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Solubility Enhancement and Formulation of Mouth Dissolving Tablet of Clonazepam with Solid Dispersion Technology

Swati C. Jagdale[1]*; Ajay S. Bhadoriya[2]; Aniruddha R. Chabukswar[3]

[1]Professor, Head of Department, Department of Pharmaceutics, MAEER's Maharashtra Institute of Pharmacy, MIT Campus, S.No124, Kothrud, Pune 411 038, Maharashtra, India.
[2]MAEER's Maharashtra Institute of Pharmacy, MIT Campus, S.No124, Kothrud, Pune 411 038, Maharashtra, India.
[3]Professor, Head of Department, Department of Pharmaceutical Chemistry, MAEER's Maharashtra Institute of Pharmacy, MIT Campus, S.No124, Kothrud, Pune 411 038, Maharashtra, India.

ABSTRACT

Clonazepam (CLZ) is an anticonvulsant benzodiazepine widely used in the treatment of epilepsy. CLZ is a BCS Class II drug and its bioavailability is thus dissolution limited. The objective of the present study was to prepare solid dispersions (SDs) of CLZ by various techniques, using the amphiphilic carrier Gelucire 50/13 in various proportions, to increase its water solubility. Drug-polymer interactions were investigated by Fourier-transform infrared (FTIR) and Ultra-Violet (UV) spectroscopy. The SDs were characterized physically by differential scanning calorimetry (DSC) and X-ray diffraction (XRD). A phase solubility study was performed and the stability constant (Ks) was found to be 275.27, while the negative Gibbs free energy (ΔG^{o}_{tr}) indicated spontaneous solubilization of the drug. The dissolution study showed that the SDs considerably enhanced the dissolution rate of the drug. The FTIR and UV spectra revealed no chemical incompatibility between the drug and Gelucire 50/13. XRD patterns and the DSC profiles indicated the CLZ was in the amorphous form, which explains the improved dissolution rate of the drug from its SDs. Finally, mouth dissolving tablets (MDTs) were prepared from the optimized batches (kneading method) of solid dispersion, using crospovidone and Doshion P544 resin as superdisintegrants. The tablets were characterized by *in-vitro* disintegration and dissolution tests. The study of the MDTs showed disintegration times in the range 32.0±0.85 to 20.0±1.30 sec and dissolution was faster than for the commercial preparation. In conclusion, this investigation demonstrated the potential of solid dispersions of a drug with Gelucire 50/13 for promoting the dissolution of the drug and contributed to the understanding of the effect of a superdisintegrant on mouth dissolving tablets containing a solid dispersion of a hydrophobic drug.

Keywords: Clonazepam, Gelucire 50/13. Solid Dispersions. Kneading. Tablet. FTIR.

Corresponding Author: Dr. Jagdale, Swati C. - Professor Head of Department Department of Pharmaceutics - MAEER's Maharashtra Institute of Pharmacy - MIT Campus - S.No124 - Kothrud - Pune 411038 Maharashtr-India-tel.:919881478118-e-mail:jagdaleswati@rediffmail.com

INTRODUCTION

Clonazepam (CLZ) is an anticonvulsant chloro-nitrobenzodiazepine that enhances the gamma-aminobutyric acid (GABA) receptor response. CLZ exerts its action by binding to the benzodiazepine site of the GABA receptors, causing an enhancement of the electric effect of GABA on the neuron when it binds to the receptor. CLZ is a light yellow crystalline powder which is practically odorless. It is freely soluble in methanol, ethanol and acetone, but practically insoluble in water (at 25°C<0.1 mg/mL) (Moneghini et al., 2001). According to the biopharmaceutical classification (BCS), CLZ is in class II, which consists of drugs of low solubility and high permeability.

Various techniques have been used to improve the solubility/dissolution rate of poorly water-soluble drugs. Among them, the solid dispersion technique (Duncan, 2002) and complexation with cyclodextrin (Caron et al., 2007) are most frequently used. In solid dispersions, hydrophilic polymers have commonly been used as carriers (Ruan et al., 2005).

Solid dispersion (SD) is defined as the dispersion of one or more active ingredients in inert carriers in the solid state, by fusion, solvent or solvent-fusion methods. The physical state of the drug is often transformed in solid dispersions from crystalline to amorphous and the dissolution surface increases because of particle size reduction (Duncan, 2002).

Gelucire 50/13 (G50), an amphiphilic excipient composed of mixed glyceride esters of polyethylene glycols, is widely used in the preparation of solid dispersions (Fini

et al., 2005; Lee et al., 2008). In the present study, G50 was chosen as a vehicle, for its low melting point and its favorable dispersibility. Gelucires are characterized by two numbers (e.g. 50/13), the first corresponding to the approximate melting point of the material and the second to the HLB number (hydrophile-lipophile balance), which reflects the proportion of water-soluble to lipid-soluble moieties in the matrix (Damian et al., 2000).

To solve the problem of inability to swallow conventional dosage forms, such as tablets, capsules and powders, it is helpful to develop solid dosage forms that disintegrate rapidly or dissolve even when taken orally without water.

The aim of the present study was to prepare and characterize various solid dispersions of CLZ with G50, so as to improve its dissolution and to prepare mouth-dissolving tablets (MDTs).

MATERIALS AND METHODS

Clonazepam (CLZ) was kindly supplied by Piramal Health Care, (Baddi, India); Gelucire 13/50 (G50) was purchased from Colorcon Asia Pvt. Ltd. (Mumbai, India). All other chemicals and solvents were of analytical reagent grade.

Preparation of physical mixture and solid dispersions

Solid dispersions (SDs) were prepared by various techniques, such as physical mixture (PM) (Nokhodchi et al., 2007), solvent evaporation (SE) (Sugimoto et al., 1998; Verheyen et al., 2002), co-grinding (COG) (Van den Mooter et al., 1998; Dong et al., 2008), co-precipitation (COP) (Shah et al., 2009), kneading (KN) (Tran et al., 2009) and closed melting (CM) (Biswal et al., 2009), with drug : carrier ratios of 1:0.25, 1:0.5 and 1:1.

SDs were stored in screw-capped glass vials in desiccators.

Physical mixture (PM)

Physical mixtures of clonazepam were prepared by mixing accurately weighed amounts of CLZ and the carrier, G50, in a mortar and passed through a 35-mesh sieve. The PMs were subsequently stored at room temperature in screw-capped glass vials until use.

Solvent evaporation method (SE)

Solid dispersions were prepared by dissolving accurately weighed amounts of CLZ and G50 in ethanol. After complete dissolution, the solution was sonicated for 20 minutes and the solvent was evaporated under reduced pressure at room temperature in a desiccator. The solid mass was ground and the particle size fraction of <250 µm was separated by sieving. The sieved ground powder was stored in an oven at 40ºC for at least 48 h. All SDs were kept at room temperature in screw-capped glass vials until use.

Co-grinding method (COG)

A known weight of CLZ was ground with a minimal quantity of ethanol in a glass mortar until it dissolved. Weighed carrier was then added and the suspension ground rapidly at room temperature until the solvent evaporated and passed through a 40-mesh sieve.

Co-precipitation Method (COP)

Accurately weighed amounts of G50 and CLZ were dissolved in water and ethanol, respectively. After complete dissolution, the aqueous solution of the carrier was then poured into the ethanolic solution of the drug. The solvents were then evaporated under reduced pressure at room temperature in a desiccator. Subsequently, the solid dispersions were stored in an oven for 48 h at 60ºC. All dispersions were then pulverized with a pestle and mortar, sieved (<250 µm) and dried in an oven for at least 48 h. The SDs were kept at room temperature in screw-capped glass vials until use.

Kneading method (KN)

A mixture of G50 and CLZ (in ratios 1:1, 1:0.5 and 1:0.25 by weight) was wetted with water and kneaded thoroughly for 30 minutes in a glass mortar. The paste formed was dried under vacuum for 24 hours. The dried powder was passed through a 60-mesh sieve and stored in a desiccator until further use.

Closed melting method (CM)

One gram of PM was weighed into a glass ampoule (20mL), which was then sealed and heated for 30 min. in a water bath, to melt the mixture. After slow cooling, the ampoule cap was opened and the SD collected. All dispersions were pulverized with a pestle and mortar, sieved (<250 µm) and dried in an oven for at least 48 h. Dispersions were kept at room temperature in screw-capped glass vials until use.

Characterization of solid dispersions of clonazepam:

Percentage drug content and percent yield

Accurately weighed aliquots of each SD, equivalent to 2 mg of drug, were dissolved in methanol and the solution was ultrasonicated for 10 min. After that, the volume was adjusted to 100 mL with methanol and the solution was filtered through Whatman filter paper and suitably diluted. The absorbance was measured at 245 nm in a double-beam Cary 100 UV-Vis spectrophotometer (Varian, Australia). The CLZ content was read from the calibration curve (Rao et al., 2010).

After preparation, each solid dispersion was weighed and the yield was calculated by the following formula

$$[\%\ \text{Yield} = (a/b) \times 100] \ldots\ldots\ldots (1),$$

where ‘a’ is the actual weight of sieved solid dispersion and ‘b’ is the starting weight of the ingredients.

Phase solubility

An excess of CLZ was added to aqueous solutions of the carrier containing various concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5% w/v). The flasks were sealed and shaken at 37^{0}C for 48 h in a thermostatically controlled water bath and the samples were filtered through a 0.45 µm membrane filter. The filtrate was suitably diluted and analyzed spectrophotometrically at λmax 245 nm (Shirsand et al., 2009).

Saturation solubility

Excess quantities of pure CLZ, physical mixtures and SDs were placed separately in glass-stoppered flasks containing 10 mL distilled water. The flasks were maintained at 25°C for 48 h. The resulting saturated solutions were sonicated for 20min, centrifuged and filtered through Whatman filter paper no. 41. The filtered supernatants were suitably diluted in distilled water and assayed spectrophotometrically at 245 nm (Ahuja et al., 2007).

Drug:carrier interaction

All prepared solid dispersions were subjected to Fourier-transform infrared and UV-Vis spectroscopy analysis, to determine drug carrier interaction.

Fourier transform infrared spectroscopy

FTIR spectra of the pure drug and G50, and of SDs and physical mixtures, were recorded in a scanning range of 4000 to 400 cm^{-1}, with a 640-IR spectrophotometer (Varian, Australia), using KBr disks (Leonardi et al., 2007).

Stability of dispersion in solution

The stability of the drug in solution was assessed by UV-visible spectroscopy (Jagdale et al., 2009).

Physical characterization

Physical characterization was performed by differential scanning calorimetry (DSC) and powder X-ray diffractometry (pXRD) on CLZ, G50 and some SDs, selected on the basis of the saturation solubility and dissolution results.

Differential scanning calorimetry:

DSC curves were recorded, representing the rate of heat uptake from each sample (Janssens et al., 2008). A sample of about 2-10mg was weighed on a standard open aluminum pan of a differential scanning calorimeter (DSC 823e, Mettler Toledo, Switzerland), while being heated from 20°-450°C, at a rate of 10°C/min, and purged with dry nitrogen.

Powder X-ray diffractometry:

Powder X-ray diffraction (pXRD) patterns were traced with a PW 1729 diffractometer (Philips, Netherlands), employing a Ni filter, Cu Kα radiation, a voltage of kV, a current of 20 mA and receiving slit of 0.2 in. The samples were analyzed over a range of 2θ = 5° to 60°, with a scan step size of 0.020° (2θ) and scan step time of 1 second (Cirri et al., 2004).

***In-vitro* dissolution test**

Dissolution was tested by the basket method (USP Apparatus I) at 75 rpm.

Preparation of clonazepam tablet

Mouth-dissolving tablets (MDTs) were prepared by the direct compression method from an optimized batch of kneaded 1:1 CLZ-G50 SD (designated G50-KN1), (on the basis of the dissolution and saturation solubility results for the SDs), by adding one of two different superdisintegrants (crospovidone or Doshion). The tablets were prepared separately in the proportions given in Table 1, using a Minipress II 8-station rotary tablet machine (Rimek Ltd.), equipped with 8-mm flat-faced punches (Sammour et al., 2006).

Table I. Formulae of G50-KN1 tablets

S.No.	INGREDIENT	FORMULATION CODE*			
		$KN1_{CP8}$	$KN1_{CP12}$	$KN1_{DO8}$	$KN1_{DO12}$
1	CP (mg) (%)	20.8 (8%)	31.2 (12%)	-	-
2	DO (mg) . (%)	-	-	20.8 (8%)	31.2 (12%)
3	G50-KN1** (mg)	4	4	4	4
4	Mannitol (mg)	125.2	114.8	125.2	114.8
5	MCC (mg)	100	100	100	100
6	Talc (mg)	5	5	5	5
7	Aspartame(mg)	5	5	5	5
8	Magnesium Stearate(mg)	2	2	2	2
9	Flavor (orange) (mg)	2	2	2	2
10	Total (mg)	260	260	260	260

*KN1 = G50-KN1, CP = crospovidone, DO = Doshion P544, 8 or 12 = % content of superdisintegrant
**G50-KN1 4mg, equivalent to 2mg of CLZ

Assessment of the prepared tablets

Properties of powder blend

The loose bulk density, tapped density, compressibility (Carr's) index and Hausner ratio of the

blends were calculated by the formulae given below (Lachman et al., 1991).

{ note replacement of V with D (for density) below }

$$Bulk\ Density(D_b) = \frac{Mass}{Bulk\ Volume} \quad (g/cm^3) \quad (2)$$

$$Tapped\ Density\ (D_t) = \frac{Mass}{Tapped\ Volume} \ (g/cm^3) \quad (3)$$

$$Hausner's\ Ratio\ (H) = \frac{D_t}{D_b} \quad (4)$$

$$Carr's\ Index\ (I) = \frac{D_t - D_b}{D_t} \times 100 \quad (5)$$

Properties of tablets

Tablets were tested for hardness (Monsanto tablet hardness tester), friability (**Roche"friabilator**), uniformity of drug content and weight variation, as per US Pharmacopeia (USP, 2009).

Study of *in vitro* disintegration, wetting time and water absorption ratio

To determine the *in vitro* disintegration time, one tablet was placed in a beaker containing 10mL of pH 6.8 phosphate buffer at 37±0.5°C and the time required for complete dissolution (with mild shaking) was measured (Abdelbary et al., 2005).

On the other hand, the wetting time was measured by placing a sample of the final tablet in a Petri dish (10cm in diameter) containing 10mL water at room temperature. The wetting time is that necessary for the complete wetting of the tablet (Shirsand et al., 2008). The wetted tablet was then weighed and the water absorption ratio, R, was determined by the following equation (Fini et al., 2008):

$$R = 100\ (W_a - W_b)/W_a \quad (6)$$

where W_a and W_b are the weights before and after water absorption, respectively.

***In vitro* dissolution**

In vitro dissolution studies of MDTs (containing solid dispersion) and commercial tablets of CLZ (containing 2 mg), Lonazep (LNZ: Sun Pharmaceutical Ind. Ltd., Silvassa, India), were carried out with 900 mL of pH 6.8 phosphate buffer as the dissolution medium at 37°±0.5°C, using the basket method at 75 rpm.

Short-term stability studies

The MDTs prepared from the best formulation (G50-KN1) were packed in Alu–Alu pouches and stored under the following conditions for periods prescribed by International Conference on Harmonisation (ICH) guidelines for accelerated studies.

(i) 30° ± 2°C and RH 65% ± 5%

(ii) 40° ± 2°C and RH 75 % ± 5%.

The tablets were withdrawn after periods of 7, 14 days, 1, 2 and 3 month and subjected to physicochemical characterization (visual defects, hardness, disintegration, percentage drug content, etc.)

Data analysis

Phase-solubility studies

The apparent stability constant, Ks, of each drug–carrier combination was computed from the slope and intercept of the phase-solubility profile, which is a plot of solubility of CLZ against concentration of carrier:

$$Ks = \frac{Slope}{Intercept(1 - Slope)} \quad (7),$$

where the *intercept* is the solubility at 0% carrier and the *slope* is from the linear portion of the plot.

The values of Gibbs free energy of transfer (ΔG^o_{tr}) of CLZ from aqueous solution to the carrier solutions were calculated from the following relationship:

$$\Delta Go_{tr} = -2.303RT.\log\frac{S_o}{S_s} \quad (8),$$

where S_o and S_s are the molar solubilities of CLZ in 1% w/v aqueous solution of the carrier and in water, respectively.

Studies of drug release from SDs

Dissolution kinetic modelling

The Zero order, First order, Higuchi's, Hixson-Crowell, Korsmeyer and Peppas models were fitted to the *in vitro* drug-release data for the solid dispersions (Cutrignelli et al., 2008).

Dissolution efficiency (DE)

The drug-release profiles were characterized by calculating the DE, which is defined as the area under the dissolution curve up to a certain time t_1, expressed as a percentage of the area of the rectangle arising from 100% dissolution at the start, extended over the same time period. DE can be calculated as a fraction (from 0 to 1) by the following equation:

$$\boxed{DE = \frac{\int ydt}{100t}} \quad (14)$$

where y is the percent drug dissolved at time t_1 (Jagdale et al., 2010).

Similarity factor (f_2)

The similarity factor between the two formulations (LNZ and test) was determined from the data collected in the drug release studies. The percent similarity was computed by the following formula:

$$f_2 = 50\log\left\{100 \cdot \left[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5}\right\} \qquad (15)$$

where n is the number of withdrawal points, R_t is the percentage of reference substance dissolved at time point t and T_t is the percentage of test substance dissolved at t. A value of 100% for f_2 suggests that the test and reference profiles are identical; $50<f_2<100$ shows that the dissolution profiles are similar and a smaller value implies greater dissimilarity between the release profiles (Jain et al., 2009).

RESULTS

Phase solubility studies

The current study showed that G50 has a significant solubilizing effect on CLZ. The values of the stability constant (Ks) and slope were found to be 275.27 M^{-1} (?) and 0.9865, respectively, with R^2 value 0.984.

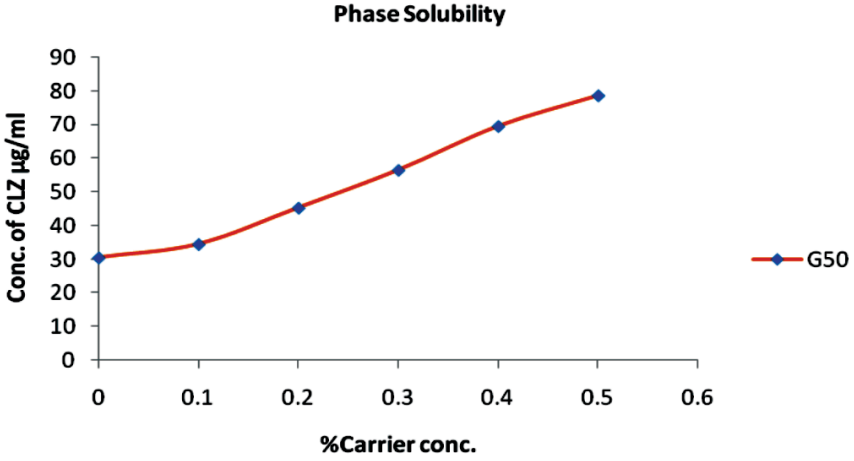

Figure 1. Phase-solubility study of CLZ-G50 SDs.

Saturation solubility study

The solubility of CLZ in water at room temperature was 30.54 µg/mL. It was found that solubility increased (Table 2) with increasing carrier proportion (up to 1:1 w/w) in solid dispersions prepared by all five methods and that further enhancement of the carrier concentration did not affect solubility but hindered the release of drug from the dispersion, owing to formation of a viscous layer around the drug particle.

Characterization of solid dispersions

Content uniformity

The % drug content of the prepared CLZ-G50 SDs was in the range 86.48 to 112.78 % w/v. The percent yield was found in the range 86.53 to 97.54 % w/w (Table 2). However, for the KN and COP dispersions, the ranges were narrower: drug content 98.06 to 99.77% and yield 92.46 to 97.43%.

Drug-carrier interactions

UV spectroscopy

In the UV spectra of the dissolved dispersions, the position of λ max and the form of the curves (Figure 2) were identical to those of CLZ, indicating the absence of interaction between the SD constituents and confirming the stability of CLZ in solution with G50.

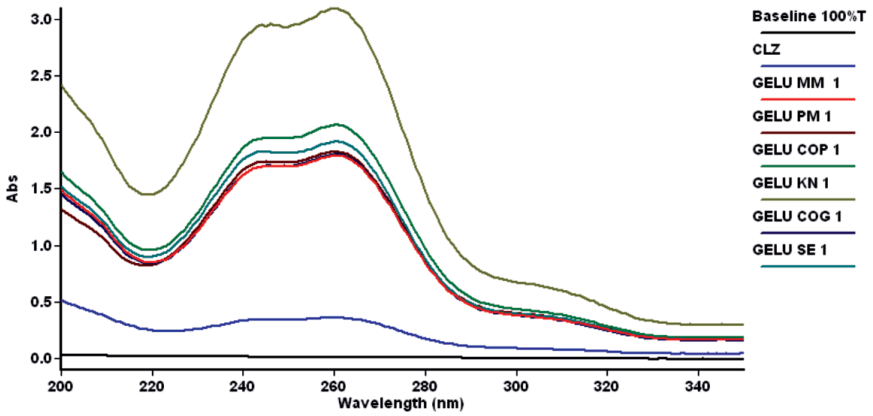

Figure 2. Drug-carrier interaction study by UV spectroscopy.

Fourier-transform infrared spectroscopy

FTIR spectroscopy was used to assess the interaction between G50 and CLZ molecules in the solid state. The chemical interactions between the drug and the carrier often lead to identifiable changes in the infrared profile of complexes. The spectrum of pure CLZ shows characteristic peaks at 3221 cm^{-1} (N-H stretching), 3,076, 3,056 cm^{-1} (aromatic C-H stretching), 1765 cm^{-1} (carbonyl stretching), 1623, 1582 cm^{-1} (aromatic ring), 1356 cm^{-1} (symmetric NO_2 stretching), 766 cm^{-1} (four adjacent free Hs and aromatic C-H out of plane bending) and 850 cm^{-1} (two adjacent free Hs and aromatic C-H out of plane bending). G50 exhibited peaks of C-H stretching at 2890 cm^{-1} and the C-O (ether) stretching at 1125 cm^{-1}, 450, 710 and 1232 cm^{-1}, denoting CH in plane deformation (Figures 3a, 3b & 3c).

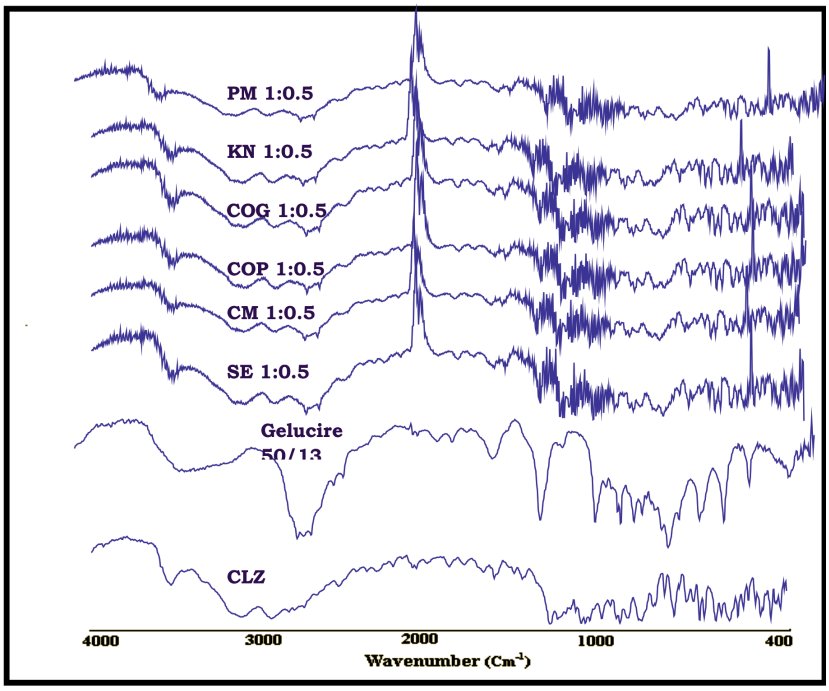

Figure 3a. FTIR spectra of Clonazepam solid dispersions in Gelucire 50/13 (1:0.5).

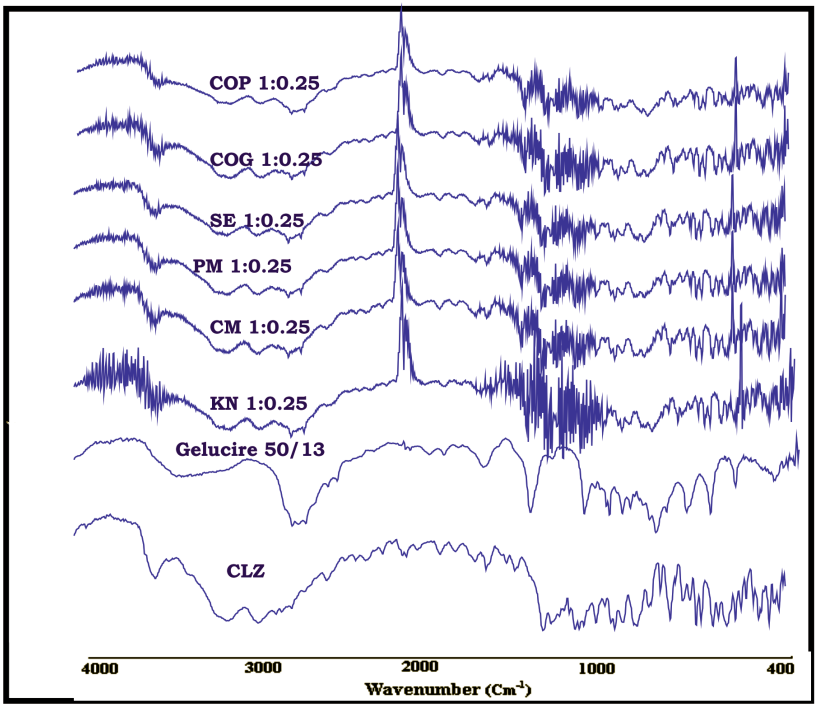

Figure 3b. FTIR spectra of Clonazepam solid dispersions in Gelucire 50/13 (1:0.25).

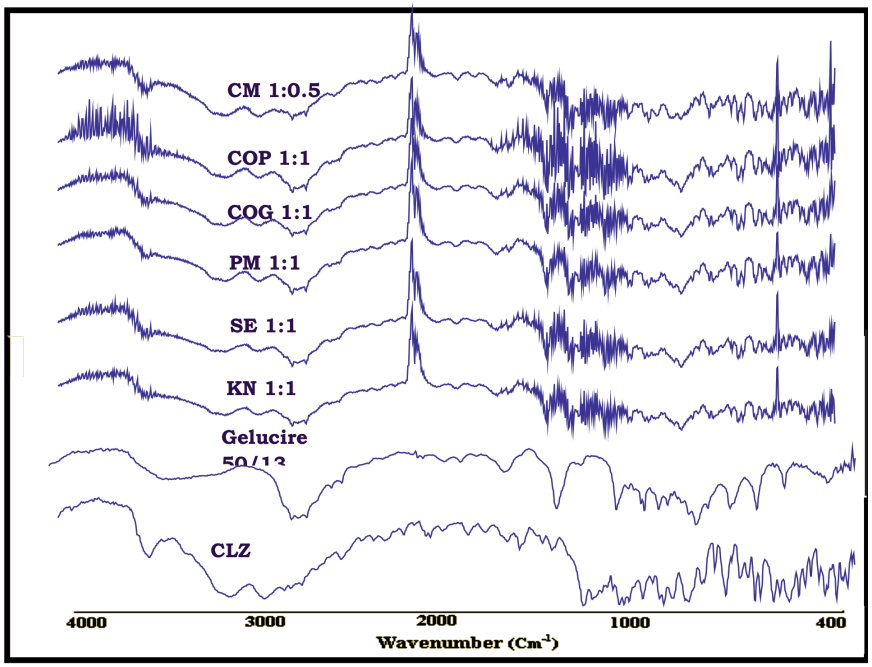

Figure 3c. FTIR spectra of Clonazepam solid dispersions in Gelucire 50/13 (1:1).

Differential scanning calorimetry

The DSC curve for CLZ showed an endothermic (melting) peak at 238.46°C (Figure 4, curve a). The thermogram for G50 (curve b) also showed an endothermic peak, at 50^{0}C, corresponding to its melting point.

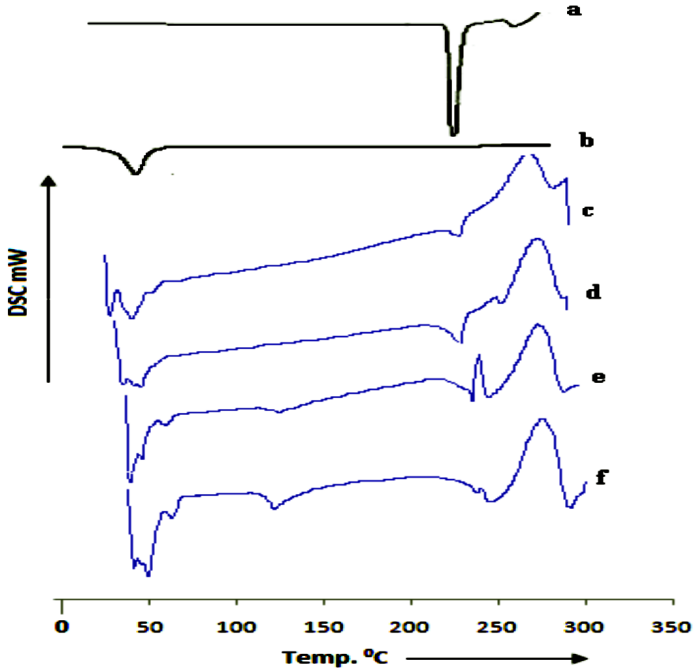

Figure 4. DSC Thermograms of: (a) CLZ, (b) Gelucire 50/13, c) G50-KN 1, d) G50-COP 2, e) G50-COP 1 and f) G50-KN 2

Powder X-ray diffraction

Powder X-ray diffraction analysis was used to assess the degree of crystallinity of the SD constituents and some KN and COP SDs. CLZ showed major peaks at 2θ values of 11.84, 14.75, 14.98, 18.22, 18.50, 20.03, 20.45, 22.98, 23.91, 24.32, 26.07, 27.14, 27.38, 27.80 and 30.22° (Figure 5, curve a). The pXRD pattern of the carrier showed the typical peaks for triglycerides, at 2θ = 19.18 and 23.38° (curve b).

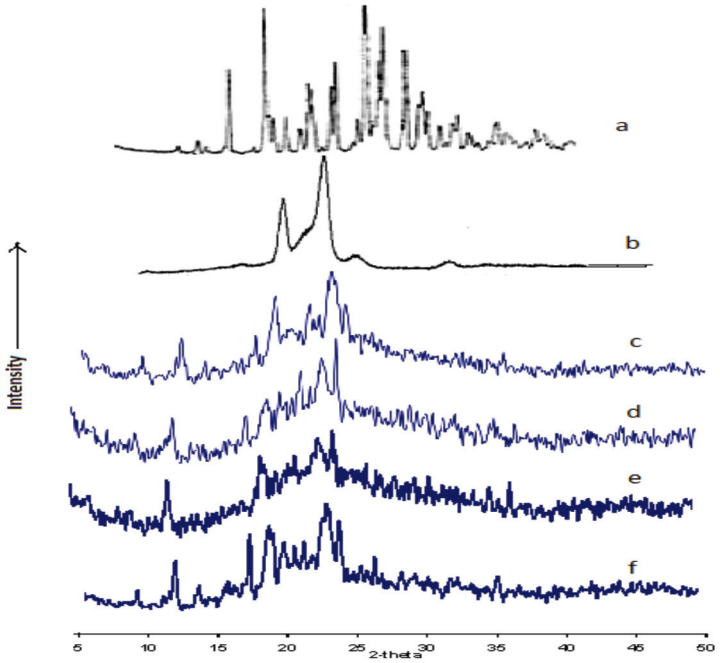

Figure 5. X-ray diffraction patterns: a) CLZ, b) Gelucire 50/13, c) G50-KN 2, d) G50-COP 1, e) G50-COP 2 and f) G50-KN 1

Dissolution studies of CLZ-G50 solid dispersion

In-vitro release studies revealed that there was a marked increase in the dissolution rate of CLZ from all SDs, compared to pure CLZ (Figures 6, 7 and 8). The cumulative % drug released at 120 min was found in the range of 23.86 to 102.97 % w/v for the CLZ–G50 SDs batches (Table 2). Both the percent drug dissolved (DP) and dissolution efficiency (DE), after 60 min and 120 min, indicate that the SDs performed better than pure CLZ (Table 2).

Table 2. Dissolution Parameters of Solid Dispersions

Solid Dispersion	DP		%DE		Best Fit Model	n=	% Drug content ±SD*	f2 (%)	Mean % Yield ±SD*	Saturation Solubility (µg/ml)
	DP60	DP120	DE60	DE120						
CLZ	15.38±1.25	27.82± 0.37	6.41±0.56	15.47±0.68	Zero order	0.9758	-	-	-	-
G50-PM1	58.58±0.98	64.28±0.16	49.99 ±0.126	56.96±0.42	Peppas	0.1563	98.26±0.44	19.78	93.73±0.56	187.27
G50-PM 2	43.23±1.50	53.76±0.78	34.39 ±0.29	44.01±0.17	Peppas	0.2394	96.72±0.64	29.03	94.78±1.02	178.34
G50-PM 3	34.75±0.67	62.05±0.85	30.22±0.34	40.55±0.39	Matrix	0.6731	97.53±0.42	31.06	97.54±1	172.83
G50-KN1	76.72±0.83	102.05±0.61	42.61±	56.96±0.25	Peppas	0.1703	98.52±1.34	10.52	97.43±1	272.26
G50-KN2	90.09±0.58	98.39±0.97	76.55 ±	88.02±0.22	Peppas	1.0552	98.49±0.56	8.63	96.85±0.52	270.0
G50-KN3	23.05±0.08	38.61±0.22	76.56±0.05	88.02±0.43	Hix.Crow. Peppas	0.9866	98.13±0.4	8.63	94.77±1.22	213.04
G50-CM1	44.78±0.99	49.11±0.37	30.36±0.71	44.24±0.79		0.2410	96.48±0.37	27.62	94.33±0.88	269.23
G50-CM2	23.24±.038	33.58±0.51	12.65±0.61	22.12±0.56	1st Order	0.3959	87.11±0.26	59.16	86.53±0.77	265.69
G50-CM3	26.59±0.61	37.83±0.13	28.16 ±0.32	43.6 ±0.19	Peppas	0.9015	86.48	52.09	93.41±0.42	179.54
G50-COG1	97.09±0.78	100.37±0.61	87.46 ±0.11	95.08±0.26	Peppas	0.1265	91.12±0.73	5.86	89.35±0.4	136.78
G50-COG2	71.40±0.34	83.34±0.94	55.18±0.84	68.87±0.13	Peppas	0.1522	89.97±0.53	15.84	90.41±0.68	99.02
G50-COG3	35.49±0.57	44.72±1.43	27.07±	35.02±0.08	Peppas	0.2773	112.78±0.51	36.00	89.62±0.45	97.52
G50-COP1	99.33±0.69	101.91±0.50	94.02 ±0.27	99.22±0.51	Peppas	0.0541	98.18±0.5	4.16	92.46±1.55	252.52
G50-COP2	89.22±0.28	91.39±0.31	76.97 ±0.67	85.32±0.41	Peppas	0.1522	99.77±0.84	8.95	95.57±0.64	248.56
G50- COP3	75.61±1.50	102.97±0.28	46.78 ±0.33	52.21±0.29	Peppas	0.1666	98.06±0.94	12.22	97.31±0.55	233.64
G50-SE1	13.64±0.66	23.86±0.57	8.14±0.51	14.50±1.34	Peppas	0.8927	96.73±0.56	76.84	88.42±0.86	127.02
G50-SE2	40.26±0.14	50.72±0.95	31.22 ±0.20	40.15±0.47	Matrix	0.4329	87.58±0.49	31.78	87.16±0.33	94.93
G50-SE3	38.09±0.05	49.30±0.43	28.86 ±0.43	38.34±0.67	Peppas	0.3929	90.40±0.58	33.81	91.63±0.71	87.18

* Average of three determinations. ±SD - Standard deviation. n = 3

1, 2, 3, - ratio 1:1, 1:0.5 and 1:0.25 ratio of drug:carrier. DP= cumulative percent drug dissolved, DE= dissolution efficiency, f2= similarity factor, n= release exponent, G50= Gelucire 50/13 , PM= physical mixture, SE= solvent evaporation, KN= kneading, CM= closed melting, CP= co-precipitation, CG= co-grinding.

The release profiles of KN and COP SDs with ratios 1:1 and 1:0.5 show uniformly high percent drug release after 120 minutes and the drug release profiles were almost the same for these two methods at these ratios (Figures 6 7, and 8). However, in the case of ratio 1:0.25, this uniformity in drug release was not found. This high loading of the drug prevented the carrier from solubilizing all the drug.

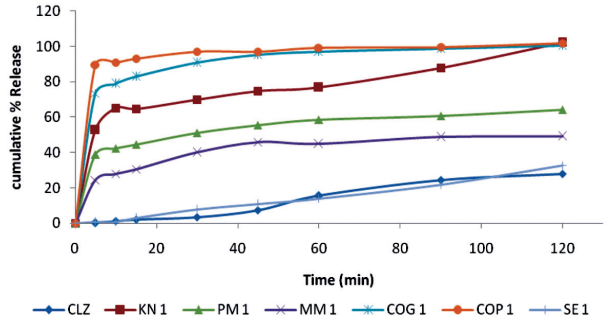

Figure 6. Dissolution profile of CLZ-G50 1:1

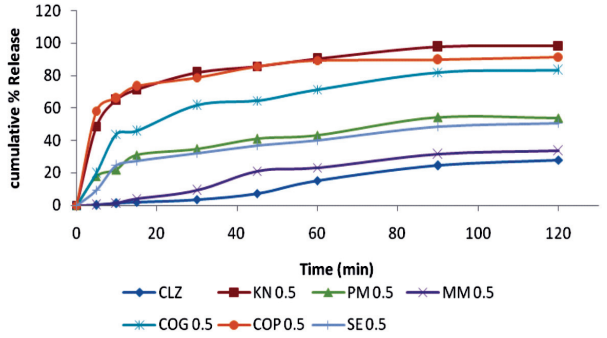

Figure 7. Dissolution profile of CLZ-G50 1:0.5

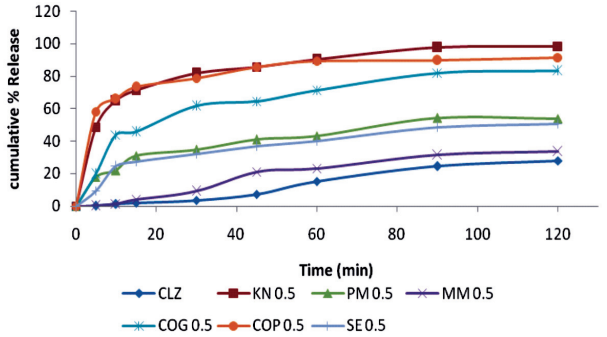

Figure 8. Dissolution profile of CLZ-G50 1:0.25

Tablet characterization and dissolution

When all the SDs had been tested, it was found that formulation G50-KN1 showed the best overall performance, so this SD was selected for the formulation of MDTs. For this purpose, two different superdisintegrants, crospovidone (CP) and the ion-exchange resin Doshion-P544 (DO), were tried at contents of 8% and 12%. The trial formulations were thus denominated $KN1_{CP8}$, $KN1_{CP12}$, $KN1_{DO8}$, $KN1_{DO12}$.

Table 3. Evaluation of Fast Dissolving Tablet

TEST PARAMETER	FORMULATION CODE			
	$KN1_{CP8}$	$KN1_{CP12}$	$KN1_{DO8}$	$KN1_{DO12}$
Bulk density (g/cm³)	0.406	0.382	0.419	0.406
Tapped density (g/cm³)	0.500	0.448	0.52	0.50
Compressibility index	18.75	14.70	17.14	18.75
Hausner's ratio	1.23	1.17	1.24	1.23
Hardness** (kg/cm²)	3.2±0.211	3.3±0.468	3.0±0.612	3.4±0.348
Friability (%)	0.35	0.37	0.56	0.48
Thickness(mm)**	3.2±0.02	3.3±0.24	3.1±0.15	3.5±0.25
Average Weight (mg)*	260±1.06	263±0.58	262±0.94	259±0.43
In-vitro disintegration time** (s)	35.0±0.67	20.0±1.30	40.0±0.91	32.0±0.85
Wetting Time** (s)	40.0±2.54	28.61±0.72	51.0±1.67	31.29±0.62
Water absorption Ratio** %(w/v)	95.4±0.97	128.0±1.64	129.0±0.84	140.0±1.21
Percent Drug Content*	99.35±026	100.5±0.73	99.01±0.81	101.07±0.61

* Average ± SD n = 10, ** Average ± SD, n =6;
KN= kneading, CP= crospovidone; DP= Doshion P544; 8 & 12 = % content.

Bulk densities of MDTs were found to be between 0.38 and 0.42 g/cm³ and tapped density between 0.45 and 0.52 g/cm³. From the density data, % compressibility and Hausner's ratio were calculated and found to lie in the ranges 18.75%-14.70% and 1.24-1.17; hence, it was concluded that the tablets showed good to fair flow properties (Table 3).

All the formulations were white, odorless, and smooth-surfaced flat discs. Hardness and friability of all the formulations were within acceptable limits. The hardness of the MDTs prepared by direct compression was in the range 3.2 to 3.4 kg/cm², while the friability of all formulations was less than 0.6 %. The average weights of the MDT batches were between 259 to 263 mg and the weight variation in each batch was within 1.06%–0.43 % (w/w). The thickness of the tablets varied in the range 3.1 to 3.5 mm. Their drug content was in the range 99.35 to 101.07 mg per tablet (Table 3).

Table 4. Dissolution Parameters of Tablets

Formulation Code	%Drug Release (DP)					Similarity Factor (f_2 %)	DE_5*
	Time(min)						
	1	2	3	4	5		
LNZ	72.45	91.48	102.92	102.67	102.41	-	85.93
$KN1_{CP8}$	71.99	94.15	99.30	99.26	99.22	82.29	73.57
$KN1_{CP12}$	79.49	93.89	101.70	99.72	99.72	78.08	74.43
$KN1_{DO8}$	77.92	91.08	101.07	100.91	100.71	69.96	75.60
$KN1_{DO12}$	71.17	91.38	98.66	98.59	98.53	75.36	71.87

*DE_5 Dissolution Efficiency after 5 min.
LNZ = Lonazep, a commercial CLZ tablet.

The dissolution test gives an idea of the bioavailability of a prepared formulation, relative to an existing one, and is thus a quality control tool. The data from the similarity factor (f_2) analysis showed an average similarity of 73.86% among the formulations $KN1_{CP8,}$ $KN1_{DO8,}$ $KN1_{CP12}$ and $KN1_{DO12,}$ that is a value between 50 to 100 % (Table 4). This

confirms the similarity between the drug release profiles of the test and commercial formulations.

Stability testing of the best formulation:

On the basis of the dissolution test results, four batches of SDs were selected for the stability studies: G50-KN1, G50-KN2, G50-COP1 and G50-COP2 and all the MDT formulations ($KN1_{CP8,}$ $KN1_{DO8,}$ $KN1_{CP12}$ and $KN1_{DO12}$).

DISCUSSION

Phase solubility

The negative values of Gibbs free energy of transfer (ΔG^{o}_{tr}) (-13.65, -18.74 and -21.13 kJ/mol) for carrier contents of 0.3 %, 0.4 % and 0.5 % w/v, respectively, indicate the spontaneity of the solubilizing process.

Fourier transform infrared spectroscopy

In the spectra of the SDs (Figure 3 a,b,c), the peaks characteristic of G50 were present at almost the same positions, whereas CLZ peaks were also present, but at a reduced intensity of absorption, indicating the trapping of CLZ inside the carrier matrix. None of the spectra showed any peaks other than those assigned to CLZ and G50, which indicates the absence of any well-defined chemical interactions.

Differential scanning calorimetry

The DSC curves of all the SDs exhibited an endothermic peak corresponding to G50 melting. The sharp endothermic peak corresponding to the melting of CLZ became rounded and broad in the DSC thermograms of the SDs. This might be due to an amorphous form of CLZ in the solid dispersion or dissolution of crystalline CLZ into the molten carrier during the DSC scan. The DSC curve of the co-precipitated SD (G50-COP1) showed a weak sharp exothermic peak near the CLZ melting point (Figure 4e). The exothermic peak around 250-275 ^{0}C was indicative of thermal degradation of CLZ.

The CLZ peak was absent from the DSC curve of G50-KN1, indicating that the drug in this SD was less crystalline (more amorphous).

Powder X-ray diffraction (pXRD)

Analysis of the pXRD patterns of all the SDs (Figure 5c-f) indicated that the degree of crystallinity of CLZ was decreased by addition of the polymer, G50. The degree of crystallinity decreased the most in the case of G50-KN1, as a number of peaks were markedly reduced, corroborating the DSC data. A fall in the degree of crystallinity means an improvement in the amorphousness of a sample. Hence, from the above discussion, it can be concluded that the kneading method resulted in an amorphous dispersion of CLZ with G50, which led to improved dissolution, relative to other samples.

Data analysis

Dissolution kinetic modeling

Kinetic models were employed to interpret the release kinetics and determine the mechanism of drug release from solid dispersions. The coefficient of determination (r^2) was considered the main parameter for assessing the models.

The value of the release coefficient, n, gives an indication of the release mechanism: when n = 1, the release rate is independent of time (zero-order) (case II transport), n = 0.5 for Fickian diffusion and when 0.5 < n <1.0, diffusion and non-Fickian transport are implicated. Lastly, when n > 1.0, super case II transport is apparent.

Kinetic treatment for PEG 4000 solid dispersions:

Most of the formulations followed Peppas release kinetics, with 4 exceptions, namely G50-KN3, G50-PM3, G50-CM2 and G50-SE2 (Table 2).

All but one of the formulations showed values of exponent **n** in the range 0.0541 to 0.98662, indicating Fickian diffusion and non-Fickian transport as the predominant mechanisms of drug release, while n was 1.0552 for formulation G50-KN2, which is an indication of Super Case-II transport.

Dissolution efficiency and similarity factor

Percent dissolution efficiency (%DE) at 60 min ($\%DE_{60}$min) and 120 min ($\%DE_{120}$min) was computed for each preparation. The similarity factor was used to compare the dissolution profiles (or bioavailability) of the pure drug and prepared solid dispersion.

The resulting dissolution efficiency at 60 and 120 min and the times required to release 50% and 90% of the drug indicate the superiority of solid dispersions over the pure drug (Table 2). The calculated similarity factors indicate that dispersions G50-CM2, G50-CM3 and G50-SE1 followed similar patterns of dissolution to that of CLZ, as their f_2 values exceed 50 (59.16, 52.09 and 76.84, respectively), but the other SDs were dissimilar in their drug release behavior. The results for total drug released (DP) after 60 and 120 min confirmed that the percent released from the dispersions was much more than that from pure CLZ (15.38 and 27.82%, respectively), while the highest amounts of drug were released from the SDs made by kneading and co-precipitation.

Tablet characterization & dissolution

Disintegration time is an important parameter for MDTs, which, according to the European Pharmacopoeia (EP), should be within 3 min. This rapid disintegration

assists swallowing and also plays a role in drug absorption in the buccal cavity, thus promoting bioavailability. The *in vitro* disintegration times of the prepared MDTs were in the range 20-40 s, in the order $KN_{DO8} > KN1_{CP8} > KN_{DO12} > KN1_{CP12.}$ These superdisintegrants accelerate the dispersion of tablets by virtue of their ability to absorb a large amount of water when exposed to an aqueous medium.

As the concentration of superdisintegrants in the formulation increased, the disintegration time was found to decrease and, at the same concentration, tablets made with crospovidone disintegrated faster than those with Doshion. Wetting time is used as an indicator of the ease with which a tablet disintegrates in the mouth. The observed wetting times of the tablets were in the range of 28.61 to 51.0 s, in the order $KN_{DO8} > KN1_{CP8} > KN_{DO12} > KN1_{CP12}$. On comparing superdisintegrants, formulations containing Doshion took more time to wet than crospovidone. This may be due to the fact that crospovidone is disintegrated by capillary action, a mechanism leading to faster wetting, while Doshion perform its disintegrating action by swelling, with minimal gelling.

The dissolution efficiency of the formulated MDTs after 5 min was in the range of 71.87 to 75.60% (compared to 85.93% for the commercial MDT, Lonazep). Maximum percent dissolution was found after 3 min and was in the range 98.66 - 101.70 % w/v. The best result (101.7%) was for KN_{CP12}, very close to that for Lonazep (LNZ) (102.92).

Stability testing of the best formulation

There were no significant changes in drug release profile for the batches stored at 30^0C (±2 ^{0}C) and 65% R.H. (±5%) and 40^0C (±2 ^{0}C) and 75% RH (±5%), relative to the initial batch. From the stability data it can be concluded that there were no changes in any parameter tested in the formulations and thus that the optimized batches of both SDs and MDTs were stable.

SDs of CLZ prepared with G50 by the KN1, KN2, COP1 and COP2 methods resulted in the greatest increases in drug dissolution. As demonstrated by both X-ray diffraction and DSC, a decreased crystallinity of CLZ and the surface morphology of the polymeric particles explained this improved dissolution rate. The prepared MDTs had drug dissolution profiles that were better than those of Lonazep tablets. Moreover, the flow properties of the powder, as well as the disintegration analysis and technological parameters of the tablets, indicated that the powder blend of excipients used was suitable for the development of CLZ- MDTs.

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