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CLOZAPINE-SODIUM STARCH GLYCOLATE DISPERSIONS: IN VITRO DISSOLUTION BEHAVIOUR, PHYSICOCHEMICAL CHARACTERISATION AND RELEASE KINETIC MODEL FITTING

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Clozapine (CLZ) is an atypical antipsychotic agent used in the management of schizophrenia. It exhibits extensive first pass metabolism with poor oral bioavailability (27%–50%) limiting its therapeutic efficiency. The present study involved an attempt to enhance its aqueous solubility by formulating as solid dispersions (SDs) using sodium starch glycollate (SSG) as a carrier. The dispersions were formulated by dispersion method and evaluated by phase solubility, drug content, in vitro release and mathematical modelling. Solid state characterisation of samples was carried out by X-ray diffraction (XRD), differential scanning calorimetric (DSC), Fourier transform infrared spectrophotometry (FTIR), near infrared (NIR), Raman analysis and wettability studies. The phase solubility and thermodynamic parameters indicated the spontaneity and solubilisation effect of carrier. The release rate from the dispersions was higher than pure drug and found to increase with an increase in carrier content. The optimised dispersions were selected based on release studies, profiles and dissolution parameters. XRD, DSC, FTIR, NIR and Raman analysis proved the crystallinity reduction, changes in crystal quality and compatibility between drug and carriers. Wettability studies proved the increased wettability in selected dispersions. Based on the findings, possible mechanisms that would have contributed to dissolution enhancement of CLZ were suggested. Such findings could be extrapolated to enhance the aqueous solubility of other poorly water-soluble drugs.

Keywords: Solid dispersions, Sodium starch glycollate, Crystallinity, Wetting

INTRODUCTION

Modern drug discovery techniques result in increasing number of highly lipophilic drug candidates. More than 40% of compounds identified as potential drugs are classified as poorly soluble. Slow dissolution of such drugs results in poor oral bioavailability. Improvement of oral bioavailability and solubility behaviour of poorly water-soluble drugs is still one of the most challenging aspects of drug development (Panchagnula and Thomas 2000). Based upon their permeability characteristics, the biopharmaceutics classification system (BCS) categorises such drugs into two major classes, i.e., Class II and IV. The BCS Class II drugs are poorly water-soluble entities with high permeability.

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Attempts to enhance drug solubility of these therapeutic agents correlate well with enhancement in their bioavailability (Lipinski 2002).

Various methods have been introduced to enhance the bioavailability of poor water-soluble drugs, which can be summarised as the methods used with physical and chemical modifications (Hamsraj, Vikram and Moorthy 2006). Decrease in the particle size, modification of the crystal habit, complexation, dendrimers for drug solubilisation, spray drying, nano approaches, use of surfactants and drug dispersions in carriers [eutectic mixtures, solid dispersions (SDs) and solid solutions] are among the methods used in physical modification. The application of soluble pro-drugs and salt formation is the most frequently used methods in chemical modification. The preparation of SDs has become one of the most active areas of research in pharmaceutical field to improve the bioavailability of poorly soluble drugs (Krishnaiah 2010).

SD is defined as a dispersion of one or more active ingredients in an inert carrier or matrix in solid state prepared by a melting (fusion), solvent or melt-solvent method. Dispersions obtained through a fusion process are often called melts, and those obtained by solvent method are frequently referred to as co-precipitates or co-evaporates. On the basis of their fast-release mechanisms, SDs are classified in to six representative types: (a) simple eutectic mixtures, (b) solid solutions, (c) glass suspensions, (d) amorphous precipitates in crystalline carriers, (e) compounds or complexes and (f) combinations of the previous five types (Serajuddin 1999).

This technique provides a means of reducing particle size to a nearly molecular level, offers a variety of processing and excipients options that allow for flexibility when formulating oral delivery systems of poorly water soluble drugs with significant reduction in dose and cost. It has been widely demonstrated that hydrophilic carrier dissolves rapidly exposing the drug particles to dissolution medium as fine particles for quick dissolution and absorption (Ansu and Jain 2011; Serajuddin 1999) .

Clozapine (CLZ) is an atypical antipsychotic agent with a molecular weight of 326.8 g/mol and log P of 2.5. CLZ functions by blocking dopamine receptors (D_4, and to a lesser extend D_1 and D_2) thereby countering schizophrenic effects. It is rapidly absorbed but exhibits an extensive first pass metabolism having two metabolites with minimal pharmacological activity, with oral bioavailability of about 27%–50%. It is highly lipophilic and highly bound to plasma protein (97%). Its main adverse effects include agranulocytosis which is mainly associated with its high dose. Frequent blood monitoring is very essential in treatment with CLZ to avoid such complications (Dinunzio and Williams 2008; Cheng, Illum and Davis 2000; Jann 1999) . Of late, few attempts to enhance the aqueous solubility and dissolution of CLZ have appeared in literature with various carriers.

The selection of carriers in formulating SDs plays a significant role in development of successful dosage forms without major limitations in formulation processing at large scale. Polymers, superdisintegrants, surfactants are extensively studied in recent years for dissolution enhancement of drugs (Ansu and Jain 2011).

Superdisintegrants belong to the recent class of pharmaceutical excipients used widely in food, confectionary and pharmaceuticals. They owe their function to their hydrophilic nature. Several insoluble drugs have been shown to exhibit improved aqueous solubility, dissolution rate and oral absorption when formulated as SDs utilising such carriers (Rajshree *et al*. 2007; Caramella 1990).

The primary objective of the present work is to enhance the aqueous solubility of CLZ by dispersing in sodium starch glycollate (SSG) and to investigate the mechanism of drug release through mathematical modeling and solid state characterisation.

METHODS

CLZ was procured *ex-gratis* from M/s Orchid Pharma Ltd. (Chennai, India). SSG, microcrystalline cellulose [directly compressible (DC) grade], magnesium stearate, potassium dihydrogen orthophosphate and sodium hydroxide were all procured from M/s SD Fine Chemicals Ltd. (Mumbai). All the other reagents used were of analytical grade**.**

Phase Solubility Studies

The effect of SSG on aqueous solubility of CLZ was investigated using phase-solubility method (Arias, Gines and Moyano 1996; Higuchi 1965). Twenty five mg of CLZ was added to distilled water containing various concentrations of SSG at different drug: carrier ratio (i.e., 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10). The solutions were placed in screw capped bottles, sealed and shaken in Orbital incubator shaker (Remi Lab Equipments, Mumbai) for 24 h at 37°C and 24°C. The container with pure drug and water was used as control. After the study period the solutions were filtered through 0.45 µm membranes, suitably diluted and analysed spectrophotometrically (UV Vis 1700 spectrophotometer, Shimadzu, Kyoto) at 237 nm. The solubility of CLZ in various carriers was calculated using the standard curve [optical density (OD) = 0.0772 x concentration + 0.0039]. Subsequently, the data was subjected to phase solubility analysis to calculate various thermodynamic parameters like Gibbs free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) (Singh *et al*. 2007).

Phase Solubility Data Analysis (Biswal *et al*. 2008; Singh, Naveen and Katare 2007)

Stability Constant

The value of apparent stability constant, Ka between drug–carrier combinations were computed from the phase solubility profiles using the equation (Eq.) (1):

$$Ka = \frac{Slope}{Intercept\ (1 - Slope)} \tag{1}$$

The values of Gibbs free energy, ΔG were calculated by following Eq. (2):

$$\Delta G = -RT\ \ln\ Ka \tag{2}$$

where,
R – gas constant (8.313 J/mol K)
T – temperature (K).

Enthalpy

The enthalpy change in the physical mixture systems was calculated from Van't Hoff equation and on rearrangement enthalpy, ΔH is calculated from Eq. (3):

$$\Delta H = \frac{-RT \ln Ka}{dT(K)} \quad (3)$$

where,
dT – difference in temperature (K).

Entropy

The entropy, ΔS of the system was calculated from the Gibbs free energy and enthalpy of the physical mixtures by using the following Eq. (4):

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (4)$$

Preparation of SDs

A series of SDs were prepared by keeping the level of CLZ constant and varying the ratio of SSG in samples. The drug: carrier ratios were 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10. CLZ was dissolved in acetone to form a clear solution. The carrier was powdered well in a mortar. The CLZ solution was then poured on to the powdered carrier with constant trituration. The wet solid mixture was dried at 60°C for 6 h in hot air oven. The dried mass was kept in dessicator for 12 h. Next, the dried mass was powdered and sifted through sieve no. 100 (SS Pharma Equipments, Mumbai). The samples was then stored in dessicator until further use (Chowdary and Rao 2000).

Drug Content Determination

Weighed amount of SDs (equivalent to 25 mg of CLZ) was dissolved in 10 mL of 0.1 N HCl. The solution was filtered using Whatman Filter paper (0.45 µm, 13 mm, Whatman, Pittsburgh, USA) and further diluted such that the absorbance value was within the standard curve range. The content was estimated spectrophotometrically (UV-1700, Shimadzu, Kyoto) at 237 nm using standard curve.

In vitro Dissolution Studies

The in vitro dissolution of SDs was determined at 37±0.5°C employing United States Pharmacopeia (USP) apparatus Type II (M/s. Campbell Electronics, Mumbai) at 50 rpm using acetate buffer pH 4.6 (900 mL) as the dissolution medium [Food and Drug Administration-Center for Drug Evaluation and Research (FDA-CDER) 2007]. Dissolution studies were performed on CLZ (50 mg) and SDs (equivalent to 50 mg of CLZ). The samples (5 mL) withdrawn at specific time intervals were diluted and analysed spectrophotometrically at 239 nm. The withdrawn sample was replenished with equal volume of fresh dissolution medium to maintain sink condition. The content of CLZ was

calculated from the standard curve [OD = 0.0696 x concentration – 0.0039, R^2 = 0.9987, p>0.001]. Three such determinations were carried out for each formulation.

Dissolution Data Analysis

Dissolution Parameters

The following dissolution parameters are calculated (Cirri *et al*. 2007, 2004; Gohel *et al*. 2007; Gohel, Parikh and Mukesh 2000) from the dissolution data. The amount of drug released at 5 min and 30 min was taken as Q5 and Q30 values.

Dissolution Efficiency

It can be defined as the area under the dissolution curve up to a certain time. It is estimated using the trapezoidal method and is expressed as a percentage of the area of the rectangle divided by the area of 100% dissolution in the same time. Percent dissolution efficiency (%DE) was calculated by using Eq. (5). It was computed to compare the relative performance of various carriers in solid dispersion formulations (Khan 1975).

$$\% DE = \left(\frac{\int_0^t y \, . \, dt}{y_{100} . \, t} \right) 100\% \tag{5}$$

Y is the percent drug release as the function of time and t is the total time of drug release. Y_{100} is 100% drug release.

Relative Dissolution Rate (RDR)

It is the ratio of the drug released from the samples with respect to pure CLZ at specific time intervals like 5 min (Q05) and 30 min (Q30).

Dissolution Rate Constant (DRC)

A plot of log percentage drug unreleased versus time was drawn and the slope was calculated using MS-Excel 2007 computer programme. Dissolution rate constant was calculated from the slope by using Eq. (6):

$$DRC = Slope * 2.303 \tag{6}$$

The time taken by the samples to release 50% and 85% of drug are taken as dissolution half-life ($t_{50\%}$) and $t_{85\%}$ respectively (Cirri *et al*. 2004).

Fitting of Release Kinetic Model

The in vitro drug release data were fitted in to various release kinetic models viz. Zero order, First order, Higuchi (Higuchi 1965, 1963), Hixson-Crowell cube root (Hixson and Crowell 1931) and Korsemeyer–Peppas model (Ritger and Peppas 1987; Peppas 1985) employing the following set of equations (Eq. 7–11):

Zero order model

$$M_0 - M_t = K_1\ t \tag{7}$$

First order model

$$In(M_0 - M_T) = K_1\ t \tag{8}$$

Higuchi model

$$M_t = K\sqrt{t} \tag{9}$$

Hixson Crowell model

$$(W_0)^{\frac{1}{3}} - (W_t)^{\frac{1}{3}} = K_{\frac{1}{3}}\ t \tag{10}$$

Korsemeyer-Peppas model

$$\frac{M_T}{M_\infty} = k\ t^n \tag{11}$$

Where M_o, M_t and M_∞ correspond to the drug amount taken at time equal to zero, drug dissolved at a particular time, t and infinite time, respectively. The terms W_0 and W_t refer to the weight of the drug taken initially and at time, t, respectively. Various other terms viz. k, k_o, k_1, $k_{1/3}$ and K refer to the release kinetic constants obtained from the linear curves of the Korsemeyer-Peppas, Zero order, First order, Hixson Crowell cube root law and Higuchi equation, respectively (Hamid *et al*. 2006; Costa and Lobo 2001).

Selection of Optimised Dispersions

Based on in vitro release data, release profiles and dissolution parameters namely amount released at different time intervals like Q05 and Q30, %DE, RDR, dissolution rate constant (DRC), $t_{50\,\%}$ and $t_{85\%}$, the best releasing samples were selected from the lot.

Solid State Characterisation

X-ray Diffraction Studies

Powder X-ray diffraction (XRD) patterns were traced by employing X-ray diffractometer (Philips, Cambridge, England) for drug, physical mixtures at 1:1 ratio and selected SDs using Ni filtered Cu K (α) radiation, a voltage of 40 kV, a current of 20 mA and receiving slit of 0.2 inch. The samples were analysed over 2Ø range from 2°C–50°C at the rate of 2°C per min at 0.02° at 2Ø step size.

Differential Scanning Calorimetry Studies

Differential scanning calorimetric (DSC) analysis of CLZ, carrier and selected SDs were carried out on the samples using differential scanning calorimeter (Q 10 DSC TA, Instruments, Waters Inc., Newcastle, USA) with liquid nitrogen accessory. The analysis was performed under purge of nitrogen gas (50 mL/min). High purity indium was used to calibrate the heat flow and heat capacity of the instruments. Sample (5–10 mg) placed in flat bottomed aluminium pan was firmly crimped with lid to provide an adequate seal. Sample was heated from ambient temperature to 400°C at pre-programmed heating rate of 10°C/min.

Fourier Transform Infrared Spectroscopic Studies

Fourier transform infrared (FTIR) spectroscopy was employed to further characterise the possible interactions between drug and the carriers in solid state on an FTIR spectrophotometer (Jasco-FTIR-1700, Tokyo) by the conventional KBr pellet method. FTIR spectra of CLZ, carriers, along with their physical mixtures (1:1) and optimised SDs (from each carrier) were analysed in a similar manner. Physical mixtures were prepared by blending individual component in glass mortar.

Near Infrared Analysis

Near infrared (NIR) analysis spectra of drug and selected samples were recorded in FTIR spectrometer (Jasco-FTIR-1700, Tokyo) in diffuse reflectance mode (DRS). The samples were scanned in the wavelength range of 800–2000 nm and absorbance was measured in transmittance mode.

Confocal Raman Spectroscopic Analysis

The Raman spectra of samples and drug were recorded in Confocal Raman spectrophotometer [WITEC Alpha 300, Confocal Raman Nd: YAG laser (532 nm), Tennessee, USA].

Wetting Studies

Formulation of Tablets

The tablets of CLZ and optimised SDs were formulated using 25 mg of pure CLZ and SDs equivalent to 25 mg of CLZ. Sufficient quantity of microcrystalline cellulose (diluent) and magnesium stearate (lubricant) was added and mixed well in a mortar. The mixture was directly compressed in a 10-station rotary tableting machine (Rimek Ltd., Mumbai) at a compression pressure of 5 kg/cm^2. Each tablet weighed around 350 mg.

Wetting Time Studies

Circular tissue papers (10 cm in diameter) were placed in a petri dish. Ten mL of water containing 0.5% methylene blue (a water-soluble dye) was added to the petri dish and a tablet was carefully placed on the surface of the tissue paper at ambient temperature. The

time required for water to reach the upper surface of the tablets and to completely wet them was noted as the wetting time. These measurements were carried out in replicates of three. Wetting time was recorded with digital watch (Mohapatra, Parikh and Gohel 2008; Adel, Semreen and Mazen 2005).

Water Absorption Ratio

The weight of the tablet prior to placement in the petri dish was noted (W_b), utilising a Metler Toledo Digital (Greifensee, Switzerland) balance. The wetted tablet was removed and reweighed (W_a). Water absorption ratio, R, was then determined using the following equation (Sunilkumar *et al*. 2007; Fukami *et al*. 2006):

$$R = \frac{W_a - W_b}{W_b} \times 100 \quad (12)$$

In vitro Tablet Dispersion Studies

A tablet was added to 10 mL of acetate buffer pH 4.6 at 37°C. The time required for complete dispersion was noted. Three such determinations were carried out (Mohapatra *et al*. 2008).

Statistical Evaluations

The relevance of difference in the in vitro dissolution profile and pharmacokinetic parameters was evaluated statistically. The data were tested by two way analysis of variance using Systat Statistical Software, Bangalore, India.

RESULTS

Phase Solubility Studies

The phase solubility parameters (Table 1) showed a linear increase in drug solubility with increased carrier levels and temperature. The values of apparent stability constant (Ka) were computed and all the values of Gibb's free energy were negative (Table 2) and associated with negative enthalpy and positive entropy at all levels of carrier.

Dissolution Rate Studies

The dissolution rate of CLZ was found to be low (about 54) and nearly 45% of the drug remained unreleased in the dissolution study period (1 h). SDs formulated with SSG showed a higher release rate (87.34%) than CLZ. The release profiles of SDs are illustrated in Figure 1. The release rate of CLZ was found to show a linear increase with an increase in carrier level in all SDs.

For comparative analysis of all SDs, the parameters, the amount of drug released at Q05 and Q30, $\%DE_{60}$, RDR at 5 and 30 min, DRC, $t_{50\%}$ and $t_{85\%}$ values were compared (Table 3). The value of $\%DE_{60}$ was enhanced from a low value of 12.80 for CLZ to a high value of 76.62% for CLZ-SSG systems. The $t_{50\%}$ and $t_{85\%}$ values were found to be more than

60 min (for pure CLZ) whereas the values of the sample SDs was found to be less than that of CLZ. The %DE was found to increase whereas $t_{50\%}$ was found to decrease with an increase in the amount of carrier in SDs and this phenomenon was illustrated in the form of correlation plots (Fig. 2). The dissolution efficiency and $t_{50\%}$ data showed a significant difference among the test products (p<0.05).

Furthermore, the parameters like the amount of CLZ released (mg) at Q05 and Q30, and RDR were found to increase whereas DRC, $t_{50\%}$ and $t_{85\ \%}$ values (min) were found to decrease with an increase in carrier content.

Release Kinetic Model Fitting

The in vitro release data of samples were fitted in to various kinetic models and the data are shown in Table 4. The goodness of fit for various kinetic models was assessed for SDs.

Solid State Characterisation

To investigate the mechanistic of dissolution rate enhancement of CLZ from its SDs, the most promising SDs (CLZ-SSG) was subjected to the solid state characterisation studies.

X-Ray Diffraction Analysis

X-ray diffractogram of CLZ, SSG, physical mixtures (1:1 ratio) and optimised SDs (CSSG10) are compared in Figure 3. Numerous distinctive sharp, narrow peaks appeared in CLZ diffractogram at 10.5, 17.4, 19.7 and 23.7 at 2θ positions, with high peak heights indicating the crystalline nature of CLZ (Sridhar *et al*. 2011; Qian *et al*. 2008; Aminabhavi and Agnihotri 2004). The amorphous nature of carriers was indicated by presence of few distinct peaks in carrier spectra.

Table 1: Phase solubility data of CLZ-SSG physical mixtures.

Drug carrier	Solubility (mg/100 mL)	
	25°C	**37°C**
01:00	1.819	2.270
01:01	2.401	3.886
01:02	2.450	4.257
01:04	3.849	5.569
01:06	4.765	6.188
01:08	5.000	6.683
01:10	5.619	7.302

Table 2: Thermodynamic parameters of CLZ-SSG physical mixtures.

S. no.	Carrier	Temp (°C)	Slope	Intercept	Ka (M^{-1})	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS J/mol K
01	SSG	25	563.51	–21.512	0.047	–2.596	–2.596	2.587
		37	504.2	–17.11	0.058	–2.733	–2.733	2.724

Note: Temp – temperature, Ka – stability constant, ΔG – Gibbs free energy, ΔH – enthalpy, ΔS – entropy

Table 3: Dissolution parameters of CLZ-SSG SDs.

Code	Composition CLZ: carrier	Q05 (mg)	Q30 (mg)	DE %	RDR 05	RDR 30	DRC	$t_{50\%}$ (min)	$t_{85\%}$ (min)
CLZ	1:0	10.43 (1.52)	15.56 (0.81)	12.81 (1.12)	–	–	0.026	–	>60
CSSG2	1:2	15.87 (0.39)	21.09 (1.04)	30.93 (2.13)	1.25	1.01	0.072	>60	>60
CSSG4	1:4	19.94 (1.04)	25.50 (0.65)	36.98 (0.86)	1.44	1.24	0.072	>60	>60
CSSG6	1:6	23.32 (0.52)	27.71 (0.54)	36.58 (0.64)	1.50	1.20	0.070	>60	>60
CSSG8	1:8	26.36 (0.40)	29.55 (0.39)	51.36 (1.04)	2.20	1.70	0.069	10	>60
CSSG10	1:10	36.59 (0.68)	39.92 (1.20)	76.62 (0.94)	3.45	2.55	0.063	4	57.5

Notes: Values in parenthesis indicates standard deviation, Q05 – amount released at 05 min, Q30 – amount released at 30 min, DE – dissolution efficiency, DRC – dissolution rate constant, RDR – relative dissolution rate at specific time intervals, $t_{50\%}$ – dissolution half-life, $t_{85\%}$ – time taken to release 85% of drug from dispersions

Table 4: Release kinetic parameters of CLZ-SSG SDs.

Batch	Mathematical models for drug release										
	Zero order		First order			Higuchi		Hixson Crowell		Korsemeyer Peppas	
	r^2	K_0	r^2	K_1	Slope	r^2	Slope	r^2	Slope	r^2	"n"
CLZ	0.744	0.949	0.011	0.025	0.145	0.950	6.354	0.411	0.019	0.975	0.063
CSSG1	0.844	0.634	0.173	0.012	0.028	0.885	5.871	0.787	0.010	0.985	0.178
CSSG2	0.670	0.727	0.147	0.010	0.023	0.847	6.791	0.759	0.012	0.976	0.209
CSSG4	0.480	0.868	0.074	0.006	0.014	0.729	8.885	0.637	0.019	0.967	0.292
CSSG6	0.436	0.912	0.016	0.002	0.005	0.689	9.518	0.636	0.023	0.946	0.318
CSSG8	0.448	0.947	0.002	–0.001	0.002	0.693	9.781	0.699	0.027	0.954	0.327
CSSG10	0.436	0.939	0.002	–0.001	0.002	0.682	9.746	0.686	0.027	0.951	0.326

Differential Scanning Calorimetry Studies

The thermograms of optimised SDs compared with carrier and CLZ are shown in Figure 4. A sharp narrow endothermic peak appeared at 187.20°C in thermogram of CLZ with high peak area and enthalpy value.

It was observed that the endothermic peak of CLZ was found to reduce in all optimised dispersion thermograms. The peak intensities of the optimised dispersions are compared (Table 5) with that of CLZ.

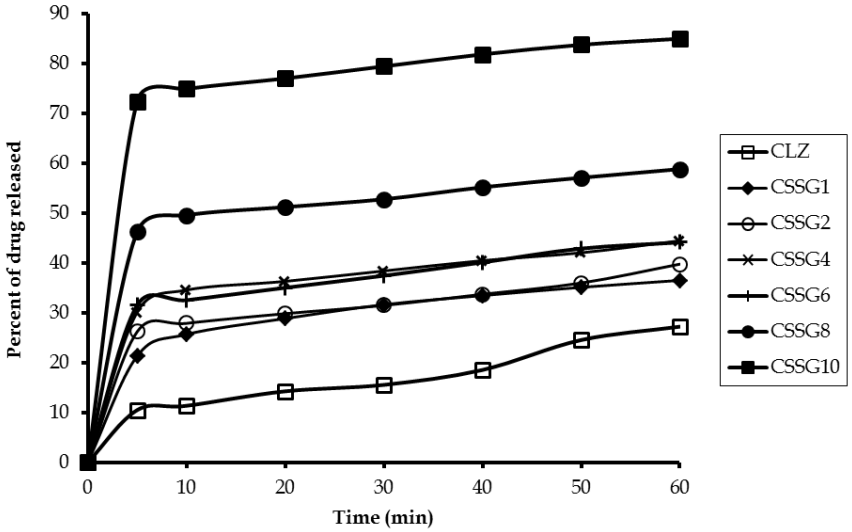

Fig. 1: Dissolution profiles of CLZ-SSG SDs compared with pure CLZ.
Note: All data points represent the mean of 3 values, n=3

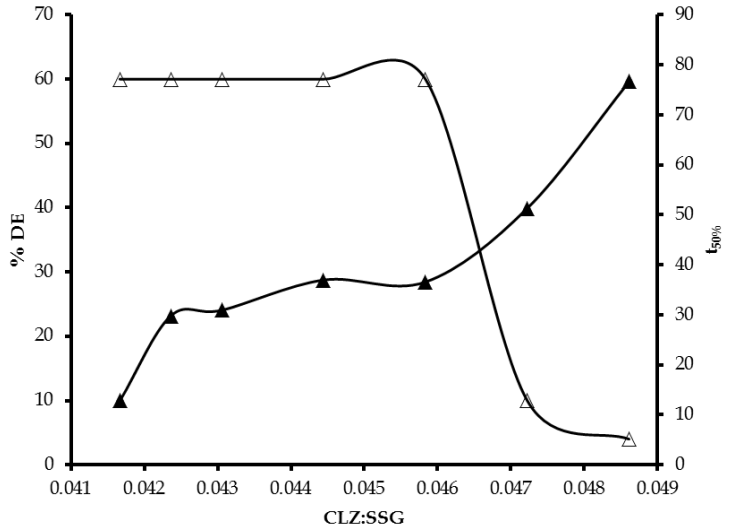

Fig. 2: Correlation plot of %DE and dissolution half-life of CLZ-SSG SDs compared with pure drug.
Note: All data points represent the mean of 3 values, n=3, ▲– %DE and Δ – $t_{50\%}$

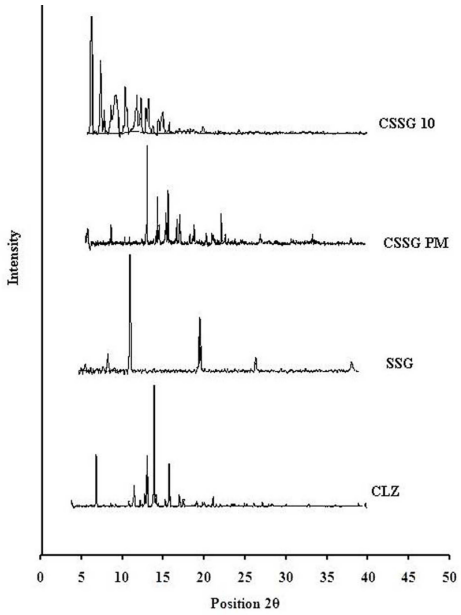

Fig. 3: XRD spectra of pure CLZ, SSG, physical mixtures (PM) at 1:1 ratio and selected SD (CSSG10).

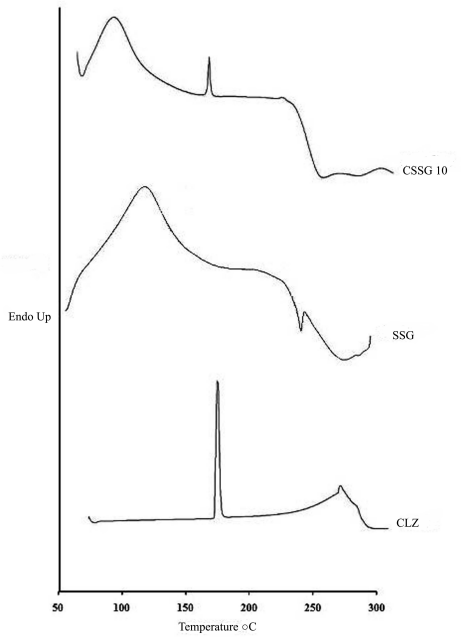

Fig. 4: DSC thermograms of pure CLZ, SSG and selected SD (CSSG10).

Table 5: DSC peak parameters of CLZ and selected SDs.

Batch	Onset (°C)	Peak (°C)	Area (mJ)	ΔH (J/g)
CLZ	183.69	187.20	150.060	50.020
CSSG10	181.61	183.68	7.790	3.387

Fourier Transform Infrared Spectroscopic Analysis

The FTIR spectra of CLZ, physical mixtures and all SDs are compared in Figure 5. The spectra of CLZ showed the following characteristic peaks: 3294 cm^{-1} (N-H stretching), 2968 and 2931 cm^{-1} (aliphatic C-H stretching); 1590 and 1551 cm^{-1} (C=N stretching), 1462 and 1431 cm^{-1} (aromatic C=C stretching); 820 cm^{-1} (C-Cl stretching) (Sridhar *et al*. 2011; Masareddy, Kadia and Manvi 2008).

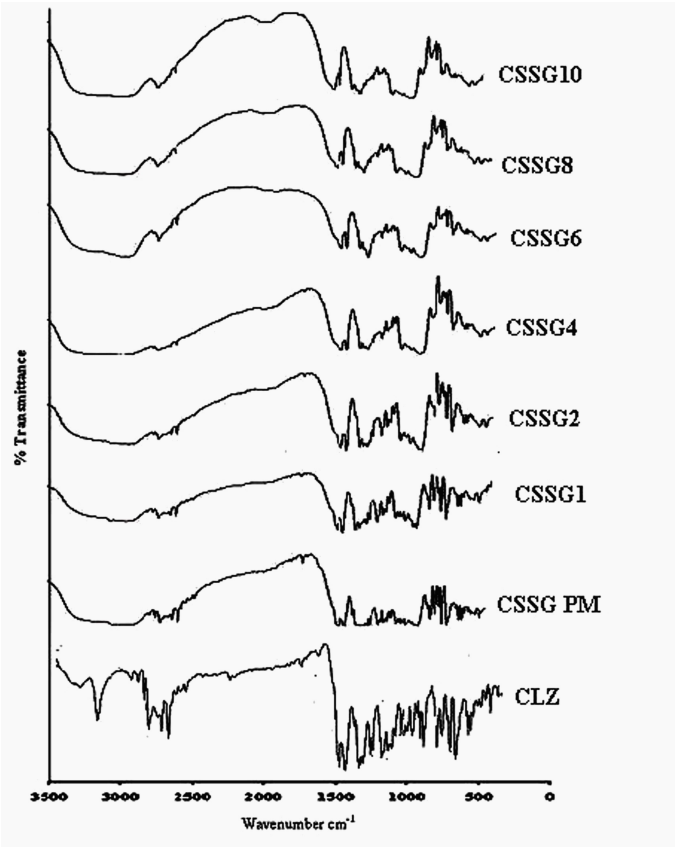

Fig. 5: FTIR spectra of pure CLZ, physical mixtures (PM) at 1:1 ratio and SDs CSSG1, CSSG2, CSSG4, CSSG6, CSSG8 and CSSG10.

Near Infrared Analysis

The NIR spectra of CLZ and optimised SDs (CSSG10) are compared (Fig. 6). The prominent peaks of CLZ were found to appear at 1590 and 1430 nm (Sridhar *et al*. 2011; Qian *et al*. 2008).

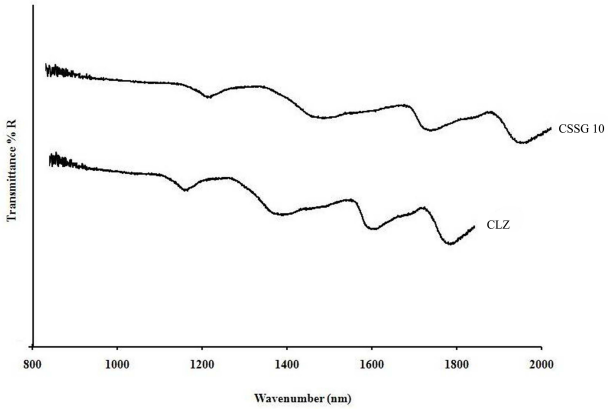

Fig. 6: NIR spectra of pure CLZ and selected SD (CSSG10).

Raman Analysis

The Raman spectra of CLZ and selected SDs are compared in Figure 7. The characteristic peaks of CLZ was found to appear at 3421, 2948, 2441, 2124, 1773, 1294, 483 and 335 cm^{-1} (Sridhar *et al*. 2011; Qian *et al*. 2008).

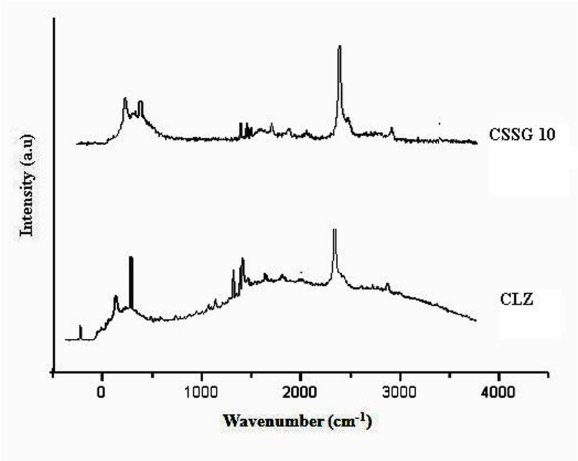

Fig. 7: Raman spectra of CLZ and selected SD (CSSG10).

Wettability Studies

The wetting time, water absorption ratio and in vitro dispersion time of pure CLZ and the optimised SDs were compared (Table 6). The water absorption ratio was found to be low (12.80) due to its high hydrophobicity. It was also noticed that tablets prepared with CLZ did not show any sign of structural changes and its compactness was maintained throughout the study. These observations clearly indicate the high hydrophobicity and poor wettability of CLZ (Sridhar *et al*. 2011; Masareddy, Kadia and Manvi 2008).

DISCUSSION

The phase solubility results were computed and given in Tables 1 and 2. The slopes of straight linear relationship between concentration of carrier and solubility is indicative of the solubilising efficiency of carrier (Biswal *et al*. 2008; Levine 2007; Singh, Naveen and Katare 2007) and spontaneity of the solubilisation process. The enhancement of drug solubility in hydrophilic carrier was also attributed to its co-solvency effect. Hydrophilic carriers are known to interact with drug molecules mainly by electrostatic forces like Van der Waals forces and occasionally by other types of forces like hydrogen bonds and this would have led to the formation of weak soluble complexes. These findings were found to be in accordance with the well-established formation of weak soluble complexes (Biswal *et al*. 2008).

Drug Content

The drug content in samples (98%–103%) clearly indicates the uniform distribution of drug in dispersions and suitability of the method used for its formulation.

Dissolution Rate Studies

The dissolution of CLZ from its SDs was found to be higher than CLZ. The dissolution enhancement could be related to micronisation of drug particles on the large surface area of carrier. The unique fibrous nature of SSG acts as hydrophilic channel to facilitate water uptake in to the dispersions and due to this the total contact area of drug with the dissolution medium increases enormously, resulting in increased wettability and high release rate. It was also suggested that the carrier also form a hydrophilic diffusion layer around the drug particles changing the surface hydrophobicity of drug particles in the medium (Ganesh *et al*. 2008; Balasubramaniam *et al*. 2002).

Increased dissolution of CLZ from SDs could also be ascribed to micronisation of drug particles on large surface area of carrier, change in crystal quality, prevention of aggregation or agglomeration of drug particles in dissolution medium, solubilisation effect of carrier and augmentation of aqueous solubility by SSG (Ganesh *et al*. 2008; Sunilkumar *et al*. 2007). These postulations were well supported by the results of water uptake and wettability studies. It was noticed that there was significant difference (p<0.05) in release rate between the pure CLZ and SDs with varying concentration of carrier. Hence, it can be inferred that the samples are different in their formulations.

On analysing and comparing the release rate, release profiles and various dissolution parameters of all SDs, it was noticed that SDs with highest concentration of

SSG was selected as the best releasing sample (CSSG10) from the lot. The dissolution parameters were found to fit aptly for the findings of the in vitro release studies (Ganesh *et al*. 2008; Balasubramaniam *et al*. 2002).

Release Kinetic Model Fitting

The results of kinetic analysis indicated that the Korsemeyer Peppas model described the drug release kinetics in the most befitting manner than other models for the samples. Further, the values of diffusion exponent "n" obtained from the slopes of the Korsemeyer–Peppas Model, ranged between 0.21–0.35 which clearly indicates the Fickian diffusion characteristics as the values of "n" were found to be lower than the standard value i.e. 0.45. The correlation was also found to be statistically significant with the Korsemeyer-Peppas model. The fitting of the release data and the findings was found to be in consonance with the possible mechanisms suggested for higher release rate from the dispersions. The hydrophilic polymer on absorbing the dissolution medium forms a hydrophilic diffusion layer around the drug particle and the drug has to diffuse across the hydrophilic layer to reach the bulk of dissolution medium. These observations were found to be in accordance with the earlier published reports (Ganesh *et al*. 2008; Sunilkumar *et al*. 2007; Hamid *et al*. 2006; Costa and Lobo 2001).

Solid State Characterisation

X-ray Diffraction Analysis

The presence of numerous distinctive sharp, narrow peaks in CLZ diffractogram indicated the crystalline nature of CLZ (Sridhar *et al*. 2011; Qian *et al*. 2008; Aminabhavi and Agnihotri 2004). The amorphous nature of carriers was indicated by presence of few distinct peaks in carrier spectra.

The peaks in physical mixture spectra of CLZ with SSG at 1:1 ratio were found to be in the same positions as that of CLZ suggesting that the drug was preserved as such in physical mixture and lack of interaction between drug and the carrier. The diffraction spectrum of SDs vis-a-vis drug, carrier and physical mixture indicates the changes produced in crystal structure of CLZ. It was observed that the prominent peaks in optimised SDs spectra exhibited reduced intensity with broad base and high full width half maximum (FWHM) values than the corresponding peaks of CLZ. Relative reduction of diffraction intensity of CLZ peaks in optimised SDs vis-a-vis physical mixtures at these angles suggests that either the quality of the crystals is reduced, or a change is induced in the crystal orientation. These observations clearly confirm the change in crystal quality or crystallinity reduction in CLZ drug molecule. This factor might have assisted in increasing the dissolution rate of CLZ (Rajshree *et al*. 2007).

Differential Scanning Calorimetry Studies

A sharp narrow endothermic peak at 187.20°C with high peak area and enthalpy value in CLZ thermogram indicated its high crystalline nature (Sridhar *et al*. 2011; Aminabhavi and Agnihotri 2004). A broad single halo endothermic peak in carrier thermograms proved its amorphous nature (Sridhar *et al*. 2011; Aminabhavi and Agnihotri 2004).

It was noticed that the peak height, peak area and enthalpy values of the optimised SDs were found to be less than the peak properties of CLZ. From these findings, it can be inferred that the drug's crystal quality or crystal orientation might have changed or phase transition would have occurred in the drug molecule during the formulation process. These structural changes in the drug molecule would have assisted in enhancing the release rate of CLZ from SDs (Ansu and Jain 2011; Leuner and Dressman 2000).

Fourier Transform Infrared Spectroscopic Analysis

The FTIR spectra of CLZ, physical mixtures and all SDs are compared (Fig. 5) and it was noticed that prominent peaks at 2968 and 2931 cm^{-1} (aliphatic C-H stretching); 1590 and 1551 cm^{-1} of CLZ were present in physical mixture and in sample SDs. These findings indicate the absence of interaction between drug and carrier. Thus, these results confirm the compatibility between drug and the carrier used in formulation of SDs.

It was also noticed that the sharpness of peaks at 3294 cm^{-1} was found to reduce and bands at 2967 and 2931 cm^{-1} (aliphatic C-H stretching and OH stretching) were found to be broader in nature as the amount of carrier was increased in all SDs. The infrared spectral behaviour of SDs may be attributed to change in crystal quality of CLZ or due to change in the orientation of crystal lattice of CLZ. Thus, the FTIR spectral study results conclude that some structural changes had taken place in drug molecule without any interaction between drug and the carrier. These changes would have played a significant role in enhancing the drug release from dispersions (Ganesh *et al*. 2008; Rajshree *et al*. 2007).

Near Infrared Analysis

The prominent peaks (Fig. 6) of CLZ at 1590 and 1430 nm (Sridhar *et al*. 2011; Qian *et al*. 2008) were found to appear in sample SDs too, but with increased broadness and slight shift towards the lower wavelength. These findings indicate the reduction of crystallinity and a slight change in crystal quality of CLZ in SDs. It was suggested that this phenomenon would have played a key role in improving the dissolution rate of CLZ from its SDs (Ansu and Jain 2011).

Raman Analysis

From the Raman spectra (Fig. 7) of CLZ and sample SDs, it was observed that the prominent peaks of CLZ at 3421, 2441, 1773, 484 and 339 cm^{-1} were present in spectra of optimised SDs. This indicated the compatibility between drug and the carrier. The prominent peak at 2124 cm^{-1} in CLZ was present with broader base and slight shift toward their lower wave numbers. These observations also prove the crystallinity reduction in drug molecule when dispersed in such carrier. These conclusions were found to correlate well with the published reports of similar works (Biswal *et al*. 2008; Singh, Naveen and Katare 2007; Craig 2002).

Wettability Studies

The wetting time and in vitro dispersion time (Table 6) of optimised SDs were found to be much lower than corresponding values of CLZ (more than 60 min). These findings prove

the increased wettability in optimised SDs. The water absorption ratio of the selected SDs was also found to be higher than CLZ and these observations clearly confirm the water absorption potential of the carrier. These observations may be owed to the nature of hydrophilic carrier used in the formulation of SDs and it also provides a clear insight in to the role of such carrier in dissolution enhancement process (Ganesh *et al*. 2008; Sunilkumar *et al*. 2007; Zhao and Augsburger 2005).

Mechanisms for Enhanced Release

Based on the various characterisation technique findings, the following possible mechanisms were postulated for increased release rate of CLZ from it SDs in diverse carriers *viz*. solubilisation effect of the hydrophilic carriers, particle size reduction, change in surface hydrophobicity due to carriers, change in crystal quality or disorientation of CLZ crystal lattice and increased wettability of hydrophobic drug particles (due to increased water absorption by the hydrophilic carriers). The foresaid mechanisms were found to be in accordance with earlier published reports utilising such hydrophilic carriers (Ansu and Jain 2011; Krishnaiah 2010; Masareddy *et al*. 2008; Rajshree *et al*. 2007; Craig 2002; Corrigan 1985).

CONCLUSION

The water-soluble carrier SSG investigated in the current study enhanced the solubility and dissolution characteristics of the poorly soluble drug CLZ to varying degrees, as a function of carrier concentration. The Korsemeyer–Peppas model most aptly fits the in vitro dissolution data and gives an insight into the possible drug release mechanisms invariably predominated by Fickian diffusion. Solid state characterisation studies clearly provide an opportunity to understand the underlying mechanism and the factors involved in enhancement of CLZ release from its binary systems.

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REFERENCES

ADEL, M. A., SEMREEN, M. & MAZEN, K. (2005) Superdisintegrants for solid dispersions to produce rapidly disintegrating tenoxicam tablets via camphor sublimation, *Pharmaceutical Technology*, 1, 68–74.

AMINABHAVI, T. M. & AGNIHOTRI, S. A. (2004) Controlled release of clozapine through chitosan microparticles prepared by novel method, *Journal of Controlled Release,* 96, 245–259.

ANSU, S. A. & JAIN, C. P. (2011) Solid dispersion: A promising technique to enhance solubility of poor water soluble drug, *International Journal of Drug Delivery,* 3, 149–170.

ARIAS, M. J., GINES, J. M. & MOYANO, J. R. (1996) Study by DSC and HSM of the oxazepam-PEG 6000 and oxazepam-D-Mannitol systems, application to the preparation of solid dispersions, *Thermochimica Acta,* 321**,** 33–41.

BALASUBRAMANIAM, J., ANUJ, G., RAO, V. U., SINGH, S. & BINDU, K. (2002) Solid state interaction of raloxifene HCl with different hydrophilic carriers during co-grinding and its effect on dissolution rate, *Drug Development & Industrial Pharmacy,* 35**,** 455–470.

BISWAL, S., SAHOO, J., MURTHY, P. N., GIRADKAR, R. P. & AVARI, J. G. (2008) Enhancement of dissolution rate of gliclazide using solid dispersions with polyethylene glycol 6000, *American Association of Pharmaceutical Scientists Pharmaceutical Sciences and Technology Journal (AAPS Pharm Sci Tech),* 9, 563–570.

CARAMELLA, C. (1990) Disintegrants in solid dosage forms, *Drug Development & Industrial Pharmacy,* 16, 2561–2577.

CHENG, Y. H., ILLUM, S. S. & DAVIS, S. (2000) Schizophrenia and drug delivery systems, *Journal of Drug Targetting,* 2, 107–117.

CHOWDARY, K. P. R. & RAO, S. K. (2000) Investigation of dissolution enhancement of itraconazole by solid dispersion in superdisintegrants, *Drug Development & Industrial Pharmacy,* 26, 1207–1211.

CIRRI, M., MURA, P., RABASCO, A. M., GINES, J. M. & MOYANO, J. R. (2004) Characterization of Ibuproxam binary and ternary dispersion with hydrophilic carriers, *Drug Development & Industrial Pharmacy,* 30, 65–74.

CIRRI, M., MAESTRELLI, F., CORTI, G., MURA, P. & VALLERI, M. (2007) Fast dissolving tablets of glyburide based on ternary solid dispersions with PEG 6000 and surfactants, *Drug Delivery,* 14, 247–255.

CORRIGAN, O. I. (1985) Mechanisms of dissolution of fast release solid dispersions, *Drug Development & Industrial Pharmacy,* 11, 697–724.

COSTA, P. A. & LOBO, J. M. S. (2001) Modeling and comparison of dissolution profiles, *European Journal of Pharmaceutical Sciences,* 13, 123–133.

CRAIG, D. Q. M. (2002) The mechanisms of drug release from solid dispersions in water-soluble polymers, *International Journal of Pharmaceutics,* 231, 131–144.

DINUNZIO, J. C. & WILLIAMS, R. O. (2008) CNS disorders - Current treatment options and the prospects for advanced therapies, *Drug Development & Industrial Pharmacy,* 34, 1141–1167.

FOOD AND DRUG ADMINISTRATION-CENTER FOR DRUG EVALUATION AND RESEARCH (FDA-CDER). (2007) *Guidelines* http://www.accessdata.fda.gov/scripts/cder/dissolution/dsp-search. (12 March 2007).

FUKAMI, J., YONEMOCHI, E., YOSHIHASHII, Y. & TERADA, K. (2006) Evaluation of rapidly disintegrating tablets containing glycine and carboxymethylcellulose, *International Journal of Pharmaceutics*, 310, 101–109.

GANESH, C., PIYUSH, P., SHARWAREE, H., MUKUL, K., ASHOK, B. & SAGAR, B. (2008) Formulation and evaluation of solid dispersions of furosemide and sodium starch glycollate, *Tropical Journal of Pharmaceutical Research,* 8, 43–51.

GOHEL, M. C., PARIKH, H. & MUKESH, K. (2000) Comparison of in vitro dissolution profiles using a novel model dependant approach, *Pharmaceutical Technology,* 24**,** 92–102.

GOHEL, M. C., PARIKH, K., BANSARI, K. B. & AAROHI, R. S. (2007) Improving the tablet characteristics and dissolution profile of ibuprofen by using novel co-processed superdisintegrant: A technical note, *AAPS Pharm Sci Tech,* 8, E1–E6.

HAMID, A. M., HARRIS, A. S., JAWERIA, T. & JAWARIA, I. Y. (2006) Once daily tablet formulation and in-vitro release evaluation of cefodoxime using hydroxypropylmethyl cellulose tablet: A technical note, *AAPS Pharm Sci Tech,* 7, E1–E6.

HAMSRAJ, K., VIKRAM, S. S. & MOORTHY, R. S. R. (2006). Industrially feasible alternative approaches in the manufacture of solid dispersions: A technical report, *AAPS Pharm Sci Tech,* 7, 87–96.

HIGUCHI, T. A. (1965) Phase-solubility techniques, *Advances in Analytical Chemistry and Instrumentation,* 4, 117–212.

———. (1963) Mechanism of sustained-action medication: Theoretical analysis of rate of release of solid drugs dispersed in solid matrices, *Journal of Pharmaceutical Sciences*, 52, 1145–1149.

HIXSON, A. W. & CROWELL, J. H. (1931) Dependence of reaction velocity upon surface and agitation, *Industrial & Engineering Chemistry,* 23, 923–931.

JANN, M. W. (1999) Clozapine, *Pharmacotherapy,* 11, 179–195.

KHAN, K. A. (1975). The concept of dissolution efficiency, *Journal of Pharmacy & Pharmacology,* 27, 48–49.

KRISHNAIAH, Y. S. R. (2010). Pharmaceutical technologies for enhancing oral bioavailability of poorly soluble drugs, *Journal of Bioequivalence & Bioavailability,* 2, 28–36.

LEUNER, C. & DRESSMAN, J. (2000) Improving drug solubility for oral delivery using solid dispersions, *European Journal of Pharmaceutical Sciences,* 50, 47–60.

LEVINE, I. N. (2007). *Fundamentals of physical chemistry,* vol 1, 5th edition, pp. 123–126 (New Delhi: Vallabh Prakasham).

LIPINSKI, C. A. (2002) Poor aqueous solubility: An industry wide problem in drug discovery, *American Pharmaceutical Review,* 5, 82–85.

MASAREDDY, R. S., KADIA, R. D. & MANVI, F. V. (2008) Development of mouth dissolving tablets of clozapine using two different techniques. I, *Indian Journal of Pharmaceutical Sciences,* 70, 526–528.

MOHAPATRA, A., PARIKH, R. K. & GOHEL, M. C. (2008) Formulation and evaluation of patient friendly dosage forms of metformin hydrochloride. Part I. Orally disintegrating tablets, *Asian Journal of Pharmaceutics,* 7–9, 167–171.

PANCHAGNULA, R. & THOMAS, N. T. (2000) Biopharmaceutics and pharmacokinetics in drug research, *International Journal of Pharmaceutics,* 201, 131–150.

PEPPAS, N. A. (1985). Analysis of fickian and non-fickian drug release from polymers, *Pharmaceutica Acta Helvetica*, 60, 110–117

QIAN, Y., ZHANG, L., LONG, C. & CHEN, Y. (2008) Systematic procedures for formulation design of drug loaded solid lipid microparticles: Selection of carrier material and stabilizer, *Industrial Engineering & Chemical Research,* 47, 6091–6100.

RITGER, P. L. & PEPPAS, N. A. (1987). A simple equation for description of solute release II. Fickian and anamalous release from swellable devices, *Journal of Controlled Release*, 5, 37–42.

RAJSHREE, M., YOGESH, R., MAYUR, M. S. & JOLLY, S. (2007) Effect of hydrophilic swellable polymers on dissolution enhancement of carbamazepine solid dispersions studied using response surface methodology, *AAPS Pharm Sci Tech,* 8, E1–E10.

SERAJUDDIN, A. T. M. (1999). Solid dispersion of poorly water-soluble drugs: Early promises, subsequent problems, and recent breakthroughs, *Journal of Pharmaceutical Sciences,* 88, 1058–1099.

SINGH, B., NAVEEN, A. & KATARE, O. P. (2007) Studies on dissolution enhancement and mathematical modeling of drug release of a poorly water soluble drug using water soluble carriers, *European Journal of Pharmaceuticcs and Biopharmaceutics,* 65, 36–38.

SRIDHAR, B., SIVA LAKSHMI DEVI, A., PRASUNAMBA, P. L. & RAVIKUMAR, K. (2011) Solid state characterization of clozapine monohydrate, *Structural Chemistry Communications,* 2, 101–104.

SUNILKUMAR, B., MICHAEL, A. R., SOUMYAJIT, M. & RAO, Y. (2007) Formulation and evaluation of rapidly disintegrating fenoverine tablets: Effect of superdisintegrant, *Drug Development & Industrial Pharmacy,* 33, 1225–1232.

ZHAO, N. & AUGSBURGER, L. L. (2005) Functionality comparison of 3 classes of superdistengrants in promoting aspirin tablet disintegration and dissolution, *AAPS Pharm Sci Tech,* 6, E634–E640.