolution profiles, dissolution data up to 15 min was plotted in Figures 2 to 5 and dissolution efficiency was calculated up to 90 min (Table 2). According to the results, the dissolution rates of all treated samples are remarkably faster than the untreated sample and the physical mixtures of the drug with stabilizers ($P < 0.05$).

X-ray diffraction studies

In order to investigate any changes in internal structure of crystallized gliclazide in presence of various additives, XRPD was used. Diffraction spectra of untreated gliclazide, gliclazide recrystallized in presence of additives and their physical mixtures are shown in Figure 6. The XRPD pattern of the gliclazide was very similar to that of the standard crystalline powder. Untreated gliclazide showed sharp peaks at diffraction angles of 2θ 10.5, 15, 17, 18, 22, 25 and 26.3 which is in agreement with the literature (30). Most of these peaks are present in the diffractograms of physical mixture of gliclazide-HEC (Fig. 6c) and gliclazide-CMC (Fig.6e) but with a smaller height than the standard crystalline powder. Figure 6 shows that the peak heights for recrystallized samples (Fig.6b and 6d) are pretty smaller than untreated sample and physical mixtures.

Fourier transform infrared spectroscopy

FT-IR spectra were used to further characterize the possibility of interactions between gliclazide and additives in the solid state. Figure 7 shows the FT-IR spectra of untreated drug, recrystallized in presence of PEGs and its physical binary mixtures. The spectra of all samples and all physical mixtures showed that they were identical.

DSC studies

The thermograms of untreated gliclazide and recrystallized microcrystals obtained in presence of HEC and CMC is shown in Figure 8. In order to have a better comparison between different samples in terms of enthalpy and melting point, the final results are listed in Tables 3 and 4. As the results show the T_m of the drug in all physical mixtures is almost the same but with a slight reduction in comparison with pure untreated gliclazide.

DISCUSSION

It seems that all stabilizers (additives) used in the present study acts as the crystal growth inhibitor. A stabilizing polymer usually covers the hydrophobic surfaces of the precipitated crystals and consequently due to the steric hindrance caused by the polymer prevents the crystal growth. This indicated that it is possible to reduce particle size to less than 5 μm without using milling equipment.

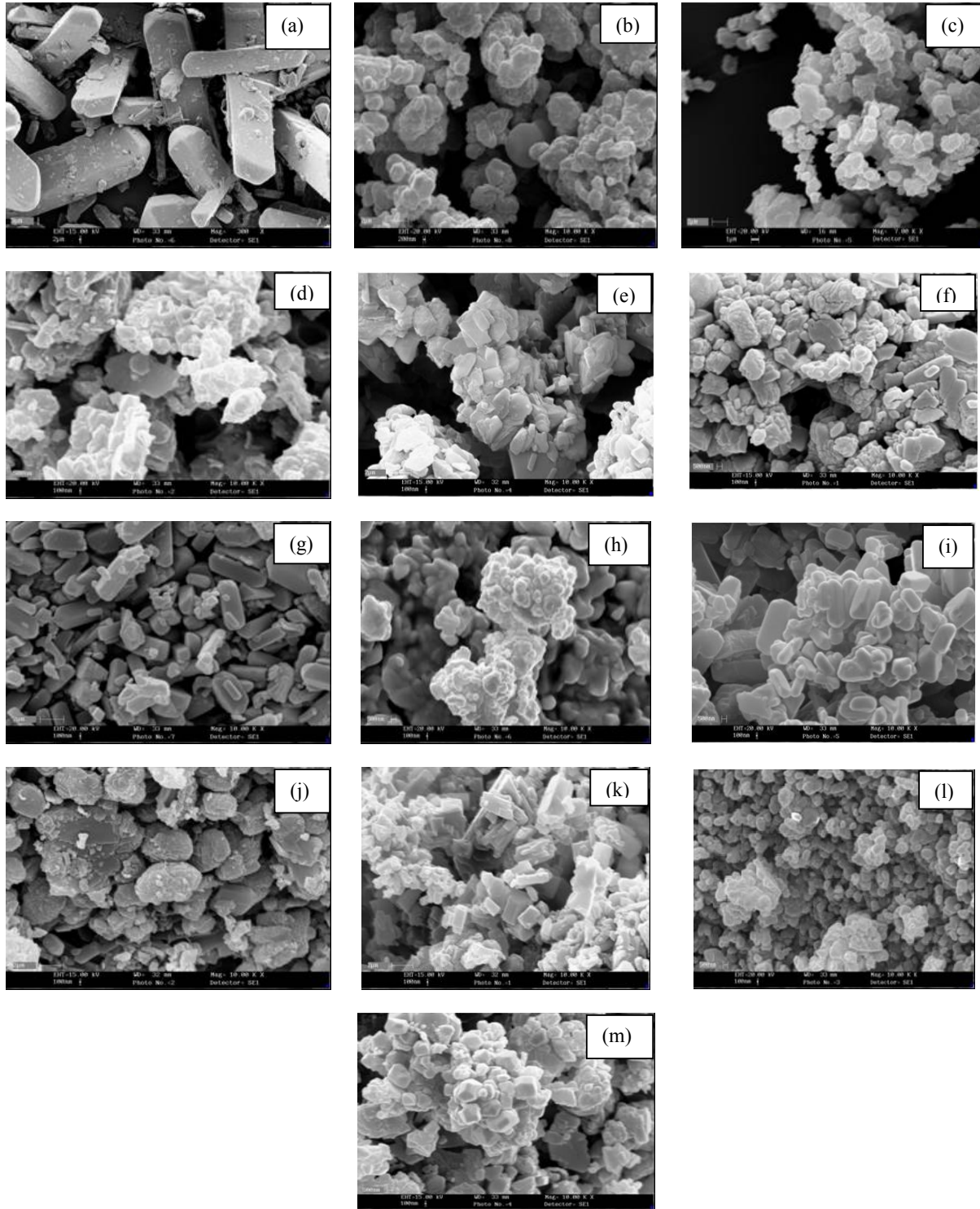


Figure 1. Scanning electron micrographs of: (a) untreated gliclazide, and microcrystals of gliclazide prepared in presence of stabilizer (b) PEG 300; (c) PEG 600; (d) PEG 1500; (e) PEG 4000; (f) PEG 6000; (g) HEC; (h)CMC; (i) Brij 35; (j) Brij76; (k) Brij 97; (l) PVP; (m) Pluronic F68.

Table 2. Dissolution and solubility data for physical mixtures of gliclazide-stabilizers and recrystallized gliclazide in presence of stabilizers.

Formulation	DE _{90min}	DE _{90min} Physical mixtures (%)	Q _{5min}	Q _{5min}	Solubility (g/lit)
	Recrystallized drug (%)		recrystallized drug (%)	Physical mixtures (%)	
Drug only*	38.7 ± 8.6	38.7 ± 8.6	12.7 ± 0.8	12.7 ± 0.8	0.025 ± 0.012
PEG 300	75.5 ± 6.0	44.5 ± 4.0	86.3 ± 10.6	20.2 ± 2.3	0.563 ± 0.041
PEG 600	54 ± 6.7	65.9 ± 11.4	88.1 ± 8.6	35.6 ± 5.5	0.407 ± 0.067
PEG 1500	79.3 ± 1.3	28.2 ± 7.4	95.8 ± 1.1	11.1 ± 2.6	0.410 ± 0.005
PEG 4000	83.2 ± 3.2	39.2 ± 10.9	76.1 ± 9.0	20.6 ± 3.3	0.453 ± 0.040
PEG 6000	80.9 ± 1.7	50.3 ± 10.6	80.2 ± 6.9	32.5 ± 6.4	0.633 ± 0.058
Brij 35	78.1 ± 0.6	16.8 ± 9.3	89.8 ± 9.6	13.7 ± 3.0	0.673 ± 0.051
Brij 76	72.8 ± 1.5	29.1 ± 2.1	72.3 ± 1.6	15.6 ± 0.7	0.437 ± 0.025
Brij 97	72.4 ± 4.1	38.2 ± 1.2	71.2 ± 4.9	15.2 ± 0.7	0.593 ± 0.050
HEC	73.7 ± 3.3	41.4 ± 1.7	71.3 ± 2.5	29.9 ± 0.1	0.480 ± 0.006
CMC	80.0 ± 7.0	30.7 ± 4.8	80.8 ± 3.4	8.9 ± 1.7	0.400 ± 0.056
PVP K30	81.3 ± 10.4	43.0 ± 1.2	66.3 ± 3.9	15.1 ± 1.1	0.540 ± 0.061
Pluronic F68	85.7 ± 12.1	40.2 ± 2.8	74.7 ± 9.8	13.4 ± 3.2	0.450 ± 0.062

*untreated drug

Table 3. Thermal analysis data obtained for untreated gliclazide and gliclazide samples produced in presence of various additives

Formulation	Onset temperature		
	(°C)	T _m (°C)	enthalpy (J/g)
Untreated drug	160.82±0.03	171.13±0.06	115.15±0.55
PEG 300	140.99±0.11	158.50±0.20	26.62±0.43
PEG 600	139.99±0.44	159.43±0.05	19.46±0.14
PEG 1500	145.52±0.05	162.63±0.01	39.57±0.42
PEG 4000	144.69±0.29	162.63±0.03	37.4±0.21
PEG 6000	151.28±0.15	166.8±0.05	51.86±0.09
Brij 35	153.76±0.05	164.28±0.01	17.26±0.25
Brij 97	149.84±0.03	166.89±0.03	37.86±0.51
Brij 76	142.54±0.13	163.00±0.04	45.23±0.41
HEC	143.61±0.15	164.02±0.05	21.37±0.62
CMC	147.12±0.08	165.69±0.04	38.31±0.53
PVP K30	149.41±0.07	161.72±0.01	30.35±0.72
Pluronic F127	154.73±0.02	167.70±0.06	78.27±0.31

An increase in Carr's index value for all treated samples is due to very high surface to mass ratio for fine particles which caused them to be more cohesive than coarser particles which are influenced more by gravitational forces. Powders having a particle size less than 10 µm are usually

extremely cohesive and resist flow under gravity. Comparing flowability (Carr's Index value) of various crystallized samples showed that gliclazide samples crystallized in the presence of PEG 6000, PVP, CMC and HEC have lower Carr's index than other treated samples.

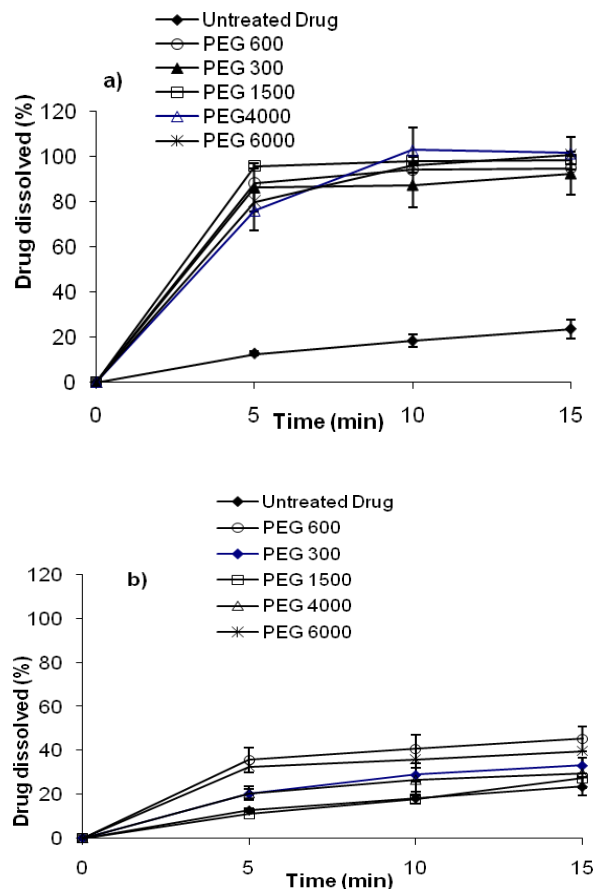


Figure 2. Dissolution behaviour of (a) recrystallized gliclazide in presence of stabilizers and (b) physical mixtures of drug-stabilizer.

This could be due to the difference in shape of drug particles obtained in presence of different stabilizers which is apparent from SEM micrographs (Figure 1). Table 1 also shows that the presence of which stabilizer in crystallization medium produced narrower particle distribution (smaller value of span indicates narrower particle size distribution). It can be seen from the Table, apart from untreated sample and gliclazide crystals treated in presence of Brij 76, HEC and PVP, the rest of gliclazide crystal samples treated in presence of other stabilizers showed very narrow drug particle size distribution.

Dissolution studies

It is clear from the dissolution profiles (Figures 2 to 5) some of the recrystallized samples dissolved 100% within the first 5 min showing at least 10 times greater dissolution rate than the dissolution rate of untreated gliclazide powders.

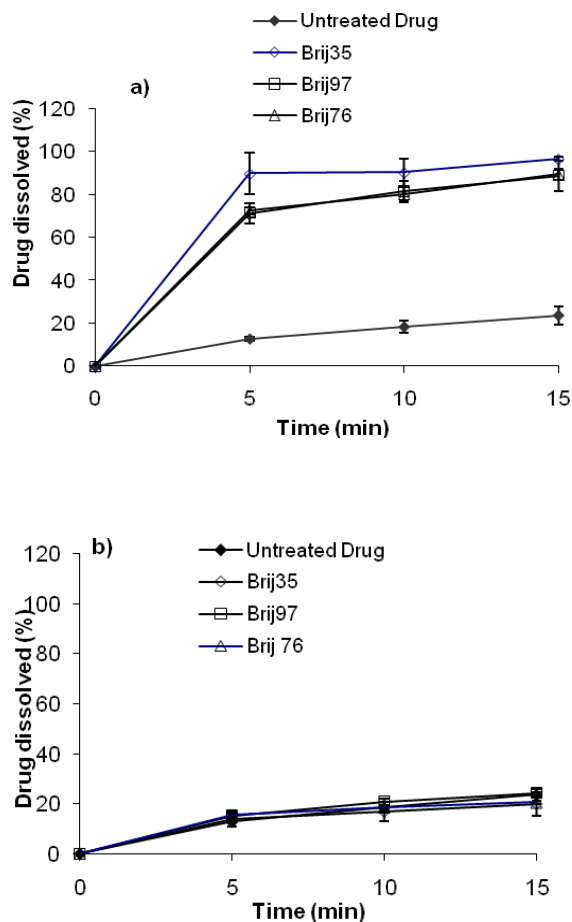


Figure 3. Dissolution behaviour of (a) recrystallized gliclazide in presence of stabilizers and (b) physical mixtures of drug-stabilizer.

In order to investigate the effect of the type of stabilizer, DE_{90min} and Q_{5min} were calculated and the results are listed in Table 2. The lowest Q_{5min} was obtained for untreated gliclazide and physical mixtures of untreated gliclazide and CMC, whereas the lowest DE_{90min} was observed for physical mixtures of untreated gliclazide-Brij 35. Comparing the results of Q_{5min} and DE_{90min} for all studied samples revealed that there is a considerable increase in Q_{5min} and DE for treated samples in presence of additives. For examples Q_{5min} for gliclazide samples recrystallized in presence of PEG 1500 was 95.8%, whereas this value for original gliclazide crystals or physical mixtures of gliclazide-PEG 1500 was 12.7 and 11.1% respectively.

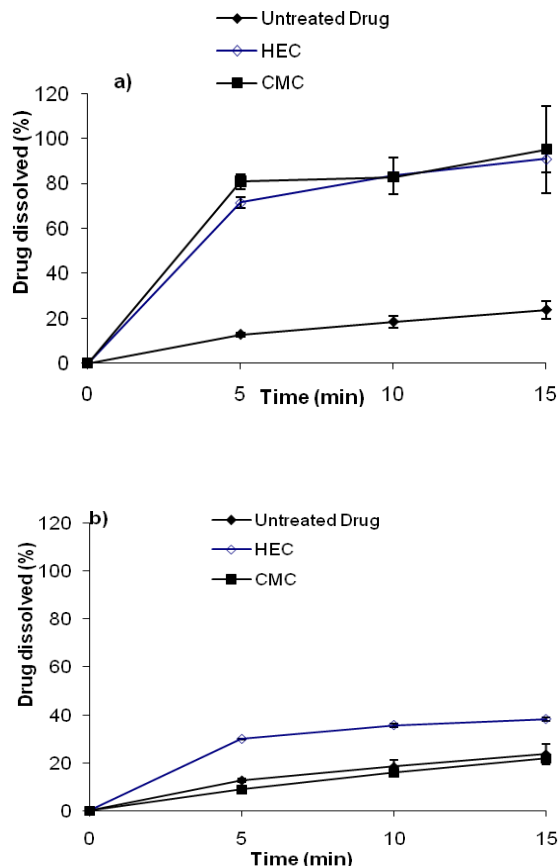


Figure 4. Dissolution behaviour of (a) recrystallized gliclazide in presence of stabilizers and (b) physical mixtures of drug-stabilizer.

By considering all data obtained in the present study it can be postulated that there should be three main factors responsible for an increase in dissolution rate of recrystallized gliclazide crystals; (a) an increase in surface area of drug particles; (b) an increase in wettability of drug particles and (c) an increase in saturated solubility of the treated drug particles.

According to Noyes and Whitney equation (31), $D_R = [(DS (C_s - C)]/h$, the drug dissolution rate (D_R) is directly proportional to its concentration gradient ($C_s - C$) in the stagnant diffusion layer, diffusion coefficient (D) and its surface (S) available for dissolution. C_s is the saturation solubility of the drug in the dissolution medium and thus, it is a constant characteristic property related to the drug and dissolving media involved.

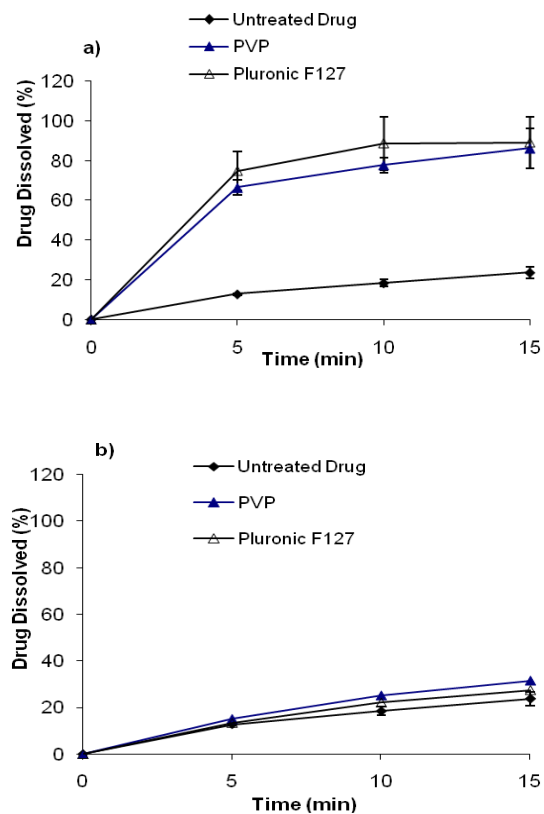


Figure 5. Dissolution behaviour of (a) recrystallized gliclazide in presence of stabilizers and (b) physical mixtures of drug-stabilizer.

Since all of dissolution tests for formulations were carried out at a constant rotational paddle speed (100 rpm) and identical dissolving media, we can assume that the thickness (h) of the stagnant diffusion layer and the diffusion coefficient (D) of the drug molecules remain almost identical.

Since treated drug powders showed higher solubility than untreated drug and also the treated drug particles have higher surface area than untreated particles (SEM pictures) due to smaller particle sizes, therefore, the observed higher dissolution rates of recrystallized gliclazide are due to the significant increase in the specific surface area and saturated solubility (Table 2) of the treated drug powders. Specific surface area was not determined for all samples. Only two treated gliclazide samples and untreated sample were selected for the surface area measurement.

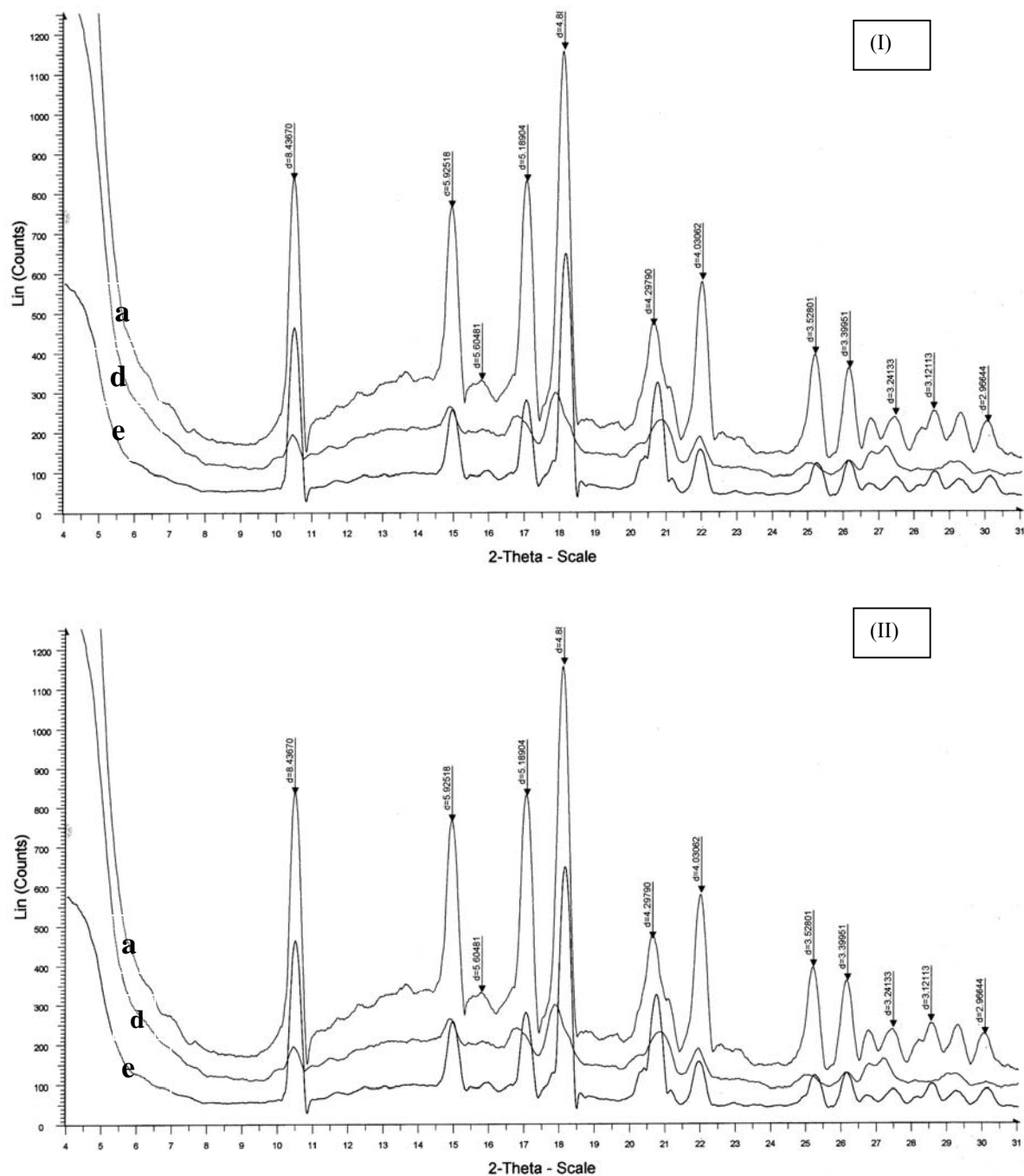


Figure 6. X-ray diffraction patterns of (a) untreated gliclazide; (b) recrystallized gliclazide in presence of HEC; (c) physical mixture of gliclazide-HEC; (d) recrystallized gliclazide in presence of CMC; and (e) physical mixture of gliclazide-CMC.

The results confirmed the above hypothesis as the specific surface area for recrystallized gliclazide in presence of Brij 35 and HEC appeared to be 18.06 and 17.56 m^2/g , whereas untreated gliclazide had specific surface area of 4.78 m^2/g . The formation of fine drug particles in

presence of stabilizers is due to the crystal growth inhibitory effect of stabilizers/additives used in the present study as described earlier in the paper (32-34). It has already been shown that wetting of the tablets or drug particle had important impact

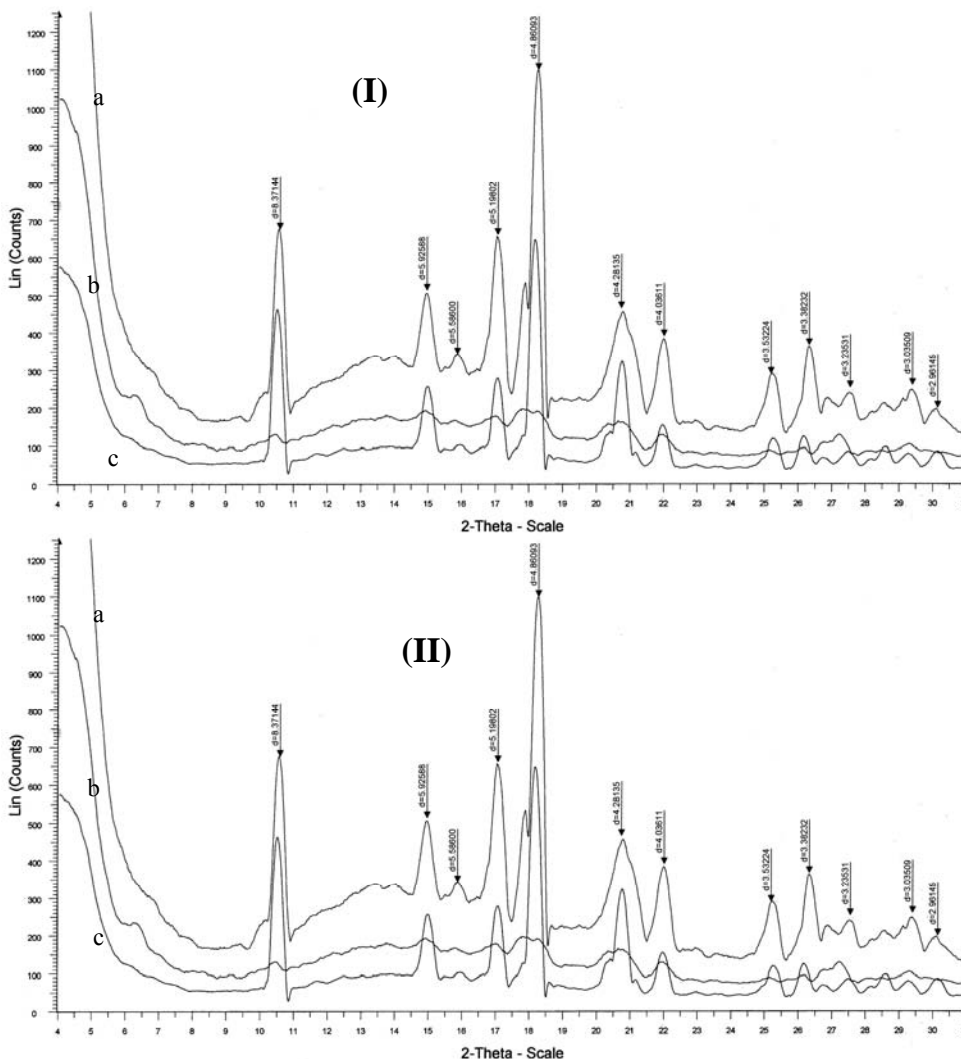


Figure.7. X-ray diffraction patterns of (a) untreated GL, (b) microcrystal and (c) physical mixture prepared by pH change method using (I) PEG 300 and (II) PEG 600 as stabilizer.

on dissolution of poorly water soluble drugs (10, 35).

Apart from crystal growth inhibitory effect of stabilizers, all of the additives used in the present study can also act as surface active agents and facilitate wetting of the drug particles by decreasing the interfacial tension between the dissolution medium and the drug. We believe that, therefore, in the present study due to significantly improved wetting properties of drug particles, the treated gliclazide samples display enhanced drug dissolution characteristics. This *in situ* micronization has several advantages over milling techniques as unlike milling process the proposed process is not a high energy process which causes disruptions in the drug's crystal lattice, resulting in the presence of disordered or amorphous

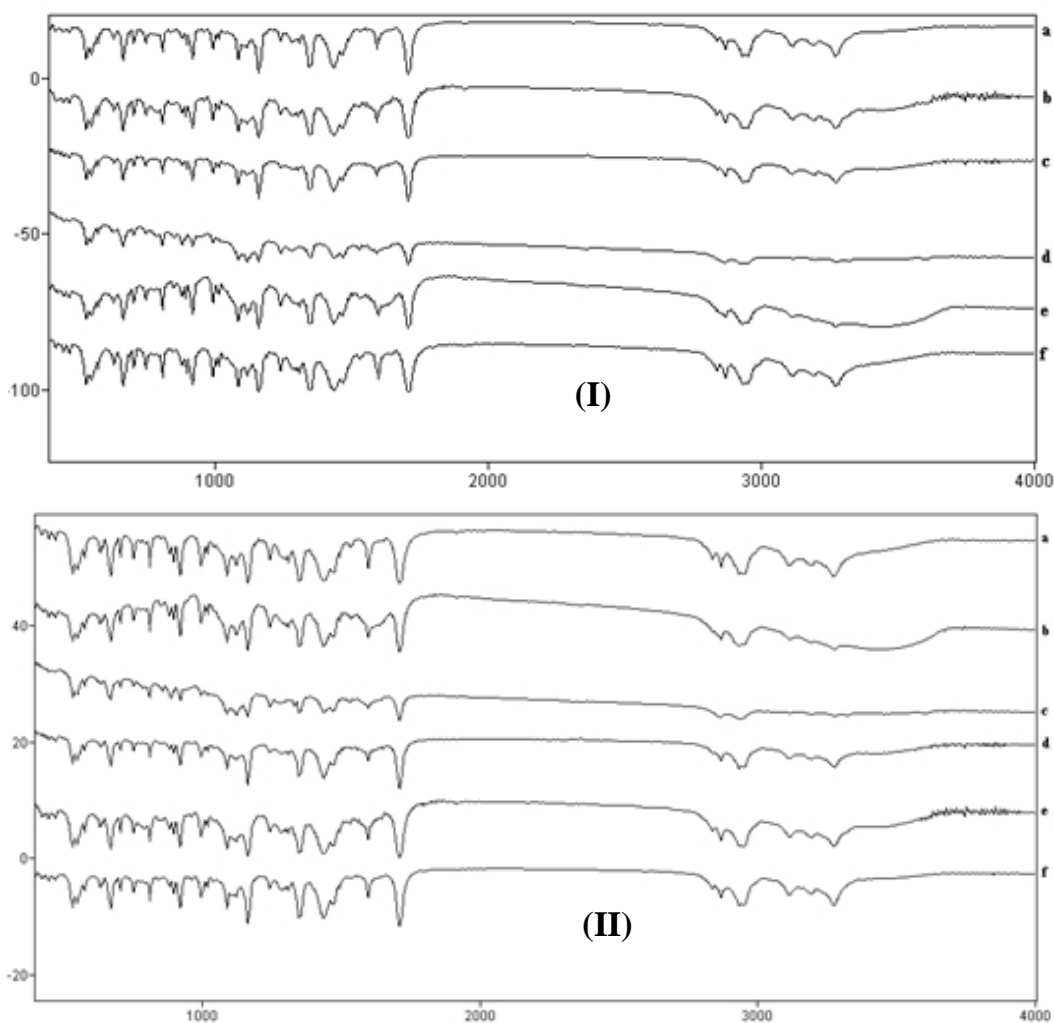
regions in the final product (36). Furthermore, milled particles often show aggregation and agglomeration which results in poor wettability and thus poor dissolution (37). The results of present study showed that agglomeration or aggregation of drug particles can be minimized by controlling the crystallization conditions as described earlier in the preliminary results.

Solid state studies

The x-ray pattern showed that different samples produced different intensity of peaks. The peak height is affected by crystal size and crystallinity (12). Therefore, the peak height of the recrystallized drug powder is expected to be smaller than that of the standard crystalline

Table 4. Thermal analysis data obtained for untreated gliclazide and physical mixtures of drug with /stabilizers

Formulation	Onset temperature (°C)	T _m (°C)	enthalpy (J/g)
Untreated drug	160.82±0.03	171.13±0.06	115.15±0.49
PEG 300	139.29±0.05	164.42±0.44	47.27±0.57
PEG 600	141.75±0.09	164.32±0.53	56.96±0.86
PEG 1500	148.84±0.51	168.49±0.75	63.97±0.11
PEG 4000	151.45±0.12	170.49±0.06	79.38±0.17
PEG 6000	151.95±0.01	170.71±0.64	73.74±1.32
Brij 35	164.8±0.35	173.88±0.43	78.97±0.44
Brij 97	167.45±0.19	175.58±0.22	95.2±0.09
Brij 76	149.37±0.53	170.22±0.45	82.17±0.05
HEC	147.65±0.08	168.76±0.64	82.21±0.75
CMC	156.01±1.52	169.29±0.09	66.82±0.47
PVP K30	160.29±1.25	173.14±0.73	75.43±0.04
Pluronic F68	156.87±0.74	171.30±0.25	85.97±1.23

**Figure 8.** FT-IR spectra of (I) recrystallized gliclazide samples and (II) physical mixtures of gliclazide in presence of (a) PEG 300 , (b) PEG 600, (c) PEG 1500, (d) PEG 4000, (e) PEG 6000, and (f) untreated gliclazide.

powder (mean particle size for untreated sample and spectrum b and c were 290, 3.69 and 3.09 μm respectively). Moreover, Figure 6 shows that the crystallinity of treated drug is lower than that of the standard gliclazide powder which is apparent from XRPD spectra (Fig. 6). The increased dissolution of treated gliclazide with respect to untreated drug powder partly can be attributed to the reduced crystallinity characterized by less intense peaks in the XRPD of treated samples. Similar results were obtained for the treated gliclazide samples in presence of other stabilizers which were not shown in the present article. As PEG 300 and 600 are liquids, any mixtures with these cosolvents may result in partial or complete solubilization of the drug. XRPD of the physical mixtures of PEG 300 and 600 with the API showed that the intensity of the peaks is only slightly smaller than physical mixtures of the API with solid excipients (compare Figures 6 and 7). This indicated that may be very little amount of gliclazide dissolved in PEG 300 or 600 as the concentration of the stabilizers was very low in these physical mixtures.

The FT-IR spectra of all samples and all physical mixtures were identical (Fig. 8) and the main absorption bands of gliclazide appeared in all the spectra in the region of C=O sulphonylurea group absorption around 1711 cm^{-1} . Similarly, the NH group located at 3265 cm^{-1} was not shifted. The sulphonyl group bands (S=O) are located at 1348 cm^{-1} and 1163 cm^{-1} in unground gliclazide, and these were not shifted in the recrystallized gliclazide samples and physical mixtures spectra. This indicated that there was no difference between the internal structures and conformation of these samples at the molecular level.

One useful technique to study of polymorphism is differential scanning calorimetry (DSC). DSC is usually combined with XRPD to determine the polymorphic composition of pharmaceutical powders, when the polymorphs present have different melting points (Figure 9). The difference in melting point of gliclazide between different recrystallized samples and untreated gliclazide is more obvious than the difference between physical mixtures and pure gliclazide (Tables 3 and 4). For example, the lowest melting point for recrystallized samples in presence of PEG 300 was $158.5\text{ }^{\circ}\text{C}$, whereas this value for untreated gliclazide was $171.14\text{ }^{\circ}\text{C}$. The lower T_m for the physical mixtures and recrystallized samples in comparison with untreated gliclazide indicates the absence of any important incompatibility between the drug and

stabilizers and suggests that the recrystallized microcrystals are not perfect crystals. Tables 3 and IV also show a significant reduction in enthalpy changes particularly for the recrystallized samples in comparison with untreated gliclazide. A reduction in enthalpy of microcrystals has been reported for other drugs (38) as the presence of dissolved impurities (additives) may change the rate of crystallization and crystal habit by adsorbing the surface-active agents to the nuclei or growing crystals. The precipitated drug is sterically stabilized against crystal growth by adsorbed polymer and the surface energy and consequently the enthalpy of the system lowered. The lower enthalpy reported for recrystallized samples in presence of additives could partly be due to crystal habit of particles (39, 40). The modification of the physical properties of phenytoin by recrystallization from methanol exhibited increase in the specific surface area of the phenytoin crystals and a progressive change of crystal habit from needles to elongated plates. They reported that XRPD did not show any gross structural changes between needle and elongated phenytoin crystals. However, the enthalpy of fusion reduced by as much as 17° reflecting a significant change in both the enthalpy and entropy of the phenytoin crystals. It was shown that a reduction in the melting enthalpy of paraffin is directly correlated to the amount of paraffin in binary mixtures of paraffin-EVA copolymer (41). Therefore, it can be concluded that the presence of small amounts of stabilizers in addition to the change in crystalline habit of the microcrystals is the cause of enthalpy reduction of the microcrystals compared to untreated drug.

CONCLUSION

The results of the present study showed that in situ micronization of gliclazide through pH change method can successfully be used to produce micron-sized drug particles to enhance dissolution rate. All recrystallized drug samples processed in presence of stabilizers showed fast dissolution rate than untreated drug and physical mixtures of drug-stabilizer. The type of stabilizer had significant effect of morphology of particles, hence on flow and particle size distribution. The results showed that crystallization of gliclazide in presence of stabilizers reduced the crystallinity of the samples as confirmed by XRPD and DSC results.

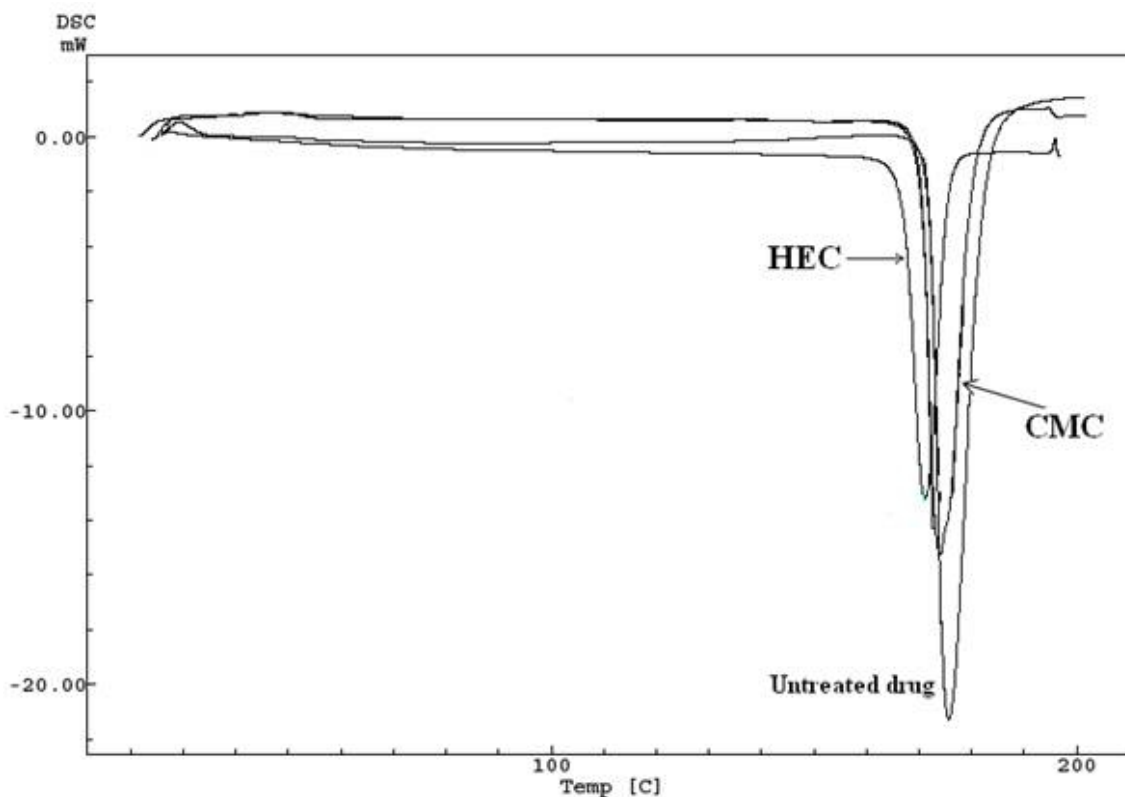


Figure 9. DSC thermograms of untreated gliclazide and recrystallized gliclazide in presence of HEC and CMC (for enthalpy and melting point data for all treated and physical mixtures refer to Tables 3 and 4).

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