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Original article

STUDIES ON CLOZAPINE-MANNITOL SOLID DISPERSIONS, PHYSICO CHEMICAL CHARACTERIZATION AND EVALUATION

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Abstract

The objective of the work is to enhance the dissolution rate of poor water soluble antipsychotic agent clozapine by using hydrophilic carrier (mannitol). The dispersions were evaluated by phase solubility studies, drug content and in-vitro release studies. The optimized dispersions were further characterized by XRD, DSC, FT-IR, Near Infrared, Raman analysis and wettability studies. The phase solubility results and the thermodynamic parameters of the physical mixture indicated the spontaneity and solubilization effect of carrier. The in-vitro dissolution rate of clozapine from the dispersions was significantly better than pure clozapine. The release rate was found to increase up to an optimum drug: carrier ratio and then it tends to be constant with further increase in carrier content. XRD, DSC, Near infra red and Raman analysis indicated the crystallinity reduction and phase transition in clozapine. FT-IR analysis proved the compatibility between the drug and carrier. The wettability studies confirmed the increased wettability in samples. The possible reasons for increased release rate from solid dispersions were postulated and it was found to correlate well with the characterization findings.

Key words: *Clozapine, Solid dispersions, Dissolution efficiency, Dissolution rate constant, Dissolution half Life and Relative dissolution rate*

Klozapin Mannitol Dispersiyonlar, Fizikokimyasal Karakterizasyonu ve Değerlendirme Çalışmaları

Bu çalışmanın amacı, suda zayıf çözünen bir antipsikotik ilaç olan klozapinin hidrofilik taşıyıcı (mannitol) kullanılarak çözünme hızının artırılmasıdır. Dispersiyonlar, faz çözünürlük çalışmaları, ilaç içeriği ve in vitro salım çalışmaları ile değerlendirilmiştir. Optimize edilmiş dispersiyonlar, üzerinde XRD, DSC,FT-IR, Yakın İnfrared, Raman analizleri ve ıslanabilirlik çalışmaları ile ileri karakterizasyonlar yapılmıştır. Faz çözünürlük sonuçları ve fiziksel karışımın termodinamik parametreleri, taşıyıcının spontanlık ve çözünürleştirici etkilerini göstermiştir. Klozapinin dispersiyonlardan in vitro çözünme hızı, saf klozapinden daha iyi bulunmuştur. Salım hızının optimum ilaç:taşıyıcı oranına kadar arttığı ve taşıyıcı içeriğinin daha da artırılması ile sabit olma eğilimi gösterdiği bulunmuştur. XRD,DSC, Yakın Infrared ve Raman analizleri klozapinin kristalinitesinde azalma ve faz geçişini göstermiştir. Islanabilirlik çalışmaları numunelerde artmış ıslanabilirliği göstermiştir. Katı dispersiyonlardan artmış salım hızının nedenleri açıklanmış ve karakterizasyon bulguları ile korele olduğu tespit edilmiştir.

Anahtar kelimeler: *Klozapin, Katı dispersiyonları, Çözünme etkinliği, Çözünme hızı sabiti, Göreceli çözünme hızı.*

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INTRODUCTION

It has been estimated that nearly 35- 40 % of drugs suffer from poor aqueous solubility and it affects the absorption of drug from gastrointestinal tract that leads to poor oral bioavailability, high intra and inter subject variability, increase in dose, reduction in therapeutic efficiency and finally failure in formulation development (1). Clozapine (CLZ) is classified as an atypical antipsychotic dibenzazepine derivative used in the management of both positive and negative symptoms of schizophrenia with a molecular weight of 326.8 g/mol and log P of 2.5. The drug undergoes extensive first pass metabolism having two metabolites with minimal pharmacological activity and providing an oral bio-availability of about 27-50 % (2-3).

Various formulation strategies like micronization, solubilization, complexation, dendrimers for drug solubilization, formation of solid solutions/dispersions with hydrophilic carriers, self micro emulsifying drug delivery systems, spray drying, nano approaches, pro-drug approaches and salt synthesis had been attempted for solubility enhancement (4). An attractive possibility could be represented by employing simple solid dispersion technique utilizing various hydrophilic carriers. Solid dispersions (SDs) are defined as the dispersion of one or more active ingredients in an inert hydrophilic carrier or matrix in a solid state, prepared by the fusion, solvent or solvent-fusion method (5). 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 poor water soluble drugs with cost effectiveness and significant dose reduction (6-7). 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 (7).

The aim of the work is to investigate the effect of hydrophilic carrier (mannitol) on dissolution enhancement of poor water soluble drug CLZ by solid dispersion technique. The dispersions were further characterized by various physicochemical characterization techniques and evaluated for the possible mechanisms for their enhanced release rate.

EXPERIMENTAL

Materials

Clozapine was generous gift sample from M/s. Orchid Pharma Ltd., Chennai, India. Mannitol, Microcrystalline cellulose (DC grade), Magnesium stearate, Potassium dihydrogen orthophosphate, sodium hydroxide was procured from SD fine Chemicals Ltd., Mumbai, India. All other solvents and reagents used were of analytical grade.

Phase solubility analysis

The drug and carrier was accurately weighed at specific drug: carrier ratio (1:1, 1:2, 1:4, 1:6,1:8 and 1:10) and added to 25 mL of water in screw capped bottles and shaken in Orbital incubator shaker (Remi, Mumbai) for 24 h at 37°C and 25°C (10). The container with pure drug and water was used as control. After 24 h the solutions were filtered, diluted and absorbance was measured at 237 nm (UV-Vis 1700 spectrophotometer, Shimadzu, Japan). The solubility of CLZ in various carriers was calculated using the standard curve The proposed method obeys Beer's law in concentration range of 1-5 µg/mL with correlation co-efficient (R^2) = 0.9986 ($p< 0.001$); intercept or constant of (a) = 0.0039±0.004 and slope (b) = 0.0736 ± 0.0042 [OD=0.0736 x concentration+ 0.003] for standard plot using 0.1 N HCL (as co-solvent) and water. Subsequently, the data was subjected to phase solubility analysis to calculate various thermodynamic parameters like ΔH, ΔS and ΔG (8-10).

Phase solubility data analysis

a) Stability Constant (8-10)

The value of apparent stability constant, Ka between drug–carrier combinations were computed from the phase solubility profiles as described below

$$Ka = \frac{Slope}{Intercept\ (1 - slope)} \qquad Eq.1$$

b) Gibbs Energy: ΔG was calculated from the Eq.

$$\Delta G = RT \ln Ka \qquad Eq.\ 2$$

Where, R - Gas constant (8.313 J/mol K)
T - Temperature
Ka - Stability Constant

c) Enthalpy

The enthalpy change in the systems was calculated from Van't Hoff equation

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

Where, R - Gas constant (8.313 J/mol K)
Ka - Stability Constant
dT - Difference in Temperature (Kelvin)

d) Entropy

The entropy of the system was calculated from Eq. 3.4

$$\Delta S = \frac{\Delta H - \Delta G}{T} \qquad Eq.4$$

Where, ΔH - Enthalpy
ΔG – Entropy

Formulation of Solid Dispersions

A series of SDs were prepared by varying the amount of mannitol while keeping the level of CLZ constant in all the formulations. The drug: carrier ratios tried 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 gradually on to the powdered carrier with constant trituration. The wet solid mixture was dried at 60 °C for 6 h. The dried mass was kept in dessicator for 12 h. Next, the dried mass was powdered and sifted through Sieve No. 100. The samples are then stored in dessicator till further use (11-12).

a) Drug content

Assay of weighed amount of samples (equivalent to 25 mg of CLZ) were carried out to determine the drug content. The weighed samples were dissolved in 10 ml of analytical media (0.1 N HCl) and the solutions were filtered using Whatman Filter paper (0.45 µm, 13 mm, Whatman, USA) and further diluted such that the absorbance falls within the standard curve range. The content was estimated spectrophotometrically (UV-1700, Shimadzu, Japan) at 237 nm using standard curve. The proposed method obeys Beer's law in concentration range of 1-5 µg/mL with correlation co-efficient (R^2) = 0.999, ($p < 0.001$); intercept or constant of (a) = 0.0039±0.004 and slope (b) = 0.077 ± 0.0006 [OD=0.077 x concentration+ 0.003] for standard plot using 0.1 *N* HCL.

b) Dissolution rate studies

Dissolution rate of the samples were in Acetate buffer pH 4.6 (900 ml) maintained at 37°C±0.5 °C using USP XXII type II dissolution apparatus (Campbell Electronics, Mumbai) at a stirring speed of 50 rpm. SDs equivalent to 50 mg of CLZ were taken for dissolution studies. Aliquots (5 mL) were withdrawn at different time intervals up to 1 h and replaced with same volume of fresh dissolution medium. Samples were filtered and estimated for CLZ dissolved by measuring absorbance at 239 nm. The proposed method obeys beers law in the concentration range of 1-5 µg/mL with corelation coefficient (R^2) = 0.9987, $p>0.001$); intercept or constant (a) = 0.005 ± 0.003 and slope (b) =0.069 ± 0.0004 [OD =0.069 x concentration + 0.005] for standard plot using acetate buffer pH 4.6.

All the dissolution measurements were performed in triplicate. The release data was subjected to data analysis to calculate various dissolution parameters namely, cumulative per cent drug release, dissolution parameters like amount released (Q), per cent dissolution efficiency (% DE), dissolution rate constant (DRC), relative dissolution rate (RDR), dissolution half life ($t_{50\%}$) and time taken to release 85% of drug ($t_{85\%}$).

c) *Dissolution parameters* (10-14)

The amount of drug released at 5 min and 30 min of the release studies were taken as Q 5 and Q 30 values.

i) Dissolution efficiency

It can be defined as the area under the dissolution curve up to a certain time. It is measured 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 the following Eq. (5), and it was computed to compare the relative performance of various carriers in solid dispersion formulations (21-23).

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

ii) *Relative dissolution rate (RDR)*

It is the ratio of the drug released from the samples with respect to pure drug at specific time intervals like 05 min and 30 min.

iii) Dissolution Rate Constant (DRC)

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

$$DRC = Slope \times 2.303 \qquad \text{Eq. 6}$$

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 (10-14).

Mathematical Modeling of Release

The *in-vitro* drug release data were fitted to various release kinetic models *viz.* Zero order, First-order, Higuchi, Hixson- Crowell cube root and Korsemeyer–Peppas model employing the following set of Eq. (7-11).

Zero- Order model:

$M_0 - M_t = K_1 t$ Eq. 7

First order model:

$ln(M_t - M_0) = K_1 t$ Eq. 8

Higuchi model:

$$M_t = \sqrt{t}$$ Eq. 9

Hixson Crowell model:

$$(W_0)\frac{1}{3} - (W_t)\frac{1}{3} = K_{\frac{1}{3}}\, t$$ Eq. 10

Korsemeyer-Peppas model:

$$\frac{M_t}{M_\infty} = K.t^n$$ Eq. 11

Where Mo, Mt and M_∞ corresponds to the drug amount taken at time equal to zero, drug dissolved at a particular time "t" and infinite time. The terms W_0 and Wt 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. Phase solubility, release data analysis and Model fitting using Eqn.1-11 were accomplished using MS –Excel 2007 computer programme (15).

Selection of optimized formulations

Based on the *in-vitro* release data, release profiles and dissolution parameters the best releasing SDs was selected and further subjected to various physico chemical characterization techniques.

SOLID STATE CHARACTERIZATION

X-ray diffraction studies (X-RD)

X-Ray Diffractometer (Philips, Finland) consisting of 40 kV, 30 mA generator with a Cu-Kα radiation tube was used. Diffraction patterns of pure drug, physical mixtures and selected SDs were scanned over 2Ø range from 2°C -50°C at the rate of 2 ° per min at 0.02° at 2Ø step size .

Differential scanning calorimetry studies (DSC)

Thermal analysis was carried out using differential scanning calorimeter (Q 10 DSC TA, Instruments, Waters Inc., Newcastle, USA) with liquid nitrogen cooling accessory. The analysis was performed under purge of nitrogen gas (50cc/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^{-1}.

Fourier transform infrared spectroscopic studies (FT-IR)

FT- IR spectra of pure CLZ, carrier, physical mixtures of drug and carrier (1:1) and dispersions were carried out using FTIR spectrophotometer with KBr disc (Jasco - FTIR -1700 spectrophotometer, Japan). Physical mixtures were prepared by blending individual component in glass mortar.

Near infrared analysis (NIR)

NIR spectra of pure drug and selected dispersion were recorded in FT-IR spectrometer (Jasco FT-IR, Japan) in Diffuse Reflectance Mode (DRS). The samples were scanned in the wavelength range of 800–2000 nm and absorbance was measured in transmittance mode.

Raman analysis

The Raman spectra of pure drug and selected sample were recorded in a WITEC alpha 300 Nd: YAG laser (532 nm) Confocal Laser Raman Spectrophotometer with the following characteristics: the laser excitation line used was the 1064 nm of an Nd: YAG laser. A secondary filter was used to remove the Rayleigh line. The scattered light was collected at an angle of 180◦. The system was equipped with a liquid N2 cooled Ge detector (D 418).

Wettability Studies

Formulation of tablets

Based on the results of the *in-vitro* release studies, solid dispersion with 1:4 concentration of mannitol (CMAN4) was selected as the best releasing solid dispersion from the samples. The tablets of pure CLZ and optimized SDs (CMAN4) were formulated by using 25 mg of pure CLZ and SDs equivalent to 25 mg of CLZ from the formulation scheme (Table 1). The mixture was directly compressed in a 10 station rotary tablet punching machine (Rimek, Ltd., Mumbai, India) at a compression pressure of 5 kg/cm^2. Each tablet weighed around 250 mg. The total solid mixture weighed around 250 mg for each tablet and about 15 tablets were compressed for pure CLZ and selected dispersion (CMAN4). The wetting studies were carried out only on the tablets made from pure drug and the selected SDs.

Table 1. Formulation scheme for tablets (Wetting Studies)

Composition	Clozapine (mg)	CMAN 4 (mg)
CLZ	25	-
Selected SD (CMAN 4)	-	≡ 25 mg of CLZ (134 mg of SD)
Microcrystalline cellulose	218	109
Magnesium Stearate	7	7
Total	250	250

Wetting time studies

Five circular tissue papers were placed in a petri dish of 10 cm diameter. Ten mL of water containing 0.5 % methylene blue, a water-soluble dye, was added to the petri dish. The dye solution was used to identify complete wetting of the tablet surface. A tablet was carefully placed on the surface of the tissue paper in the petri dish 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 (16-17).

Water absorption ratio

The weight of the tablet prior to placement in the petri dish was noted (W_b), utilizing a Metler Toledo Digital balance. The wetted tablet was removed and reweighed (Wa). Water absorption ratio R, was then determined using the following equation (16,17):

$$R = 100 \times \frac{W_a - W_b}{W_b} \qquad \text{Eq. 12}$$

In- vitro dispersion studies

A tablet was added to 10 mL of phosphate buffer pH 7.4 at 37° C. The time required for complete dispersion was noted down. Three such determinations were carried out (16-17).

Statistical evaluations

The relevance of difference in *in-vitro* dissolution profile and pharmacokinetic parameters was evaluated statistically. The data were tested by two way analysis of variance.

RESULTS AND DISCUSSION

Phase Solubility Studies

The phase solubility values (Table 2) and thermodynamic parameters of CLZ and its physical mixtures in mannitol (Table 3) are evaluated. The solubility of CLZ was found to increase with increase in temperature and carrier concentration. The free energy changes were composed of negative Gibb's free energy, negative enthalpy and positive entropy changes which indicated the process of transfer of CLZ from pure water in to aqueous solution of carriers and the spontaneous nature of solubilization process. These findings were found to be in accordance with the well established formation of weak soluble complexes (8-10). The enhancement of drug solubility in hydrophilic carrier can also be attributed to the co-solvency effect of mannitol. It was suggested that the hydrophilic carriers may interact with drug molecules by electrostatic bonds and forces like Vander Waals forces and this would have lead to the formation of weak soluble complexes on physical mixing of drug and carrier. The slopes of straight linear relationship assumed as indicative of the relative solubilizing efficiency of carrier (12-13).

Table 2. Phase solubility data of clozapine physical mixtures with mannitol.

Drug: Carrier	Solubility mg/100mL ± SD[a]	
	CMAN	
	25°C	37°C
01:0	1.819 ±0.56	2.270 ±0.25
01:01	2.129 ±0.65	3.601 ±1.22
01:02	3.391±1.43	4.220 ±1.36
01:04	3.515 ±2.33	4.257 ±2.54
01:06	3.874 ±0.84	4.310 ±0.68
01:08	4.196 ±0.36	4.344 ±0.46
01:10	4.323 ±0.36	4.579 ±0.36

[a] indicates mean ±S.D, n=3

Table 3. Thermodynamic parameters of clozapine physical mixtures with carriers

Carrier	Temp ° C	Slope[a]	Intercept [b]	Ka [c] (M^{-1})	ΔG [d] (kJ/mol)	ΔH [e] (kJ/mol)	ΔS [f] J/mol K
MAN	25	191.69 ±0.24	-5.484 ±0.35	0.183 ±0.63	-2.976 ±1.32	-2.976 ±1.24	2.966 ±2.46
	37	66.421 ±0.66	1.199 ±0.45	-0.847 ±0.36	-1.105 ±1.55	-1.105 ±1.36	1.101 ±2.56

[a, b, c, d, e, f] indicates mean ± S.D, n=3

Drug Content

The drug content data (Table 4) of the dispersions was found to be in the range of 98-104 %. These values indicated the uniform distribution of drug in dispersions and the suitability of the method used for formulation.

Table 4. Drug content data of clozapine solid dispersions

Carrier	Drug: Carrier [a]					
	1:1	1:2	1:4	1:6	1:8	1:10
MAN	98.24 ±0.67	99.23 ±0.53	98.19 0.44	104.24 ±0.33	101.30 ±0.49	98.24 ±0.98

[a] indicates mean ± S.D, n=3

Dissolution Studies

SDs (Solid Dispersions) formulated with mannitol exhibited significant improvement in the release rate than pure CLZ. The dissolution rate of pure CLZ was found to be low (about 60%) in 1 h and nearly 40 % of the drug remains unreleased in the study period (1 h) (Figure 1 and Figure 2). The percent cumulative release rate of CLZ was found to increase up to specific drug: carrier ratio values of 1:4 for SDs (CMAN4). The release rate was found to become constant on further increase in mannitol content in SDs. Dissolution parameters (Table 5) like amount of drug released, % DE and RDR values of SDs were found to increase with mannitol concentration up to specific drug: carrier values of 1:4 and tends to become constant with further increase in mannitol content. In contrast the parameters like DRC, dissolution half life, and $t_{85\%}$ values were found to decrease up to the optimized drug: carrier ratio and showed constant values on increasing the amount of mannitol in SDs. On comparing the release data, profiles and dissolution parameters of all SDs, batch CMAN4 (with 1:4 drug: carrier) was chosen as the best releasing SDs in the lot.

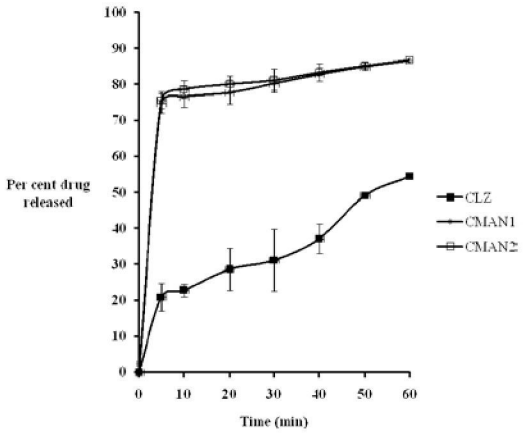

Figure 1. Dissolution profiles of batch CMAN1, CMAN2 SDs compared with pure drug. All data points represent the mean of 3 values, n=3.

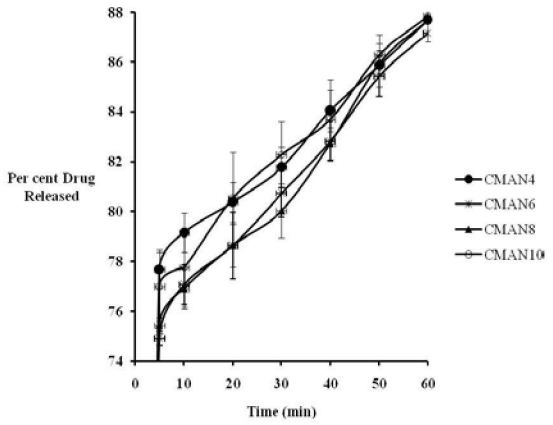

Figure 2. Dissolution profiles of batch CMAN4, CMAN6, CMAN8 and CMAN10 SDs All data points represent the mean of 3 values, n=3.

Table 5. Dissolution parameters of clozapine dispersions

Carrier	Code	Composition CLZ: Carrier	Q_{05}[a] (mg)	Q_{30}[b] (mg)	DE[c] %	RDR 05	RDR 30	DRC	$t_{50\%}$ (min)	$t_{85\%}$ (min)
Mannitol	CLZ	1:0	10.43 ±1.52	15.56 ±0.81	12.81 ±1.12	-	-	0.026	52	>60
	CMAN1	1:1	37.37 ±1.19	40.08 ±1.59	77.38 ±1.14	3.53	2.57	0.038	3.5	52.5
	CMAN2	1:2	37.80 ±0.40	40.53 ±1.17	78.4 ±0.96	3.61	2.59	0.036	3.5	46.5
	CMAN4	1:4	38.84 ±0.83	40.84 ±1.12	79.01 ±1.36	3.67	2.62	0.035	3.5	46
	CMAN6	1:6	37.45 ±0.39	40.36 ±1.08	77.85 ±2.14	3.53	2.58	0.031	3.5	46
	CMAN8	1:8	37.71 ±0.39	40.00 ±0.66	77.9 ±0.84	3.56	2.56	0.030	3.5	48
	CMAN 10	1:10	38.49 ±0.65	41.14 ±0.91	82.54 ±0.86	3.67	2.74	0.028	3.5	29

[a,b,c] indicates mean ± S.D, n=3
Q_{05} – Amount released at 05 min (mg)
Q_{30} – Amount released at 05 min (mg), 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

The possible reasons attributed for such release behavior from SDs are as follows: The carrier mannitol would have formed a hydrophilic diffusion layer around the drug particles altering the surface hydrophobic characteristics of CLZ, reducing its particle size, crystallinity, increased the wettability and prevented the drug agglomeration in dissolution medium (10-14). The behavior is typical of a carrier which brings about temporary super saturation followed by reprecipitation of part of drug and thus the drug release becomes constant after a specific drug: carrier ratio value. These observations were found to be in accordance with the earlier published reports using mannitol as carrier for solubility enhancement. The order of drug release from CLZ – Mannitol SDs was ranked in the following order: CMAN10≡ CMAN8≡ CMAN8≡CMAN4>CMAN2>CMAN> CLZ. It was noticed that there was significant difference ($p<0.001$) in release rate between the pure drug and the solid dispersions. Hence it can be inferred that the samples are not same but are different in their formulations.

Mathematical modeling of release

The *in-vitro* release data was fitted in to various release kinetic models to determine the type of drug release from dispersions. The goodness of fit for various models investigated for binary systems ranked in the order of Korsemeyer–Peppas > Higuchi > Zero order > Hixson-Crowell cube root law > First-order.

From the release kinetic data (Table 6) it was observed that Korsemeyer–Peppas model described drug release kinetics in the most befitting manner than other kinetic models since its co-efficient of correlation **"r"** values were found to be higher than other models. Overall, the values of diffusional exponent 'n', obtained from the slopes of the fitted Korsemeyer–Peppas model, ranged between 0.06-0.3. All the solid dispersions tended to exhibit Fickian diffusional characteristics, as the corresponding values of n were lower than the standard value for declaring Fickian release behavior, i.e., 0.45. The carrier (mannitol) would have formed a hydrophilic diffusion layer around the drug particles altering the surface hydrophobicity of drug particle, reducing its particle size, crystallinity, increasing the wettability and preventing the drug agglomeration in the dissolution medium. The dissolved drug has to pass through this diffusion layer to come in to contact with the bulk of the dissolution medium. The formation of diffusion layer over the surface of the drug particles by the carrier supports this release behavior of CLZ from SDs (15).

The release data of pure CLZ was found to fit in to valid correlations with the Higuchi and Zero order models. The results unequivocally point out the prevalence of diffusional mechanistic phenomena, in consonance with the results obtained while fitting Korsemeyer–Peppas model [8].

Table 6. Release kinetic parameters of clozapine-MAN solid dispersions

Code	Zero Order		First Order			Higuchi		Hixson Crowell		K-P	
	r^2	K_0	r^2	Slope	K_1	r^2	Slope	r^2	Slope	r^2	n
CLZ	0.949	0.744	0.145	0.011	0.025	0.950	6.35	0.411	0.019	0.975	0.063
CMAN1	0.616	0.810	0.094	0.007	0.015	0.623	8.66	0.506	0.019	0.934	0.237
CMAN2	0.598	0.795	0.105	0.007	0.015	0.610	8.61	0.469	0.018	0.927	0.297
CMAN4	0.593	0.795	0.102	0.007	0.015	0.602	8.63	0.464	0.018	0.923	0.299
CMAN6	0.618	0.818	0.085	0.061	0.014	0.632	8.74	0.516	0.019	0.935	0.299
CMAN8	0.617	0.817	0.086	0.006	0.014	0.630	8.72	0.514	0.019	0.933	0.298
CMAN 10	0.604	0.810	0.091	0.006	0.014	0.617	8.73	0.489	0.019	0.929	0.300

Ko –Zero order release constant ; K_1- First order release rate constant ; "n" release exponent ; K-P- Korsemeyer Peppas model

Solid State Characterization

X- Ray diffraction analysis

PXRD studies (Figure 3) shows that CLZ exhibits numerous distinctive sharp, narrow peaks appeared at 10.5, 17.4, 19.7 and 23.7 2θ positions with peak height of 1102.86, 367.89, 1081.76 and 956.3 (21-23). The presence of sharp peaks with high intensity indicates the high crystalline nature of CLZ. Few distinct peaks with slight broad base were observed in mannitol spectra indicated its nature. The spectra of physical mixture of drug and carrier at 1:1 ratio showed few peaks at respective angles of pure CLZ although with low intensity and less peak height suggesting that the drug was preserved as such in physical mixture. The peaks appeared in sample diffractogram (SDs with 1:4) exhibited less height, low relative intensity and high (FWHM) Full width half maximum values than corresponding peaks of pure CLZ. These findings prove the reduction of crystallinity of CLZ present in SDs.

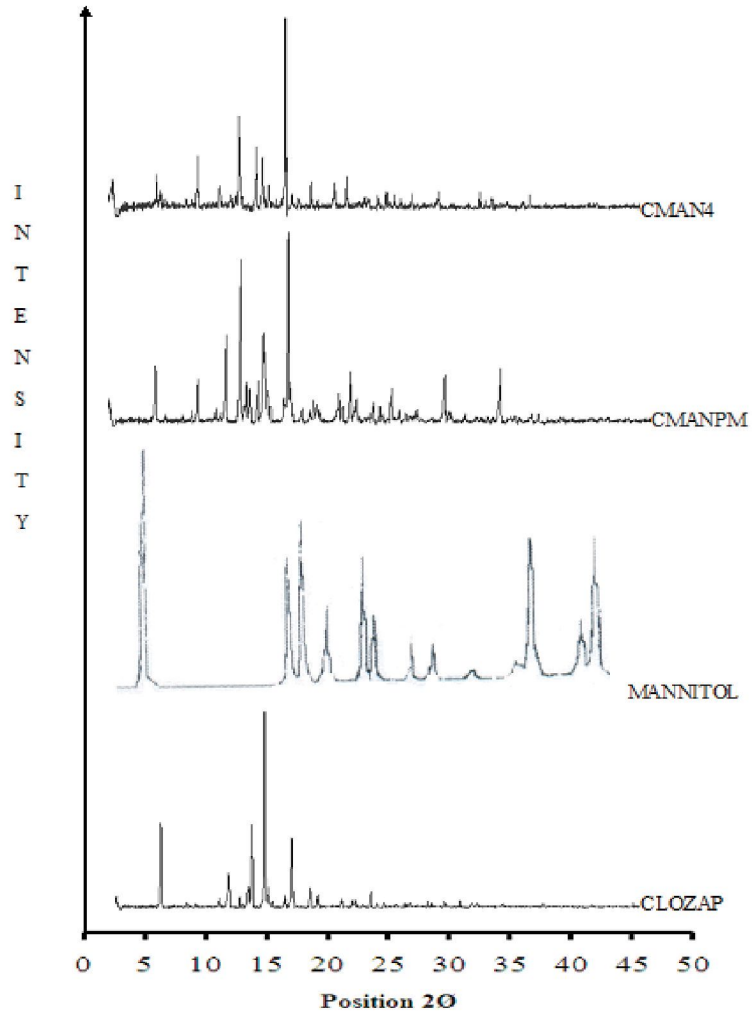

Figure 3. X-RD spectra of pure clozapine, mannitol, physical mixtures (PM) at 1:1 ratio and solid dispersion (SD) CMAN 10 at 1:4 ratio.

Differential scanning calorimetry studies

The thermal analysis was used to detect crystallinity reduction, phase transition of drug from crystalline to amorphous and to check the incompatibility between drug and the carrier used in formulation of SDs. DSC thermograms of pure CLZ, pure mannitol and SDs (CMAN4) are illustrated (Figure 4). A sharp narrow endothermic peak appeared at 187.20 ◦C in thermogram of pure CLZ with peak parameters viz: Onset (183.69 °C), peak (area (150.06 mJ) and ΔH (50.020). This thermal behavior of pure CLZ suggests its high crystalline nature. A short, broad single endothermic peak at 185 °C was observed in carrier thermogram corresponding to its melting point. Two peaks were observed in thermogram of optimized SDs (CMAN4), one peak appearing at 165.95 °C and the other with the following parameters viz.; onset (166.42 °C; peak 169.57 °C), peak area (107.573 mJ) and ΔH value of 32.456. The peak intensities were also found to be less than the peak parameters of pure CLZ. Thus these results ratify the crystallinity reduction in optimized SDs.

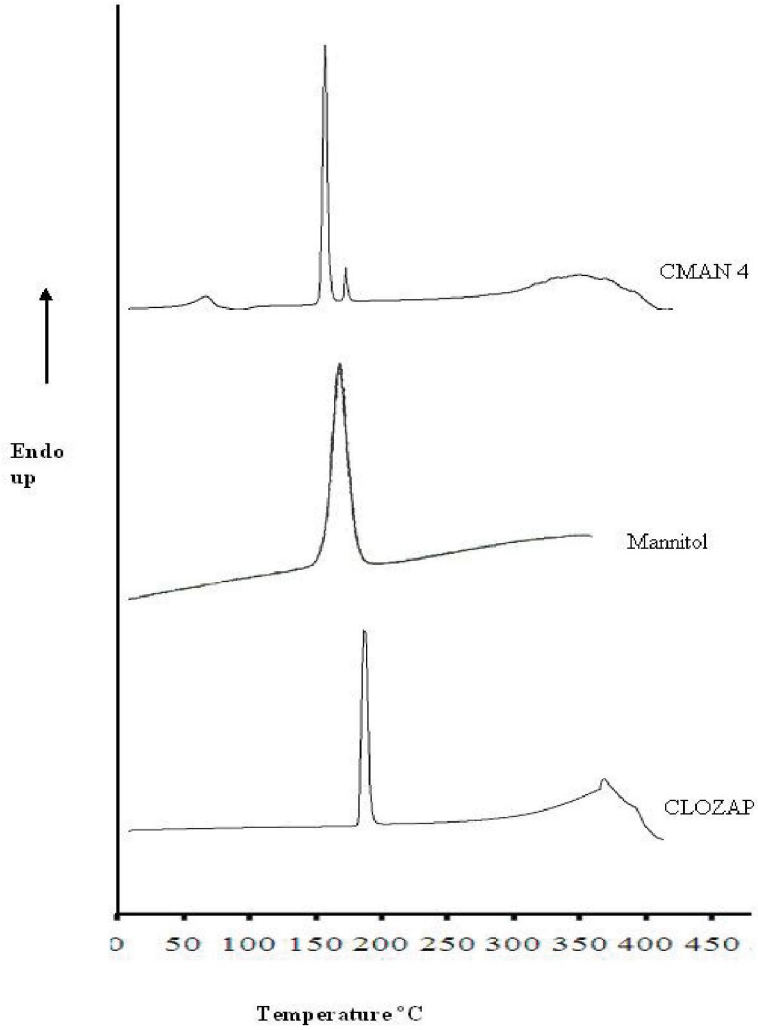

Figure 4. DSC thermograms of pure clozapine, mannitol and solid dispersions (SDs) at 1:4 ratio.

Fourier transform infrared spectroscopic analysis (FT-IR)

The spectra of pure CLZ showed the following characteristic peaks at 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) [21-23]. The IR spectra of pure CLZ, PM (physical mixture) at 1:1 and all SDs were compared (Figure 5). The characteristic peaks of CLZ were found to present in IR spectra of physical mixture and as well as in all SDs proving the compatibility between drug and mannitol. It was also noticed that, the characteristic peaks of pure CLZ were found to be in reduced form, with less sharpness and broadness in SDs. These findings prove the reduction of crystallinity in drug present in SDs (21-23).

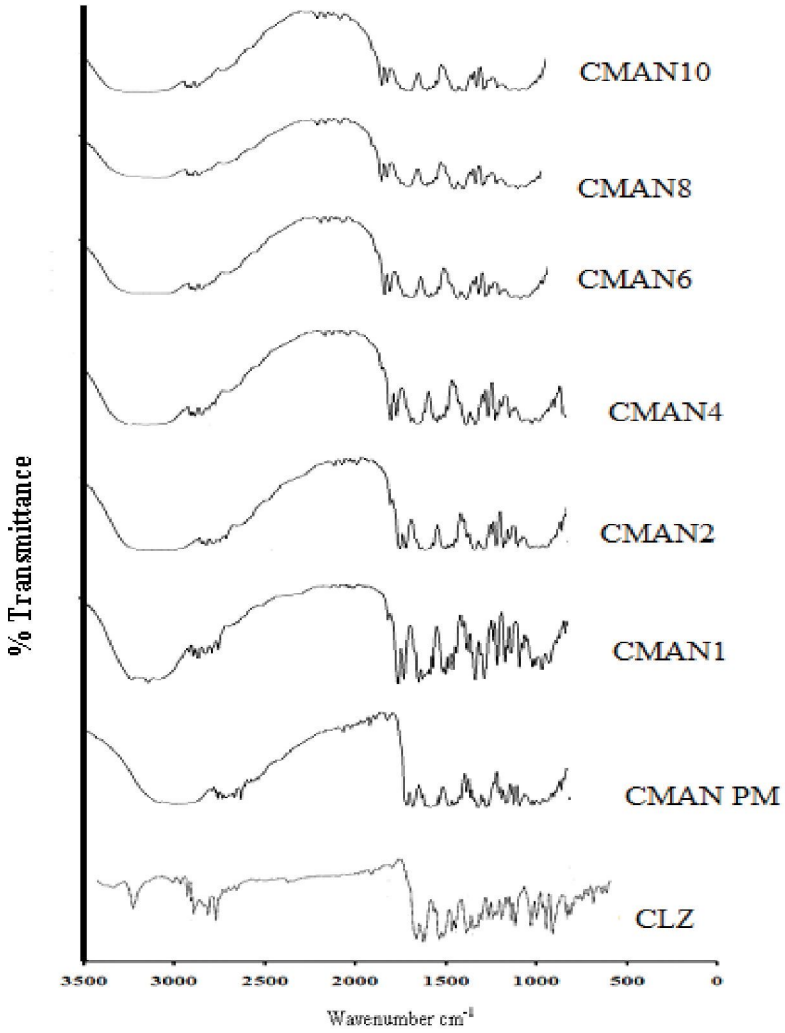

Figure 5. FT-IR Spectra of pure clozapine, physical mixtures (PM) at 1:1 ratio and solid dispersions (SDs) CMAN1, CMAN2, CMAN4, CMAN6, CMAN 8 and CMAN10.

Near infrared analysis

The near infrared spectra of pure CLZ, and optimized SDs were compared (Figure 6) and the specific peaks of pure CLZ were found to be present at 1590-1430 nm. The specific peaks of pure CLZ were found to present in spectra of optimized SDs but with increased broadness and slight shift towards the lower wavelength. These findings indicate the reduction of crystallinity of drug or structural changes in SDs. This phenomenon would have assisted in improving the drug dissolution process (21-23).

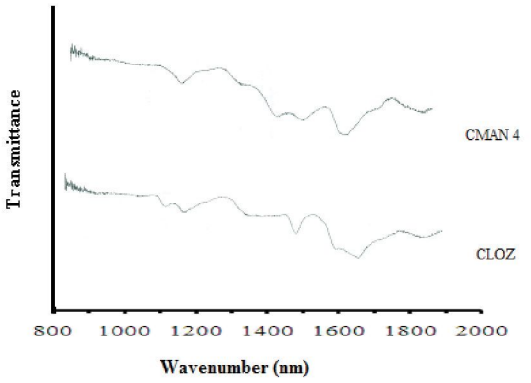

Figure 6. Near infrared spectra of pure clozapine and solid dispersions (SDs) CPGS10, CSSG10, CCCS10, CMAN 4 and CPEG6 1.

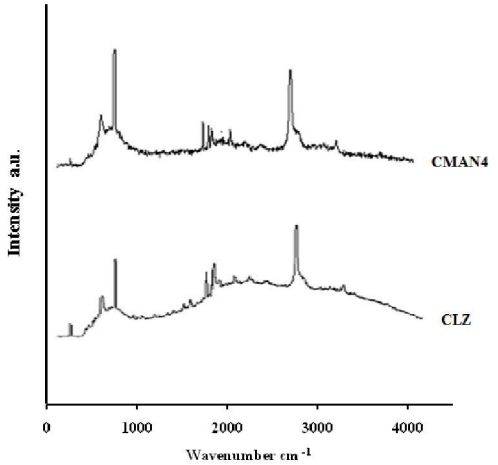

Figure 7. Raman spectra of clozapine and SDs (CMAN 4 and CPEG6 1).

Raman Analysis

The Raman spectra of pure CLZ and selected SDs (CMAN4) are compared (Figure 7). The characteristic peaks of pure CLZ were found to appear at 1590-1430 nm. It was observed that the specific peaks of pure CLZ was found to present in sample spectra but in reduced form with broad base and slight shift in their positions. These observations clearly add support to the earlier suggestions of structural changes in the drug molecule when dispersed in such carriers (21-23).

Wettability Studies

The wetting time of the pure CLZ was found to be more than 60 min. The water absorption ratio of pure CLZ was found to very low and in-vitro dispersion time was found to be more than 60 min. Due to its low water solubility it tends to absorb less amount of water during water absorption studies and it was also noticed that tablets prepared with pure clozapine alone did not showed any sign of structural changes and compactness was maintained during the study period. These observations clearly indicate the high hydrophobicity and poor wettability of the drug (16-17).

The wetting time (20 min), *in-vitro* dispersion time (22 min) and water absorption ratio (14.46) values (Table 7) were found to be less than that of pure CLZ (more than 60 min). The possible reason for this behavior may be ascribed to water absorption potential of mannitol. The dispersions process was found to be moderate in nature which then slowly disintegrates in to fragments. These observations proves that wettability has been increased significantly in samples than pure CLZ and it also explains the role of hydrophilic carriers in the formulations and this factor would have played a significant role in enhancement of dissolution rate of the model drug (16, 17).

Table 7. Wettability data of clozapine and selected solid dispersions.

Batch	Wetting Time [a] (min)	Water absorption ratio [b]	*In-vitro* dispersion time [c] (min)
CLZ	> 60 min ±3.26	12.80 ±1.16	> 60 min ±1.12
CMAN4	20 ±1.36	14.46 ±0.80	22 ±0.96

[a, b, c] indicates mean ± S.D, n=3

CONCLUSION

From the results of the studies it can be concluded that the hydrophilic carrier (mannitol) was found to enhance the aqueous solubility and dissolution characteristics of the poorly soluble drug CLZ significantly with respect to its concentration at a particular level. The Korsemeyer-Peppas model was found to fit suitably in to *in-vitro* dissolution data which was mainly dominated by Fickian diffusion. The results of physico chemical characterization of the dispersions clearly provides an insight in to the drug release process from such systems and to elucidate the mechanism and factors involved in enhancement of CLZ release from such SDs. The drawn conclusions from this study could be applied for further development of more fast release dosage forms of various poor water soluble drugs with much ease. The optimized SDs may also be evaluated for their pharmacokinetic profiles in animals and in humans to the best of their advantage.

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