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RESEARCH ARTICLE

Formulation and evaluation of clozapine solidlipidnanoparticles with natural lipid

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ABSTRACT

Solid lipid nanoparticles of poorly soluble drug (Clozapine) were prepared by using different lipids (Ghee, BBS-C, Dynasan118) with different surfactants (Acconon MC8 EP/NF, Gelucire 44/14, Labrasol, Tween 80, Capmul MCM) in the ratio 1:1. Clozapine SLNs were prepared by hot homogenization technique followed by ultrasonication and its particle size, zeta potential, morphology, entrapment efficiency and drug release in vitro were studied. The FT-IR and DSC studies of prepared formulations revealed that the drug was existed in amorphous state. SEM studies revealed that Clozapine loaded SLNs were almost spherical in shape with particle size range of below 50nm.

The optimized formulations of Clozapine loaded SLNs F1 and F5 exhibits a mean particle size of 10.59 and 9.574nm respectively. The polydispersity index values of Clozapine SLNs (F1 and F5) were found to be 0.256 and 0.231. The zeta potential of optimized Clozapine SLN formulations (F1 and F5) was found to be -2.70 and -3.14 respectively. Entrapment efficiency of Clozapine formulated with ghee as lipid ranging from 99.15 to 99.80 % was observed. The drug release of Clozapine from SLNs showed first order release kinetics, best-fitted to Higuchi equation and it follows non Fickian diffusion.

Key words: Solid lipid nanoparticles, Clozapine, Ghee, Hot homogenization method.

INTRODUCTION:

The global market for drugs for the central nervous system (CNS) is greatly under penetrated and would have to grow by over 50% just to be comparable to the global market for cardiovascular drugs[1]. The main reason is that why neurotherapeutics are unsuccessful in treating CNS disorders because they cannot be effectively delivered the required concentration of drug in the brain because of relatively impermeable Blood Brain Barrier and as a result several potential molecules are lost from the market. The blood-brain barrier (BBB) represents an insurmountable obstacle for a large number of drugs, including antibiotics, antineoplastic agents and a variety of central nervous system drugs (CNS)-active drugs, especially neuropeptides[2].

The field of novel drug delivery has been emerged as an ideal approach into existence for brain drug targeting[3]. It mainly include of use colloidal particles. The basic reason of this carrier acceptance is due to controlled profile or drug release nature as well as due to their selected targeting mechanism.

In general, colloidal drug carriers include micelles, emulsions, liposomes and nanoparticles (nanospheres and nanocapsules). It is noteworthy that only liposomes and nanoparticles have been largely exploited for brain drug delivery. The aim of using colloidal carriers is generally to increase the specificity towards cells or tissues, to improve the bioavailability of drugs by increasing their diffusion through biological membranes or to protect them against enzyme inactivation. Moreover, the colloidal systems allow access across the BBB of non-transportable drugs by masking their physico-chemical characteristics through their encapsulation in these systems[4].

Solid lipid nanoparticles[5] (SLNs) are a stable lipid-based nanocarrier with a solid hydrophobic lipid core, in which the drug can be dissolved or dispersed. Solid lipid nanoparticles possess a solid lipid core matrix that can solubilize lipophilic molecules. Solid lipid nanoparticles generally are spherical in shape and are comprised of a solid lipid core stabilized by a surfactant interfacial region.

Solid lipid nanoparticles (SLN) were developed at the beginning of the 1990s. They are colloidal lipid suspensions or submicron sized aqueous dispersions of solid lipids called also lipospheres or nanospheres and produced by replacing the liquid lipid (oil) of an o/w emulsion by a solid lipid or a blend of solid lipids, i.e. the lipid particle matrix being solid at both room and body temperature. They are stabilized by surfactants. They are

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attractive because they combine advantages of various traditional carriers.

They are made with biocompatible lipids such as triglycerides, fatty acids, or waxes. They are generally of small size (around 10–1000nm) allowing them to cross tight endothelial cells of the BBB and escape from the reticulo endothelial system (RES). During their fabrication the melted lipid, mixed with the drug, is commonly dispersed in an aqueous surfactant by high-pressure homogenization or micro emulsification. The advantages of SLN are their biocompatibility, drug entrapment efficiency comparatively higher than other NPs, and the ability to provide a continuous release of the drug for several weeks. Moreover, the composition of SLN can be controlled modifying their surface properties to target molecules to the brain and to limit RES uptake. Several reports are available describing an enhanced drug delivery to the brain mediated by SLN.

Table 1: Formulation table of Clozapine SLNs prepared by hot homogenization technique

Ingredients	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Clozapine (mg)	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Buffalo ghee (mg)	50	50	50	50	50	50	-	-	-	-	-	-	-	-	-	-
Dynasan 118 (mg)	-	-	-	-	-	-	-	-	-	-	-	50	50	50	50	50
BBS-C (mg)	-	-	-	-	-	-	50	50	50	50	50	-	-	-	-	-
Acconon MC8 EP/NF (%)	-	1	-	-	-	-	1	-	-	-	-	1	-	-	-	-
Tween 80 (%)	-	-	1	-	-	-	-	1	-	-	-	-	1	-	-	-
Capmul MCM (%)	-	-	-	1	-	-	-	-	1	-	-	-	-	1	-	-
Labrasol (%)	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1	-
Gelucire 44/14 (%)	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1
Soya lecithin (mg)	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Stearylamine (mg)	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50

MATERIALS AND METHODS:

MATERIALS:

Clozapine was a gift sample from Lupin Pvt Ltd, Mumbai. Buffalo ghee was purchased from local market. Dynasan 118 was a gift sample from Cremer care, Germany. BBS-C was a gift sample from Abitec Corp. Janesville, USA. Labrasol and Gelucire 44/14 were the gift samples from Gattefosse Pvt Ltd, France. Acconon MC8 EP/NF and Capmul MCM C8 were the Gift sample from Abitec Corp. Janesville, USA. Tween 80, Soya lecithin and Stearylamine were purchased from Merck Pvt Ltd, Mumbai. All other chemicals and solvents were of analytical reagent grade and were used without further purification.

FORMULATION DESIGN AND PREPARATION OF SOLID LIPID NANOPARTICLES:

HOT HOMOGENIZATION TECHNIQUE [6]

Solid lipid nanoparticles of Cloapine were prepared by hot homogenization technique. Poorly soluble drugs (Clozapine), Lipids (Ghee, Dynasan 118, BBS-C), Soyalecithin and Stearylamine were dissolved in 10 ml mixture of Chloroform and methanol (1:1). Organic phase was heated at a temperature above melting point of the lipid. An aqueous phase was prepared by dissolving surfactants (1% w/v) in double distilled water (sufficient to produce 25 ml of preparation) and heated to same temperature of organic phase. Hot organic phase was added to the aqueous phase, and homogenization was carried out by using a homogeniser at 5000 rpm for 30min and temperature is maintained at 70^0C for evaporation of organic solvents in the preparation. Coarse hot O/W emulsion were obtained and sonicated for 30 min for the reduction of particle size. Clozapine solid lipid nanoparticles were obtained by allowing hot nanoemulsion to cool to room temperature.

DRUG - EXCIPIENTS COMPATIBILITY STUDY:

FT-IR SPECTRAL STUDIES:

The IR Spectra for the formulation and pure drug were recorded on Bruker 1.2.4 OPUS7.0 FT-IR attached to an Attenuated total reflectance (ATR) accessory. ATR was fitted with a single bounce diamond at 45^0 internally reflected incident light providing a sampling area of 1mm in diameter with a sampling depth of several microns. Pure drugs (Clozapine), lipids and formulations were analyzed. A small amount of the sample was directly placed on the diamond disk and solid samples kept in solid sample holder. Sample was scanned for absorbance over the range from 4000 to 400 wave numbers (cm^{-1}) at a resolution of 1 cm^{-1}. IR spectra was compared and checked for any shifting in functional peaks and non involvement of functional groups.

EVALUATION OF SLNS FORMULATIONS:

MEASUREMENT OF PARTICLE SIZE & ZETA POTENTIAL:

Particle size, zeta potential and polydispersity index (P.D.I) of solid lipid nanoparticles loaded with Clozapine was determined by Zetasizer Ver 6.01 (Malvern Instruments Ltd., Malvern). Prior to the measurements all samples were diluted 1:10 with double distilled water to produce a suitable scattering intensity. All measurements were performed in triplicate. Analysis was performed at 25^0C with an angle of detection of 90^0 in a capillary cell. The polydispersity index measures the size distribution of the SLNs population. Samples were placed in square glass cuvettes and droplet size analysis was carried out for optimized Clozapine loaded SLNs formulations and F5, F1.

SEM:

The surface morphology of Clozapine loaded SLNs F5 was characterized by scanning electron microscopy (SEM) respectively. The SLN was placed on a double-side adhesive tape stuck to an aluminium stub and dried under vacuum. The samples were made conductive by sputtering thin coat of platinum under vacuum using JEOL JFC-1600 Auto fine coater and then the images were recorded. Then the sample was observed in SEM at an acceleration voltage of 15.0 KV with different magnifications.

ENTRAPMENT EFFICIENCY (EE):

The entrapment efficiencies of prepared systems were determined by measuring the concentration of free drug in the dispersion medium. The EE of Clozapine was determined by dialysis tubing method. The dialysis bag (molecular weight cut off 12000–14000Da) was soaked in deionised hot water for 12h before use. 1mL of the drug-loaded SLNs was placed into the dialysis bag. Samples of 5mL were withdrawn from the receiver medium stirred with a magnetic stirrer and replaced with equal volumes of pH 7.4 phosphate buffer. The sample was analyzed by using a UV-Visible Spectrophotometer at a λ_{max} of 285nm against a blank of phosphate buffer of pH 7.4.

The %entrapment efficiency (% EE) was calculated with the following equation:

% EE = $(DS-DE)/DS\times100$,

Where, DE was the unentrapped amount of drug and DS was the total amount of drug.

***IN VITRO* RELEASE STUDY:**

In vitro release of drug from Clozapine SLN formulations was determined by exhaustive dialysis method in pH 7.4 phosphate buffer and modified to maintain a sink condition to achieve satisfactory reproducibility. The dialysis bag (molecular weight cut off 12000–14000Da) was soaked in deionised hot water for 12h before use. 1mL of Clozapine loaded SLN dispersion was first poured into the dialysis bag with one end fixed by thread and placed into phosphate buffer pH 7.4 placed in beaker. The beaker was placed on a magnetic stirrer. At fixed time intervals of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 12 and 24 hrs, a sample was removed for analysis and equal volume of fresh buffer was added. The sample was analyzed by using a UV-Visible Spectrophotometer at a λ_{max} of 285nm against a blank of phosphate buffer of pH 7.4. Data obtained from *In vitro* release studies were fitted to various kinetic equations to find out the mechanism of drug release from SLN.

DIFFERENTIAL SCANNING CALORIMETRY (DSC):

The thermotropic properties and phase transition behaviour of Clozapine, optimised formulation F5 of Clozapine loaded SLNs were evaluated by Shimadzu Differential Scanning Calorimeter (DSC)-50 (Kelvin, Japan). Samples of about 5 mg were sealed in a 50 µl aluminum pans at a heating rate of 10°C/min throughout the analysis. Empty aluminum pans were used as references and the whole thermal behaviours were studied under a nitrogen purge (20 mL/min). The samples were accurately weighed into aluminum pan and hermetically sealed with aluminum lid. The thermograms of Clozapine, Clozapine loaded SLNs (F5) were recorded.

RESULTS AND DISCUSSION:

Clozapine (a lipophilic drug) was successfully loaded in Ghee, BBS-C and Dynasan 118. The FT-IR spectra revealed that, there was no interaction between excipients and the drug (Clozapine) indicated that the excipients were compatible with drug. SEM[13] studies revealed that Clozapine loaded SLNs were almost spherical in shape with particle size range of below 50nm.

The optimized formulations of Clozapine loaded SLNs F1 and F5 exhibits a mean particle size of 10.59 and 9.574nm respectively, which serves the required criteria of attaining the size to be used for brain delivery[10]. The

polydispersity index values of Clozapine SLNs (F1 and F5) were found to be 0.256 and 0.231, which indicates uniformity of droplet size within the formulation. The zeta potential of optimized Clozapine SLN formulations (F1 and F5) was found to be -2.70 and -3.14 respectively indicating a relatively good stability and dispersion quality[7].

Entrapment efficiency of Clozapine formulated with ghee as lipid ranging from 99.15 to 99.80 % was observed. The drug release of Clozapine from SLNs best-fitted Higuchi equation and the possible mechanisms for the drug release might be diffusion of the drug from the matrix and matrix erosion resulting from degradation of lipids. Clozapine SLNs followed first order release kinetics and anomalous (non-Fickian) diffusion where ($n < 0.89$). *In vitro* drug release of the Clozapine loaded SLN formulations prepared with ghee showed high drug release rate compared to the other formulations prepared with BBS and Dynasan 118[9,11].

DSC thermograms and FT-IR spetra of optimised SLN formulations showed that the drug (Clozapine) is dispersed in amorphous state in the lipid[10,14].

Table 2: FT-IR Spectral data of pure drug (Clozapine)

	Aliphatic C-H Stretch (cm^{-1})	C=N Stretch (cm^{-1})	Aromatic C=C Stretch (cm^{-1})	C-CL Stretch (cm^{-1})
Clozapine pure drug	2968.21-2930.78	1591.76-1549.37	1453.74-1430.03	814.86

Table 3: FT-IR Spectral data of pure drug (Clozapine) and SLNs prepared by hot homogenization technique

Formulation code	Aliphatic C-H Stretch (cm^{-1})	C=N Stretch (cm^{-1})	Formulation code	Aliphatic C-H Stretch (cm^{-1})	C=N Stretch (cm^{-1})
Clozapine	2968	1590	F8	3318.49	1637.36
F1	3332.64	1636.73	F9	3325.29	1636.61
F2	3322.39	1638.38	F10	3323.30	1637.93
F3	3308.53	1638.33	F11	3331.70	1636.69
F4	3320.04	1636.27	F12	3292.70	1638.34
F5	3330.13	1636.58	F13	3318.92	1638.11
F6	3305.97	1638.15	F14	3321.10	1637.27
F7	3327.25	1636.62	F15	3319.05	1637.89

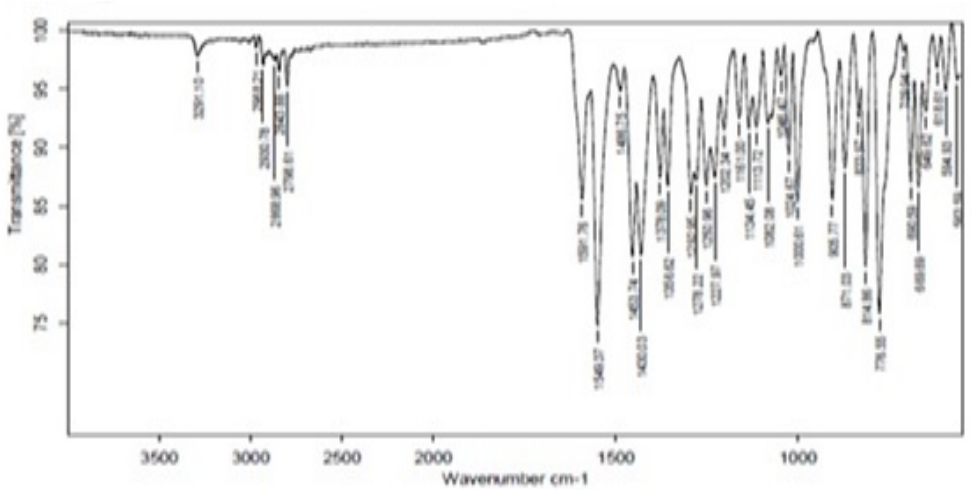

Figure 1: FT-IR spectrum of Clozapine

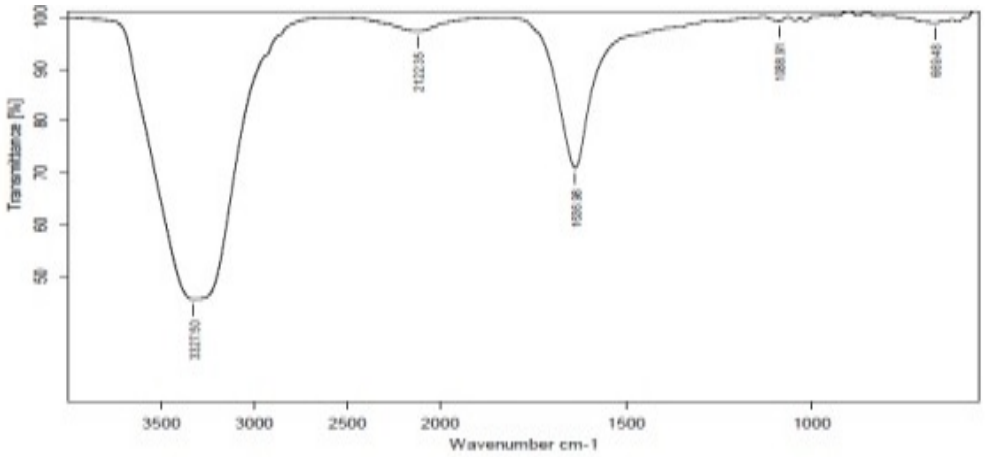

Figure 2: FT-IR spectrum of F5

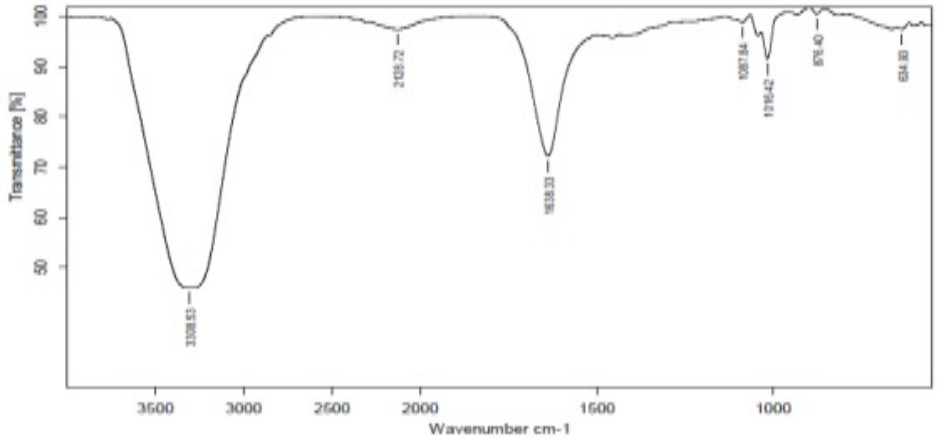

Figure 3: FT-IR spectrum of F1

Table 4: Particle size, PDI and Zeta potential values for the optimized SLNs formulations (F1, F5)

FORMULATION CODE	AVERAGE PARTICLE SIZE (nm)	POLY DISPERSITY INDEX	AVERAGE ZETA POTENTIAL (mv)
F1	10.59	0.256	-2.70
F5	9.574	0.231	-3.14

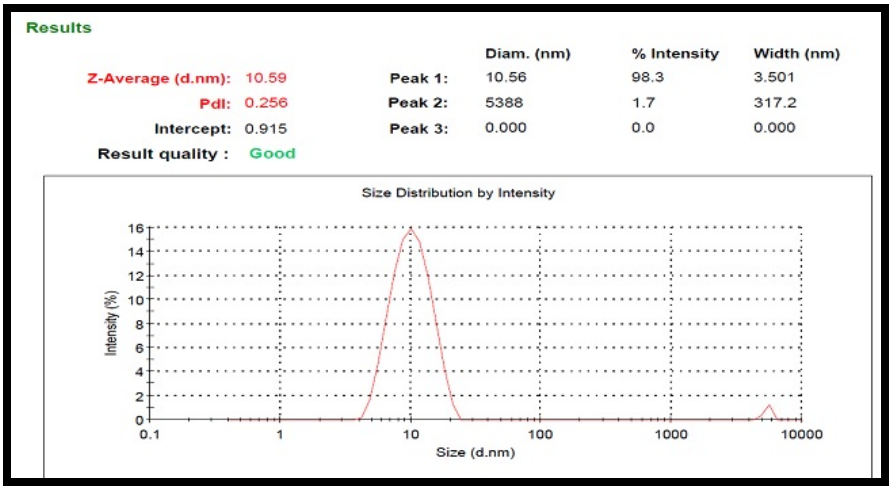

Figure 4: Particle size distribution report for CLOZAPINE SLNs F1

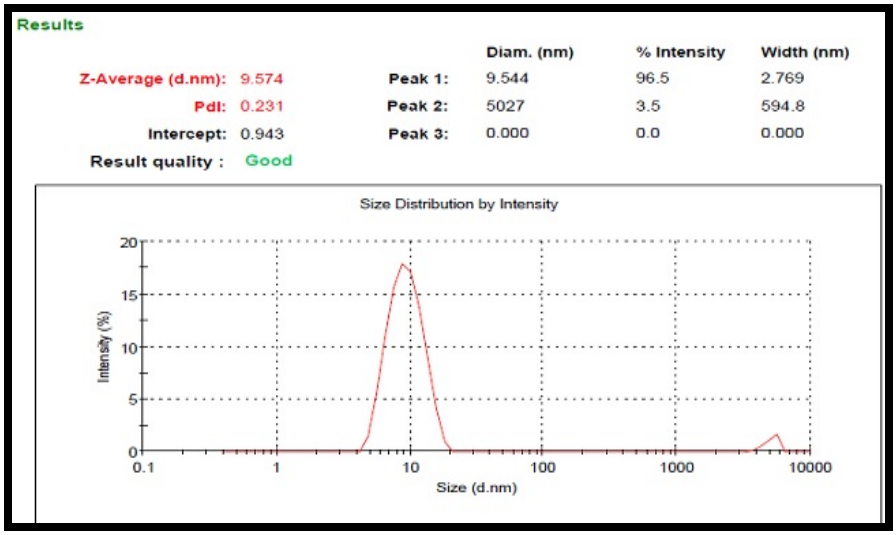

Figure 5: Particle size distribution report for CLOZAPINE SLNs F5

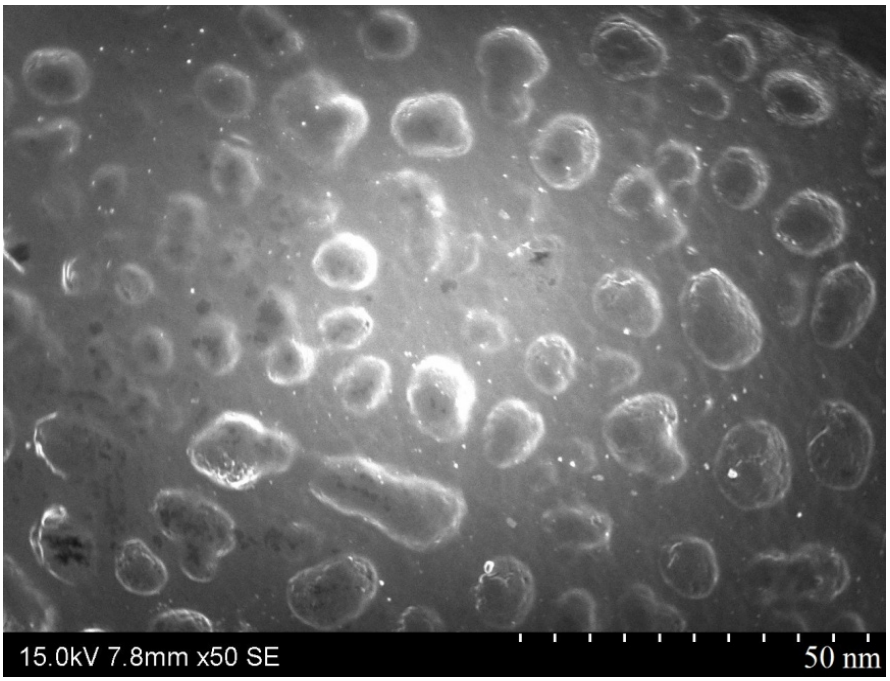

Figure 6: Scanning electron microscopic images of optimized formulation (F5) of Clozapine at 7.8mm x 5SE

Table 5: Percent entrapment efficiency of Clozapine loaded SLNs prepared with Ghee

Formulations	%Entrapment efficiency
F1	99.66 ± 0.05
F2	99.71 ± 0.03
F3	99.50 ± 0.01
F4	99.15 ± 0.04
F5	99.80 ± 0.01

Table 6: Percent entrapment efficiency of Clozapine loaded SLNs prepared with BBS-C

Formulations	% Entrapment efficiency
F6	99.72 ± 0.02
F7	99.62 ± 0.01
F8	99.62 ± 0.07
F9	99.67 ± 0.04
F10	99.79 ± 0.03

Table 7: Percent entrapment efficiency of Clozapine loaded SLNs prepared with DYNASAN118

Formulations	%Entrapment efficiency
F11	99.72 ± 0.07
F12	99.81 ± 0.05
F13	99.75 ± 0.02
F14	99.80 ± 0.04
F15	99.74 ± 0.08

All values represent mean ± standard deviation (SD), n=3

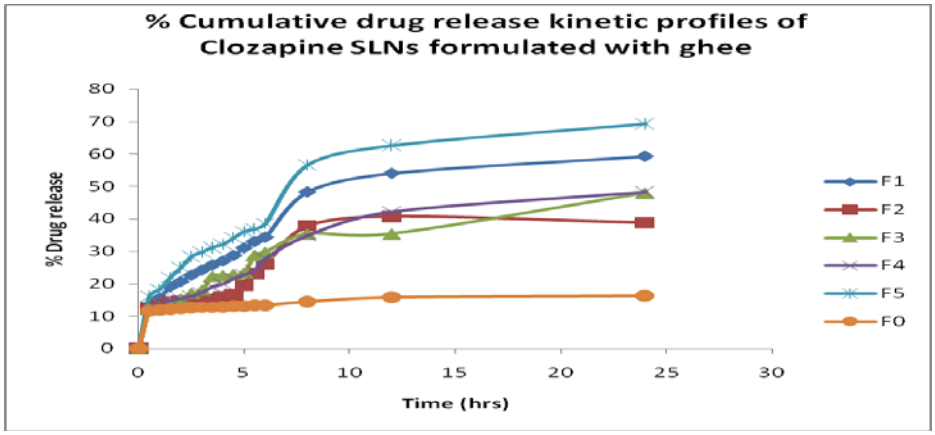

Figure 7: %Cumulative drug release kinetic profiles of Clozapine SLNs formulated with ghee in pH 7.4 phosphate buffer

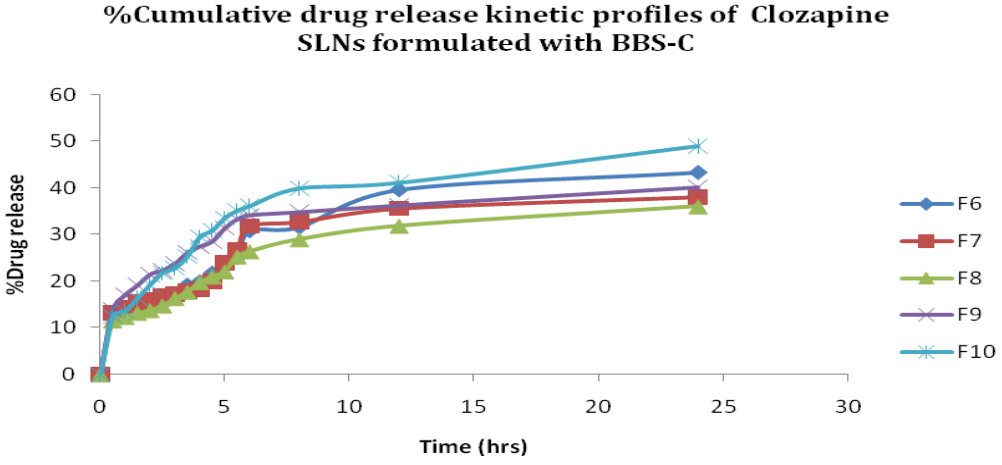

Figure 8: %Cumulative drug release kinetic profiles of Clozapine SLNs formulated with BBS-C in pH 7.4 phosphate buffer

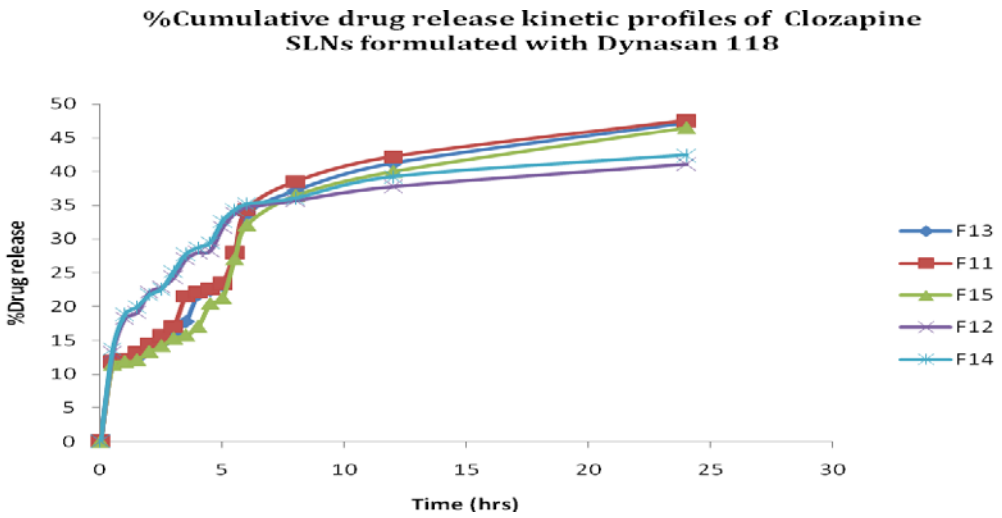

Figure 9: %Cumulative drug release kinetic profiles of Clozapine SLNs formulated with Dynasan118 in pH 7.4 phosphate buffer

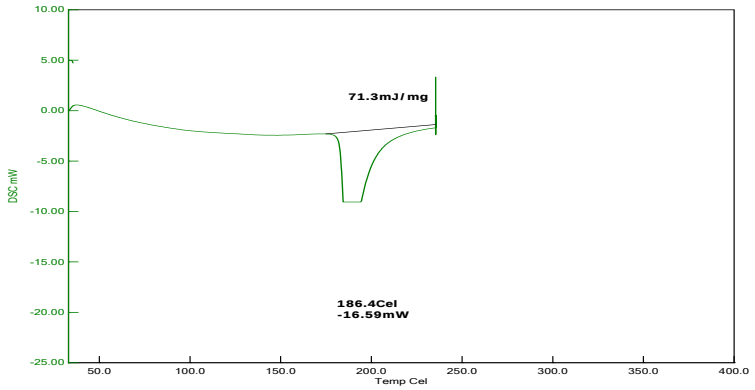

Figure 10: DSC thermogram of CLOZAPINE

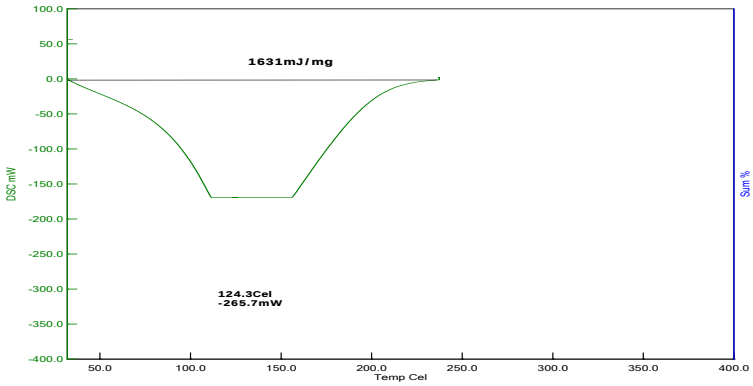

Figure 11: DSC thermogram of optimized formulation F5

CONCLUSION:

Clozapine SLNs formulated with Ghee were successfully developed to yield an optimized formulation with lowest particle size, highest entrapment efficiency and drug release characteristics that could controlled the release of drug.

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