Effects of Poloxamer and HPMC on the Dissolution of Clonazepam-Polyethylene Glycol Solid Dispersions and Tablets


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ABSTRACT

Clonazepam is an anticonvulsant and anxiolytic drug characterized by poor solubility and rapid absorption. The purpose of the study was to improve the solubility of clonazepam in solid dispersions by incorporating hydroxypropyl methyl cellulose (HPMC) and poloxamer 407. Solid dispersions were prepared by using polyethylene glycol 6000 as carrier in 1:20 ratio by melt granulation. The solid dispersions were compressed into tablets by adding microcrystalline cellulose, sodium starch glycolate and talc. The solid dispersions were characterized by differential scanning calorimetry, particle size analysis, and drug contents were measured by HPLC coupled with UV detection. The in vitro dissolution of the tablets was compared with pure drug in 900 ml water using USP dissolution apparatus (type II). HPMC improved the solubility of clonazepam by decreasing its particle size and drug dissolution from the tablets also increased significantly (p<0.05). The thermograms of poloxamer containing formulations indicated that clonazepam could be in molecularly dispersed form. Unlike HPMC, significant increase in drug solubility and dissolution (p<0.05) was observed with smaller amount of poloxamer. From the results it was concluded that both crystallization inhibitor (HPMC) and wetting agent (poloxamer) in solid dispersions can improve solubility and release profile of poorly soluble clonazepam.

KEY WORDS: Clonazepam, Solid dispersion, dissolution enhancement, Hydroxypropyl methyl cellulose, Poloxamer 407.

INTRODUCTION

Clonazepam, one of the benzodiazepines, is prescribed for the treatment of epileptic seizures and panic disorder. The drug is almost insoluble but absorbed rapidly after oral administration. Dissolution is the rate limiting step for the absorption and subsequent bioavailability for drugs like Clonazepam which are insoluble but highly permeable. Solid dispersion (SD) technique was used to improve the dissolution of benzodiazepines such as oxazepam, diazepam, temazepam but SD of Clonazepam is not yet studied. By definition SD are formulations of finely crystalline or amorphous drug dispersed in an inert matrix.

In the present study, clonazepam SD were prepared with PEG 6000 by melting method. PEG 6000 is frequently used as a carrier in SD where it improve solubility of drugs like griseofulvin, diazepam, bropirimine, tolbutamide, mequitazine, norfloxacin, indomethacin, and ibuprofen.

The aim of the present study was the enhancement of Clonazepam dissolution by PEG 6000 SDs prepared by melt granulation and comparison of the effects of a crystallization inhibiting polymer (i.e. HPMC) and a surface active polymer (micronized poloxamer) on such formulations. The differential scanning calorimetry (DSC), particle size analysis by laser diffraction, drug content determination by HPLC assay equipped with UV detector and in vitro dissolution of tablets were carried out to evaluate the characteristics of SDs.

The role of HPMC as crystallization inhibitor is well documented and SD requires them to prevent recrystallization of poorly soluble drug or PEG itself upon storage or upon contact with aqueous media. In other formulations micronized Poloxamer 407 was also mixed with PEG 6000 as a replacement of HPMC. Poloxamers are nonionic polyoxyethylene-poly-oxypropylene copolymers.
used primarily as emulsifiers, solubilizing agents, wetting agents and have been reported for enhancing the solubility and bioavailability of sparingly soluble drugs in solid dosage forms \textsuperscript{17, 18, 19}. Ferrari \textit{et al} reported the use of good amount of lactose in the tabletted SD formulations \textsuperscript{20}. In preparing SD formulations, use of lactose is very much significant. Lactose may act as filler and carrier in SD formulations. Lactose may act as binder also in case of tabletted SD formulations. The resulting SD granules were compressed into tablets after blending with suitable super disintegrants and lubricants and tested in vitro for dissolution enhancement \textsuperscript{20}.

\section*{MATERIALS AND METHODS}

\textbf{Materials}

Clonazepam USP (Centaur Pharmaceuticals, Mumbai), PEG 6000 (BASF, Germany), HPMC 6 cps (Shin Etsu, Japan), Poloxamer 407 (BASF, Germany), Lactose (New Zealand), Microcrystalline Cellulose (Mingtai Chemical Co. Ltd., Taiwan), Sodium starch glycolate (Primogel, Avebe UK Inc.). Other chemicals used were of reagent grade.

\textbf{Preparation of Clonazepam solid dispersions}

Solid dispersions were prepared by melt-extrusion method. PEG 6000 was melted in a glass beaker at 80-90°C and clonazepam was added and dissolved by continuous stirring. Polymers like HPMC and poloxamer were added in the hot mixer and thoroughly blended and mixed until solidification. Lactose was added immediately to prepare granules from the dissolved clonazepam. The granules were cooled at room temperature and screened with #40 mesh. The granules prepared from different batches (Table 1) were kept in the desiccators till further processing.

\textbf{Preparation of solid dispersion tablets}

The granules prepared by solid dispersion technique (equivalent to 2 mg Clonazepam) were blended with diluent microcrystalline cellulose (MCC) and superdisintegrant sodium starch glycolate (4% wt/wt) and finally lubricated with talc (1% wt/wt). The tablets were compressed with 16 station rotary tablet machine (Clit, Ahmedabad, India) with round, flat punches of 10 mm diameter. The theoretical tablet weight was 300 mg. The weight variation, hardness, disintegration tests, friability and drug content of the prepared tablets were tested according to USP.

\textbf{Solubility analysis}

Excess amount of pure drug and solid dispersions were added to 10 ml of distilled water in vials and sonicated for 30 minutes. The supersaturated solution was shaken manually for 10 minutes and then again sonicated for 30 min. After 24 h the resulting supernatant was filtered through membrane filter of 0.22µ pore size and assayed by HPLC method to determine drug content in the solvent.

\textbf{Tableting Properties of Clonazepam Solid Dispersion Tablet}

The tablets were evaluated for hardness, friability, disintegration, and weight variation. The crushing strength of the tablets was measured by a Monsanto hardness tester, and friability by a Roche Friabilator (Electrolab) with 20 tablets.

\textbf{Drug Content}

The solid dispersion tablets were assayed by HPLC method coupled with UV detection (Shimadzu Prominence, Japan) as described in USP. The uniformity of content of the tablets was determined by taking sample from 5 crushed tablets. The samples were diluted by water, methanol, and tetrahydrofuran at the ratio of 60:52:13 and filtered through Whatman filter paper no. 41. Then the drug content was analyzed by HPLC.

\textbf{Differential Scanning Calorimetry}

The pure drug and solid dispersions were examined by DSC Q100 (TA instruments, New Castle, USA) where 5-6 mg samples were placed in aluminum pan at a heating rate of 10°C/min with purging of dry nitrogen at a constant rate of 50 ml/min. Indium/Zinc standards were used to calibrate the DSC temperature and enthalpy scale.

\textbf{Particle size analysis}

The solid dispersions were dispersed in distilled water and the particle size of the dispersions was measured by laser diffraction technology (Mastersizer 2000, Malvern, UK).

\textbf{In vitro Dissolution of Solid Dispersion Tablet}

Pure drug and solid dispersion tablet equivalent to 2 mg Clonazepam were taken in dissolution basket and the \textit{in vitro} dissolution was carried out in USP dissolution tester (apparatus II) rotating at 75 rpm and temperature was maintained at 37±0.5°C. Samples were withdrawn from the dissolution medium (900 mL water) at 30 min and 60 min.
HPLC Analysis

According to USP methods, the filtered samples were suitably diluted and analyzed by validated HPLC system (Shimadzu Prominence, Japan) where phosphate buffer pH 8.0, methanol, and tetrahydrofuran at the ratio of 60:52:13 were used as mobile phase. The samples (20 µL) were injected onto C8 column and the mobile phase flowed at the rate of 1 ml/min. Retention times of Clonazepam standard was used to identify sample peaks at 273 nm λmax.

Statistical Analysis

The data were analyzed and graphically presented by MS-Excel. One way analysis of variance (ANOVA) was carried out while p<0.05 was considered significant.

RESULTS

The drug contents of the SD tablets were in the range of 98-99% indicating uniformity of the blend. The solubility of the formulations was in the range of 1.50 mg/100cm³ to 1.92 mg/100cm³ (Table 2). Amongst all, clonazepam solubility was the maximum in case of SD formulation of poloxamer at the ratio of clonazepam: poloxamer; 1:1.5. The tablets prepared from the different batches of SDs showed a good uniformity in total weight and had hardness value of 120±0.5 to 125±0.3 N, disintegration time of 13-15 minute, and friability of < 0.5%.

The DSC thermogram of pure Clonazepam crystals showed a sharp peak at 239.04°C. (Fig.1). All the thermograms of SDs showed peak around 49-55°C. A peak around 240°C was found in case of all the SD formulations containing HPMC and PEG. In case of HPMC comprising formulations, though the content of the polymer was increased gradually with respect to the drug at 1:0.5, 1:1, and 1:1.5 ratios, sharp peaks were observed and these sharp peaks might be the indication of the presence of clonazepam as crystalline form. But the peaks reduced gradually from formulation L1 to L3 which might be due to the crystallization inhibitory property of HPMC15, 16. Poloxamer 407 showed a characteristic melting endotherm at 60.19°C alone and in combination with the melting endotherm of PEG at the same temperature. Therefore a broad endotherm was prominent at that temperature in formulations containing poloxamer and PEG. But the formulations containing Poloxamer and PEG did not show any peak corresponding to the melting range of clonazepam. It suggest that the drug remained in molecular dispersion form in those batches.

Fig. 2 shows the particle size distribution of the clonazepam powder and clonazepam SDs. Particle sizes of the SDs were analyzed by laser diffraction immediately after diluting with water. The average particle size of clonazepam crystal was 12 µm. In the formulations containing HPMC, the mean particle size of the SDs were 10 µm, 3.3 µm and 0.72 µm respectively for 0.5 gm, 1 gm and 1.5 gm of HPMC/ g clonazepam respectively. That is, with the increment of HPMC, degree of dispersion in the water was increased resulting smaller particles. The fine particle size indicates that HPMC successfully inhibited recrystallization. In case of formulas L4-L6, poloxamer was used with PEG matrix to improve the wetting of poorly soluble drugs in aqueous media. The particle size was smaller (0.72 µm) in drug: poloxamer ratio of 1:0.5. Particle sizes of the other two SD batches were 3.8 µm where poloxamer content was 1 gm and 1.5 gm/gm clonazepam respectively.

Release rate of clonazepam from different formulations were determined using first order release kinetics (fig. 3). Release

| Table 2: Characterization of clonazepam solid dispersion tablets |
|-----------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                      | L1             | L2             | L3             | L4             | L5             | L6             |
| Weight variation (%) | ±2.0           | ±2.0           | ±3.0           | ±4.0           | ±3.0           | ±3.0           |
| Hardness (N)         | 120±0.5        | 122±0.5        | 125±0.3        | 121±0.8        | 121.5±0.2      | 120±0.3        |
| Disintegration time (min) | 13.5           | 13.4           | 14.6           | 13.1           | 13.7           | 13.5           |
| Friability (%)       | < 0.5          | < 0.5          | < 0.5          | < 0.5          | < 0.5          | < 0.5          |
| Drug content (%)     | 99.7±0.11      | 98.4±0.25      | 98.5±0.53      | 98.5±0.16      | 98.2±0.22      | 98.9±0.23      |
| Solubility (mg/100cm³) | 1.6±0.45      | 1.7±0.71       | 1.6±0.27       | 1.5±0.50       | 1.5±0.31       | 1.9±0.12       |

*Weight variation ± standard deviation (SD) from mean values (n = 10)
rate of pure clonazepam was only 0.3 %log.min⁻¹. Using SD formulations of clonazepam, this rate was found several folds increased for all the batches. In case of HPMC containing SD formulations, the highest release rate was 31.3 %log.min⁻¹ where HPMC was 1.5 times of clonazepam (formula L3). On the other hand, formula L4 showed highest release rate and it was 32.0 %log.min⁻¹ where clonazepam: poloxamer was 1:1.5.

To determine the effectiveness of dissolution enhancement by two types of SDs (formulations comprising HPMC and poloxamer), the in vitro dissolution test of SD tablets was performed for 60 minutes. Drug dissolution was improved significantly from all SD tablets in comparison to pure drug (Fig. 4) which released only 4.2% drug after 60 minutes. Whereas clonazepam release was 81.96%, 83.33% and 98.68% for drug: HPMC ratio of 1: 0.5, 1:1 and 1: 1.5 respectively from tablets. On the other hand, 98.86%, 89.4% and 87.31% clonazepam was released from the SD formulations where poloxamer was used at clonazepam: poloxamer of 1:0.5, 1:1 and 1: 1.5 respectively. The fastest and highest amount of drug was released from formula L3 (drug:PEG:HPMC::1:20:1.5) and formula L4 (drug:PEG:Poloxamer::1:20:0.5) where 83±% of drug was released within 30 minutes (Fig. 3).

**DISCUSSION**

Both HPMC and poloxamer improved the dissolution properties of clonazepam solid dispersions by changing the physicochemical properties of the formulations. Pure Clonazepam showed a sharp peak at 239.04°C in DSC thermogram (Fig. 1). PEG 6000 has a melt endotherm at 65-70°C, HPMC has a melt endotherm at 55-60°C and poloxamer has at 50-55°C (not shown in Fig. 1). In the melting thermogram of the SD formulations, characteristic changes were observed in the peak of clonazepam. In case of the formulations of clonazepam, PEG and HPMC, peaks were observed around 50-60°C which indicates the melting endotherm of PEG 6000. This type of melting endotherm of PEG was also reported previously. Small peaks were also observed around 50-60°C which indicates the presence of HPMC as crystals in the SD formulation. Around 240°C, sharp peak was also found as that of melting endotherm of pure clonazepam. It suggests the presence of drug in crystal form. After the increment of HPMC content in the formulations with respect to drug at 1:1 and 1:1.5 ratios, peak around 240°C was reduced but did not disappear. It may indicate that the drug remained in the formulations as its crystalline form.

In case of SD formulations of PEG with poloxamer, a very small peak was seen around 240°C in the endotherm for the lower content of poloxamer (L4, clonazepam: poloxamer was 1: 0.5). But in the endotherm of other two SD formulations where poloxamer content was higher (L5, clonazepam: poloxamer was 1: 1; and L6, clonazepam: poloxamer was 1: 1.5), no peak was seen around 240°C. Moreover all the peaks at this range were dissolved and a new peak was found around 60°C. Peak around the same temperature was also seen in case of L4. These peaks around 60°C were due to the presence of PEG and poloxamer. However, no peak around the melting endotherm of clonazepam was observed in the mentioned formulations and it might be explained in the following way. Firstly, drug might be entrapped inside the molten carrier (PEG) during the melting process. Secondly, it might be due to the presence of clonazepam as molecularly dispersed state in the SD formulations. Again disappearing of the peak of clonazepam around its melting endotherm might be due to the formation of eutectic mixture between the drug and the poloxamer.

Particle size dispersion of the drug as in pure form and in SD formulations after dispersing in water is shown in Fig. 2. The data suggest that SDs improve the drug dissolution by particle size reduction or wetting. The poorly soluble drugs are dispersed in SD matrix (in this case PEG) and upon dilution by aqueous media drug tends to precipitate and recrystallize.
to form aggregates. The larger particles may decrease the drug dissolution. Therefore HPMC was used in L1-L3 formulations to inhibit recrystallization. Smaller particles resulted with higher amounts of HPMC. The fine particle size indicates that HPMC successfully inhibited recrystallization. Poloxamer was also used in SD formulations for the same purpose and an effective size reduction was observed (fig. 2). Smallest particles were found with clonazepam: poloxamer at 1:0.5. But unlike HPMC, particle size did not decrease with the increment of poloxamer. It might be due to the formation of aggregates of SD formulations after being diluted with water and it was due to the presence of comparatively larger amount of poloxamer in the formulations. However, the smallest particle was found in case formulation L3 and L4. Highest dissolution was also obtained from these two formulations and it indicates that particle size after dilution is a critical factor for enhancement of solubility from SDs.

Fig.4 shows the in-vitro release of clonazepam from SD formulations prepared with HPMC and poloxamer. Theoretically the SDs improves drug dissolution by decreasing particle size, formation of amorphous forms and improved wettability. Clonazepam solubility in the dissolution media was also improved in all the batches of SD formulations. Release was 98.68% and 98.86% for HPMC and poloxamer respectively whereas it was only 4.2% ($F = 2.55$; $F_{	ext{exp}} = 7.7$; $P = 0.285$) for pure clonazepam. Moreover release was 98.68% for L3 ($F = 0.762$; $F_{	ext{exp}} = 7.7$; $P = 0.431$) and was 98.86% for L4 ($F = 0.76$; $F_{	ext{exp}} = 7.7$; $P = 0.432$) where the compositions were clonazepam:PEG:HPMC::1:20:1.5 and clonazepam:PEG:Poloxamer::1:20:0.5 respectively. It indicates the drug and polymer ratio is also critical factor for the enhancement of Clonazepam solubility.

Presence of HPMC in SDs usually retards drug release by forming a gelatinous layer around drug particles. The drug is released slowly from such matrix by diffusion process. Usually the higher molecular weight HPMC (such as HPMC K15, K100) are used for sustained release effect in tablet formulations but in case of SDs, even the low molecular weights are capable of achieving the same objective. But in the present experiment the effect of larger amount of HPMC was not prominent due to the presence of high amount of PEG which was dispersed among the drug particles. However, highest amount of clonazepam release was achieved with formulation L3 where drug: HPMC was 1:1.5. On the contrary, same amount of clonazepam release was achieved with formulation L4 where drug: poloxamer was 1:0.5. That is faster dissolution of clonazepam was achieved with poloxamer containing formulations where the poloxamer content was the least. The other two formulations of poloxamer also released the drug at a faster rate. As we know that crystalline form of drug has comparatively poor dissolution property than its amorphous form, presence of crystalline form of the drug might delay the release of the drug. Similarly, clonazepam remained as crystalline in the SD formulations of HPMC (fig. 1). And due to this reason, it was found to be released comparatively slowly from the formulations of HPMC. On the contrary, clonazepam remained as crystals in the formulations of poloxamer (Fig. 1) and release of the drug from these formulations was also comparatively faster.

**CONCLUSION**

Both HPMC and poloxamer can improve clonazepam solubility and in vitro dissolution from solid dispersions and tablets. Better solubility characteristics are necessary to increase bioavailability and to ensure faster onset of action of poorly soluble drugs like clonazepam from oral formulations. We found that particle size and crystalline state are affected by the amount of both polymers. Further studies on in vivo models are required to evaluate the potential of these solid dispersion tablet formulations.

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