



DEVELOPMENT AND EVALUATION OF CLOZAPINE PELLETS FOR CONTROLLED RELEASE

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ABSTRACT

This research work was done to design oral controlled release matrix pellets of water insoluble drug Clozapine, using blend of Hydroxypropyl cellulose and glyceryl palmito stearate as matrix polymers, methyl crystalline cellulose as spheronizer enhancer, sodium lauryl sulphate as pore forming agent. Clozapine formulations developed by the pelletization technique by drug loaded pellets were characterized with regard to the drug content, size distribution, Scanning electron microscopy, differential scanning calorimetry, fourier transform infrared spectroscopy and Xray Diffraction study. Stability studies were carried out on the optimized formulation for a period of 90 days, $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity. The drug content was in the range of 95.34 – 98.12 %. The mean particle size of drug loaded pellets was in the range 1018 to 1065 μm . SEM photographs and calculated sphericity factor confirms that the prepared formulations were spherical in nature. The drug loaded pellets were stable and compatible as confirmed by DSC and FTIR studies. XRD patterns revealed the crystalline nature of pure clozapine. Loose surface crystal study indicated that crystalline clozapine was observed in all formulation and more clear in formulation A5. Higher amount of clozapine released was observed from formulation A5 and Syclop® 25 mg tablet as compared to all other formulations and mechanism of drug release followed Fickian diffusion. It can be concluded that formulation A5 is an ideal formulation for once a day administration.

Keywords: Pelletization; Clozapine; Microporous membrane; Release kinetics

INTRODUCTION

In recent years, considerable attention has been focused on the development of novel drug delivery system (NDDS). The reason for this paradigm shift is that low development cost and time required for introducing a NDDS, as compared to new chemical entity. In the form of NDDS, an existing drug molecule can get a new life, thereby increasing its market value, competitiveness and product patent life. Among the various NDDS available in the market, oral controlled release system hold a major position because of ease of administration and better patient compliance¹. An ideal drug delivery system should be able to deliver an adequate amount of drug, preferably for an extended period of time, for its optimum therapeutic activity. Most drugs are inherently not long lasting in the body and require multiple daily dosing to achieve desired blood concentration to produce therapeutic activity. To overcome such problems, controlled release and sustained release delivery systems are receiving considerable attention from pharmaceutical industries world-wide². Controlled drug delivery systems not only prolong the duration of action, but also result in predictable and reproducible drug release kinetics³.

Clozapine [8-chloro-11-(4-methylpiperazin-1-yl)-5H dibenzo[b,e][1,4]diazepine] is a potential antipsychotic agent used in chemotherapy⁴. It is one of the most commonly used atypical antipsychotics, and is also used for treatment of resistant schizophrenia. To achieve a high level of safety and effectiveness in pharmacotherapy, quality requirements of active substances are growing^{5,6}. The starting dose of clozapine is 12.5 mg orally once or twice a day. It is practically insoluble in water, having only < 27% oral bioavailability⁷. Clozapine undergoes extensive first pass metabolism. Dosage adjustments may

be needed based upon individual patient characteristics. The use of clozapine is associated with side effects: extreme constipation, night-time drooling, muscle stiffness, sedation, tremors, orthostasis, hyperglycemia, and weight gain. The risks of extrapyramidal symptoms such as tardive dyskinesia are much less with clozapine when compared to the typical antipsychotics. Clozapine also carries eleven black box warnings for agranulocytosis, CNS depression, leukopenia, neutropenia, seizure disorder, bone marrow suppression, dementia, hypotension, myocarditis, orthostatic hypotension and seizures. To achieve maximum therapeutic effect with a low risk of adverse effects, controlled released preparations are preferred^{8,9}. The side effects could be lowered by controlling the drug release and by employing suitable modifications in the manufacturing process¹⁰. Delivering the drug from the pellet could be manipulated by suitable coating techniques¹¹. Some schizophrenic patients hide a conventional tablet under their tongue to avoid its daily dose of an atypical antipsychotic¹². To overcome this problem an attempt was made to formulate and evaluate controlled release dosage forms of clozapine. Matrix pellets were made to improve the solubility of clozapine and to enhance dissolution rate of clozapine. It may enhance the pregastric absorption of clozapine. In the present study, a novel extrusion/spheronization method was employed using inert hydrophilic and hydrophobic carriers material and non-toxic solvents to load the drug into pellets. Hydroxypropyl cellulose is a derivative of cellulose with both water solubility and organic solvent solubility¹³. It is also used in formulations containing water-insoluble drugs. HPC exhibits controlled surface erosion that provides a constant delivery of poorly

soluble drugs via multi-unit erosion matrix and drug release was found to be proportional to matrix erosion. Hence matrix erosion could be used to predict drug release. HPC has been widely exploited in pharmaceutical industry, because of its tailor – made to suit the demands of applications. HPC has the advantages of being nontoxic orally, high biocompatibility, easily in an alkaline environment. So acid sensitive drugs incorporated into the pellets would be protected from gastric juice. The chief characteristic of HPC is impermeability to gastric juices but susceptibility to intestinal juices¹⁴.

Glyceryl palmito stearate (GPS) act as an inert matrix and drug released very slowly as compared to hydrodispersible, hydrophilic matrix gelucire 50/13^{15,21}. Glyceryl palmito stearate reported as a solidifier, controls the drug release, protects the hygroscopic substances and facilitates the incorporation of liposoluble active ingredients and preservative for lipids, oils, waxes and solvents^{16,22}. MCC was incorporated in most formulations via extrusion-spheronisation, because it enhanced the rheological properties of the wetted mass, resulted good sphericity, low friability, high density and smooth surface for successful extrusion-spheronisation^{17,18,23}.

Dispersion of finely devided poorly water soluble drug in hydrophilic and lipidic carriers is an interesting technique for the production of matrix pellets¹⁹. Different methods were applied for the preparation of lipidic matrix based pellets by extrusion/spheronization²⁰. The interest in pellets as dosage forms (filled into hard gelatin capsules or compressed into disintegrating tablets) has been increasing continuously.

A thorough literature search revealed a lack of information on combination of hydrophilic hydroxypropyl cellulose and hydrophobic glyceryl palmito stearate based pellets for controlled drug release, using spheronizer enhancer methyl crystalline cellulose and Sodium lauryl sulphate (0.1 % w/v) as a leachable pore forming and wetting agent. In the present study controlled release pellets were developed by extrusion-spheronization of clozapine/ hydroxypropyl cellulose / glyceryl palmito stearate with addition of methyl crystalline cellulose with SLS to tailor drug release. The aim was to develop clozapine suitable for once daily formulation and examine the influences of various process parameters on physicochemical properties of the pellets and drug release potential.

MATERIAL AND METHODS

Clozapine was a gift sample from Microlabs, India. Hydroxypropyl cellulose (HPC) fine particle sized Klucel® Pharm Hydroxypropylcellulose (HPC) grades EF (D50 typically 100 – 150 µm with a molecular weight of 50). Glyceryl palmito stearate (GPS- Precirol ATO 5), Sodium lauryl sulphate (SLS) and micro crystalline cellulose (MCC) were procured from Loba Chemie, Mumbai, India. Solvents and chemicals were of analytical grade.

Preparation of Pellets

The pellets were prepared by pelletization technique using extrusion / spheronization. Clozapine/ hydroxypropyl

cellulose / glyceryl palmito stearate were passed through sieve No. 40 prior to pelletization and mixed uniformly in a planetary mixer. The bubble free SLS (0.1 % w/v) solution was added dropwise to the the mixture and mixed for 30 min. The obtained good dough mass was extruded using a piston extruder (1 mm orifice, Kalweka, India). The extrudates were immediately spheronized for 5 min at a rotational speed of 450 rpm and an air velocity of 1 kg/cm². The pellets were dried over night at room temperature and cured at 35°C for 24 hour in a fluid bed dryer (Kothari, India).

Particle size analysis

The particle sizes of drug loaded formulations were measured by an optical microscope fitted with an ocular and stage micrometer and particle size distribution was calculated. The Olympus model (SZX-12) having resolution of 40X was used for this purpose. The instrument was calibrated at 1 unit of eyepiece micrometer was equal to 1/30mm (33.33µm). In all measurements at least 20 particles in five different fields were studied. Each experiment was carried out in triplicate.

Micromeritic Properties

Angle of repose (θ) was assessed to know the flowability of matrix pellets, by a fixed funnel method using the formula;

$$\tan(\theta) = \text{height} / \text{radius} \quad (1)$$

Tap density and bulk density of the pellets were determined using tap density tester (TDT, Electrolab, India). The percentage Carr's index (I, %) was calculated using the formula;

$$I, \% = \{ \text{Tapped density} - \text{Bulk density} \} / \text{Tapped density} \quad (2)$$

Granule density of the pellets was determined by displacement method and calculated using the equation ;

$$\text{Granule density} = \text{Weight of pellets} / \text{Volume of petroleum ether displaced} \quad (3)$$

The Hausner ratio of the matrix pellets was calculated using the formula;

$$\text{Hausner ratio} = \text{Tapped density} / \text{Bulk density} \quad (4)$$

The friability test was performed on the pellets in a Roche Friability tester (Electro lab Friability tester, EF -2, India) and friability was calculated using the following equation; Friability (%) = [1– initial weight / weight retained after 100 rotations] × 100 (5)

Scanning Electron Microscopy Analysis (SEM)

The shape and surface characteristics were determined by scanning electron microscopy (model-LV 5600, Jeol, USA) and photomicrographs were recorded, by suitable magnification at room temperature.

Pellet Sphericity

Pellet sphericity was determined with a digital camera (Sony, DSC T-4010.Cyber shot, Japan). The obtained images were processed by image analysis software to characterize each individual pellet by mean Feret diameter (FD) (average of 180 calliper measurements with an angle of rotation of 1°), Aspect ratio (AR) (ratio of longest Feret diameter and its longest perpendicular diameter) and two-dimensional shape factor (eR)

$$eR = 2\pi r / Pm - (b/l)^2 \quad (6)$$

where r is the radius, Pm the perimeter, l the length (longest Feret diameter) and b the width (longest

perpendicular diameter to the longest Feret diameter) of the pellet.

Internal Pore Structure

To determine the internal pore structure of the pellets, computed tomography CT scanner (Phoenix nanotom-M, GE-India) was used. Combining the data of the maximum inscribed diameter (d_{max}) and the equivalent diameter (d_e) provides information about the structure of the pores.

Differential Scanning Calorimetry (DSC)

DSC studies (Du Pont thermal analyzer with 2010 DSC module) were carried out to study the thermal behaviors of drug alone and mixture of drug and polymer. Calorimetric measurements were made with the help of an empty cell (high purity alpha alumina disc) as the reference. The instrument was calibrated using high purity indium metal as standard. The DSC scans of the samples were recorded in nitrogen atmosphere at a heating rate of 10 °C/min.

Fourier Transform- Infrared Spectroscopic Analysis (FT- IR)

FTIR spectra of pure drug, empty pellets and drug loaded pellets were obtained using KBr pellet method (applying pressure of 6000 kg/cm²). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Model 8400S, Japan).

X-Ray Diffractometry (XRD)

X-ray diffraction patterns of pure clozapine and drug loaded pellets were recorded using (Phillips PW 1710, Tokyo, Japan) X-ray diffractometer with a copper target, voltage 40 Kv, current 30 MA at a scanning speed of 0.30 °C/min.

Determination of Drug Content

100 milligrams of drug loaded pellets were dissolved in 100 ml of methanol. The resulting IM concentrations were assayed using a fully validated high performance liquid chromatography with ultra violet detection (HPLC-UV) method²⁴. Quantification was achieved by the measurement of the peak area ratio of the clozapine. Diazepam was used as internal standard. The HPLC system consisted of HPLC-Shimadzu (Tokyo, Japan) LC-6A model, fitted with a μ -Bondapack C18 (4.6 X 250 mm) column of particle size 5 μ m (Supelco, Bellefonte, PA). The flow rate was maintained at 1 μ L/min, and the drug concentration was detected using a UV/visible detector (SPD-6Av). Acetonitrile-methanol-0.5% triethylamine (40:10:50) was used as mobile phase. It was found to be linear over the concentration range of 25 to 2000 ng/ml and extraction recovery was more than 80%. The coefficients of variation (CV) for intraday and interday assay were found to be less than 5%. The limit of quantification (LOQ) was 25 ng/ml. The pH of the acetate buffer was 5.5. The column was heated to 40 °C and wavelength of 250 nm was used. Calibration standards, controls, and samples were processed in batches.

Loose Surface Crystal Study (LSC)

100 milligrams of drug loaded pellets were suspended in 100 ml of methanol. The samples were shaken vigorously for 15 min in a mechanical shaker. The amount of drug leached out from the surface was analyzed by HPLC-UV method.

In Vitro Drug Release Studies

USP XXI dissolution apparatus, type II was employed to study the percentage of drug release from various formulations prepared. Accurately weighed quantities of drug loaded pellets – 25 mg equivalent to a commercial preparation – Syclop® 25 mg tablet, of each batch were taken in 900 ml dissolution medium and drug release was studied (2 hour in pH 1.2, hydrochloric acid buffer and 23 hour in pH 7.2, phosphate buffer) at 100 rpm and at a temperature of 37° ± 0.5 °C. 10 ml of samples were withdrawn periodically using guarded sample collectors at regular intervals (30 min for first 4 h and at 60 min intervals for the next 8 hour), the sample (10 ml) was withdrawn and replaced with same volume of fresh medium. The withdrawn sample was filtered through a 0.45 μ m membrane filter and after appropriate dilution estimated for clozapine concentration by HPLC – UV.

To study the drug release from polymeric mixtures (HPC, GPS & MCC), the release data in dissolution media were fitted to their well known exponential equation²⁵ (Korsmyer – Peppas equation), which is often used to describe the drug release behavior from polymeric systems.

$$M_t / M_f = k t^n \quad (7)$$

where, M_t / M_f is the drug released fraction at time t , k is a constant incorporating the structural and geometric characteristics of the matrix pellets, n is the release exponent, indicative of the drug release mechanism. In case of Fickian release (diffusion controlled-release), the n has the limiting values of 0.45 for release from sperricle particles. A differential factor (f_1) and similarity factor (f_2) were calculated from dissolution data according to the following equations;

$$f_1 = \frac{\sum_{t=1}^n (R_t - T_t)^2}{\sum_{t=1}^n R_t} \times 100 \quad (8)$$

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (9)$$

where, f_1 - differential factor, f_2 - similarity factor, n - number of time point, R_t - dissolution value of the reference at time, 't' and T_t - dissolution value of test formulation at time 't'. Differential factor, f_1 was calculated by the percentage difference between the two curves at each time point and measured the relative error between the two curves. The acceptable range for differential factor, f_1 is 0 to 15. The similarity factor, f_2 was logarithmic reciprocal square root transformation of the sum-squared error and is a measure of the similarity in the percentage dissolution between the reference and test products. If dissolution profile to be considered similar, the values for f_2 should be in the range 50 to 100.

Stability Studies of Pellets

Optimized drug contain pellets were kept for the accelerated stability studies. Accurately weighed drug contain pellets equivalent to 25 mg of clozapine were filled into a hard gelatin capsules manually. Study was performed at 40 ± 2 °C and 75 ± 5% relative humidity (RH) for up to 90 days (Thermolab, India). A visual

inspection and drug content estimation was conducted every 15 days for the entire period of stability study. Drug content was estimated by HPLC – UV.

Table 1: Optimization of process parameters for pelletization

Formulation & Parameters	Parameters	Description
A1 [clozapine:HPC:GPS:MCC w/w%]	15 : 26 : 06 : 53	Cylindrical shape
A2 [clozapine:HPC:GPS:MCC w/w%]	15 : 24 : 05 : 56	Rod shape
A3 [clozapine:HPC:GPS:MCC w/w%]	15 : 22 : 04 : 59	Egg shape
A4 [clozapine:HPC:GPS:MCC w/w%]	15 : 22 : 03 : 60	Semi spherical
A5 [clozapine:HPC:GPS:MCC w/w%]	15 : 18 : 02 : 65	Spherical
A5 & Spheronization speed (rpm)	410	Cylindrical shape
	480	Rod shape
	540	Egg shape
	610	Semi spherical
	680	Spherical
A5 & Spheronization time (min)	3	Cylindrical shape
	5	Rod shape
	7	Egg shape
	9	Semi spherical
	12	Spherical
Yield (%) A1 A2 A3 A4 A5	93.23	Cylindrical shape
	93.33	Rod shape
	94.12	Egg shape
	95.76	Semi spherical
	96.65	Spherical

Table 2: Yield, size distribution, micromeritic properties and friability of pellets

Formulation	Average size ^a (µm)	Angle of repose ^a θ ⁰	Tapped density ^a (g/cm ³)	Granule density ^a (g/cm ³)	Carr's index ^a (%)	Hausner ratio ^a (%)	Friability ^a (%)
F1	1018	24.11	0.793	1.011	7.34	1.009	0.26
F2	1027	25.02	0.832	1.049	8.61	1.075	0.31
F3	1039	25.12	0.817	1.032	8.49	1.078	0.39
F4	1048	26.43	0.808	1.027	8.48	1.045	0.41
F5	1065	26.34	0.846	1.054	8.65	1.089	0.48

^amean±standard deviation, n = 3

Table 3: Drug loading properties of matrix pellets

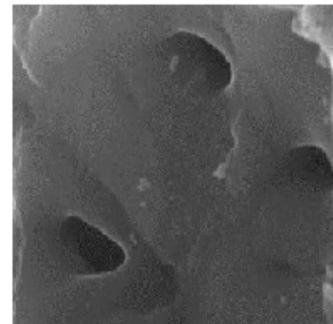
Formulation	Drug loading (mg) ^a	Encapsulation efficiency (%) ^a
F1	16.78	95.34
F2	16.95	96.52
F3	17.39	97.32
F4	17.89	97.78
F5	18.56	98.12

^amean±standard deviation, n = 3

Table 4: Analytical results of stability studies of pellets (A5)

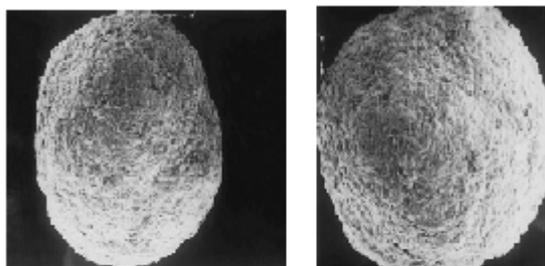
Sampling time (days)	Drug content ^a (%)
00	98.12
15	98.09
45	97.98
90	97.86

^amean ± standard deviation, n = 3



Equivalent Diameter 278 µm
Maximum Inscribed diameter 47 µm

Figure 2: Nano CT – scan showing internal pore structure of a porous pellet (A5)



20.0 kV x 300 lmm 20.0 kV x 300 lmm

1(a)

1(b)

Figure 1: SEM photomicrographs of; (a) clozapine loaded pellets in spherical shape (A5), (b) OZ loaded pellets showing surface pores (A5)

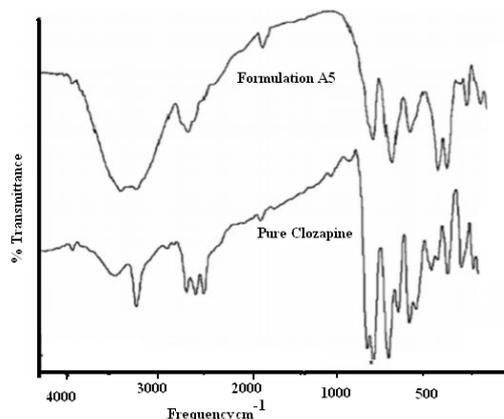


Figure 3: FTIR spectra of Pure clozapine and clozapine loaded matrix pellet (a5)

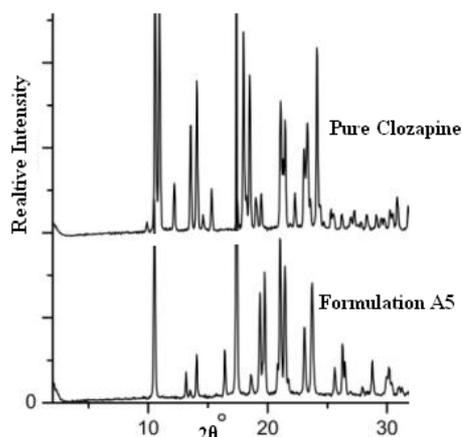


Figure 4: X-ray powder diffraction patterns of pure clozapine and clozapine loaded matrix pellet (a5)

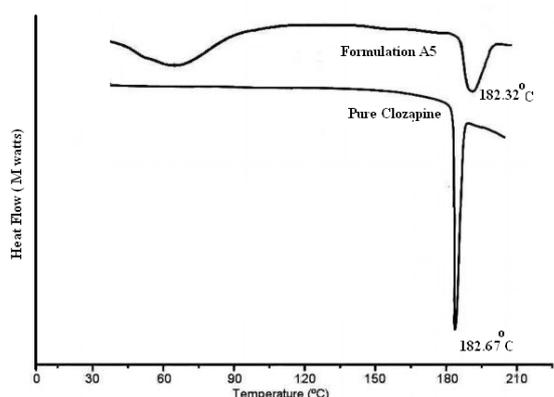


Figure 5: DSC thermograms of pure clozapine and clozapine loaded matrix pellet (a5)

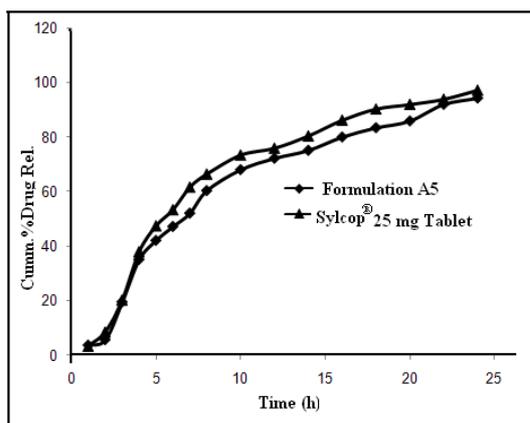


Figure 6: Cumulative % release of olanzapine from matrix pellet (A5) and Syclop® 25 mg tablet in the intestinal environment against time. Formulation A5 (—◆—) and Syclop® 25 mg tablet (—▲—)

RESULTS AND DISCUSSION

In the present study, blend of hydroxypropyl cellulose / glyceryl palmito stearate (hydrophillic and lipidic materials) formulated as pellets by different ratio, (Table 1) have the physical properties and behavior suitability to prepare matrix pellets. None of them succeeded to formulate pellets by blend of hydroxypropyl cellulose / glyceryl palmito stearate by extrusion spheronisation. The present study examines the influences of various process parameters on physicochemical properties and drug release potential from pellets.

Incorporation of drug into different ratios of hydroxypropyl cellulose blend affects the physical appearance of the pellets. In the present study the formulation A5 having the optimum drug and blend ratio (15: 18: 02: 65) suitable to produce solid, discrete, spherical, free flowing pellets and having a sufficient mechanical strength. Resultant pellets did not have any surface irregularities and they are non aggregated. It was found that the higher ratio of drug used (20, 30 and 45 % w/w) in hydroxypropyl cellulose blend, produced aggregate pellets masses which were unsuitable for pharmaceutical uses. SEM photographs also indicated the presence of the drug crystals on the surface of the pellets. Because of surface accumulation drug exhibited burst release which was impossible to control during dissolution.

In the present study, optimized ratio 18 % w/w of hydroxypropyl cellulose was used to produce spherical pellets. It was found that higher ratio of hydroxypropyl cellulose (> 18 % w/w) or decreased ratio of HPC (< 18 % w/w) was used, the produced pellets were not spherical and not easy to distinguish as individual pellets. In order to avoid the formation of irregular shaped pellets, an optimum of 18 % w/w ratio was used to prepare pellets. To obtain optimal ratio of glyceryl palmito stearate, ranging from 2 to 6 % w/w of the total formulations were investigated. In the present study, optimum concentration of 2 % w/w of glyceryl palmito stearate was used to produce better pellets.

In order to obtain optimal ratio of pore forming agent, various ratios of aqueous solution SLS ranging from 0.05 to 0.3 % w/w of the total formulations were investigated. But 0.05 to 0.9 % w/w of SLS failed to produce required pores in the pellets. In the present study, optimum ratio, 0.1 % w/w of SLS was used as pore forming agent and also wetting agent in the pellets formulation and resultant pellets contained sufficient numbers of pores. Incorporation of hydroxypropyl cellulose (hydrophillic) into glyceryl palmito stearate (lipophilic) polymer requires the addition of wetting agent at an optimum concentration of 0.1 % w/w of SLS to reduce the interfacial tension between HPC and GPS. An attempt was made to prepare wet mass without the addition of wetting agent. But the process was failed and as it resulted, in an aggregate cake like mass during the pelletization, which may due to repulsion resulting between glyceryl palmito stearate and methyl crystalline cellulose

It was found that hydrophilic and lipophilic balance (HLB) value of SLS is 40, and was found to be more suitable to increase substantial dispersion of drug in HPC/GPS blend. It was also noticed that 0.1 % w/w of SLS was used as wetting agent, produced pellets which were spherical, free flowing and free from surface irregularities. As the ratio of SLS was more than 0.1 % w/w, resultant pellets were sticky, aggregate, and impossible to produce spherical shaped pellets. As the ratio of SLS used was less than 0.1 % w/w, it required more pressure for pelletization and was difficult to separate as an individual pellets. Hence, the changes in ratio of SLS as wetting agent & pore forming agent, affects the the sphericity of the pellets.

The important factor that influences the size distribution of pellets was the spheronization speed and residence time. A spheronization speed of 680 rpm and residence time 12 min was used to obtain reproducible and uniform sized pellets. As increase in spheronization speed from 410 to 680 rpm, a change in the shape and size of the pellets were noticed. When the spheronization speed was 410, 480, 540 & 610 rpm, cylindrical, rod, egg and semi spherical shaped pellets were produced, respectively. Increased spheronization speed from 410 to 601 rpm, a reduction in the average sizes and recovery yield of the pellets was observed. Spheronization speed was lower than 410 rpm, produced larger and irregular shaped pellets were not suitable for pharmaceutical purpose. It was found that optimized spheronization speed 400 rpm was suitable to produce discrete, spherical, hard and free flowing solid pellets. Spheronization time also affects on the pellet shape and size (Table 1).

It was also observed that an increase in spheronization residence time from 3 to 12 min (at a stirring speed of 401- 610 rpm) resulted in changes in the shape and size of the pellets. From the study, optimized spheronization time was found to be 12 min, suitable to produce spherical, free flowing solid pellets having sufficient mechanical strength. However, further increases in spheronization time considerably affect the pellet shape and size. Hence, to produce required shape and sizes of the pellets, optimum spheronization speed 680 rpm, and spheronization residence time (12 min) was used & data are summarized in Table 1.

Sieve analysis data (Table 2) indicates that the prepared pellets were in the size range of 1018 to 1065 μm . In the present study, MCC posses a good extrusion aid at optimal concentration (65 % w/w) influence the diameter of the pellets. Due to good binding properties of MCC, it provides cohesiveness to a wetted mass, able to retain a large quantity of binding agent helps to provide large surface area and high internal porosity. Hence the optimal ratio 65 % w/w of MCC improved the plasticity of wetted mass and enhancing spheronisation by preventing phase separation, during extrusion spheronisation²⁶.

Generally multi particulate drug delivery systems are formulated as single dosage form (in the form of capsule or tablet), such systems posses better and adequate micromeritic properties (Table 2). The values of angle of repose (θ^0) for the pellet were in the range 24.11 – 26.34 indicating good flow potential for the pellets. The measured tapped density (0.793 to 0.846 g / cm^3), granule density (1.011 to 1.054 g/ cm^3), % Carr's index (7.34 to 8.65%) and Hausner ratio (1.009 to 1.089), were well within the limits, which indicates good flow potential for the prepared pellets.

The friability of the clozapine pellet formulations was found to be in the range 0.26 - 0.48% and it falls in the expected range (less than 5% as per FDA specification). Friability is measured to assess the mechanical strength of the pellets in terms of fragmenting or powdering during filling operation into capsule shell. It was observed that as the ratio of MCC, GPS & HPC decreases, friability of the pellets was increased (Table 2). Additionally, pellets cured at 40° C for 24 hour produces pellets with good mechanical strength, due to sufficient moisture content.

As the curing temperature increases (45° C for 24 hour), friability of the pellets found to decreases, due to drop in residual moisture content from pellets. When the pellets cured below 40° C for 24 hour, produced pellets were dumbbell shaped with protruding surfaces (confirmed from SEM photomicrographs) and these pellets not suitable for pharmaceutical purpose.

SEM photomicrographs (Figure 1 a), showed that the pellets (formulation A5) were spherical in nature and had a smooth surface when they cured at 24 hour at 40 °C. SEM photomicrographs of the pellets reveal the uniform distribution of the drug in the pellets. Figure 2 (b) shows the presence of pores on the surface of the pellets (A5). Pellets cured at 24 hour for 45°C, surface inward dents and shrinkage were observed, it might be due to drop in residual moisture content from pellets. Drug crystals were observed on the surface, because of migration of drug crystal along with moisture from inner surface of the pellet to outer surface during drying. The removal of residual moisture content from pellets during curing exerts an influence on the morphology of the final product²⁷.

From the photomicrograph image analysis, calculated Aspect ratio (AR) and two-dimensional shape factor (*eR*) was found to be 1.08 and 0.75, respectively and Feret diameter (FD) was 845 μm . The obtained AR and *eR* values of the pellets nearer to the value 1, confirmed that the prepared pellets were spherical in nature and 94 % of the pellets were in the range of 1018 to 1065. Interestingly, pellets cured for 24 hour at 40 °C the sphericity values of the pellets nearer to the value 1, whereas pellets cured for 24 hour at 45 °C, obtained sphericity values ranged between 1.03 - 1.09.

Nano CT – scanning of clozapine loaded matrix pellets (A5) containig pore structure presented in Figure 2. The porosity and median pore diameter of the porous pellets was found to be 40.12 \pm 0.2% and 0.5 \pm 0.2 μm , respectively. The equivalent diameter (278 μm) of the detected one pore structure of a pellet was found to more than the maximum inscribed diameter (47 μm).

From the FTIR studies (Figure 3), the characteristic bands for important functional group of pure clozapine and clozapine loaded pellets were identified. Peaks in IR spectra at 3293 cm^{-1} due to NH stretching, 2968 cm^{-1} due to aliphatic C-H stretching, 1462 cm^{-1} aromatic C = C stretching, 1551 cm^{-1} C = N stretching and 820 cm^{-1} due to C – Cl stretching. FTIR spectra showed that the characteristics bands of clozapine were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and carriers used. A comparison and interpretation of this region in our spectra agrees with their conclusions²⁸.

X-RD pattern of pure clozapine showed showed a characteristic peaks at $2\theta^0 = 10.52, 17.39, 19.36, 19.73, 21.05, 21.44, 23.09$ and 23.72 and OZ loaded matrix pellets (A5) showed characteristic peaks at $2\theta^0 = 10.92, 17.97, 18.51, 19.85, 20.95, 21.83, 23.49$ and 23.74 as presented in Fig.4. X-ray diffraction patterns revealed the crystalline nature of pure clozapine. The X-ray diffractogram of clozapine showed number of sharp and

intense peaks. The diffractogram of clozapine loaded matrix pellets (A5) showed broad peaks with low intensity. This may be attributed to the incorporation of clozapine between parts of the crystal lattice of the HPC, leading to a change in the degree of crystallinity of the clozapine²⁹.

DSC studies were performed on pure clozapine and clozapine loaded matrix pellets (A5). clozapine exhibits a sharp endothermic peak at 182.67° C presented in Figure 5. It was observed that presence of an endothermic peak of the drug at 182.32° C in the clozapine loaded matrix pellets (A5) indicates, that the drug is uniformly distributed in the pellets. The peak intensity corresponding to the melting of clozapine decreased in the thermograms of clozapine loaded pellets. These results indicate that only a small fraction of the drug substance existed in the crystalline state. Reduction in the melting point and enthalpy of the melting endotherm was observed. Incorporation of clozapine in the carrier results in decrease in the melting point in the formulation (A5). Small sized matrix pellets leads to high surface energy, which creates an energetically suboptimal state causing a decrease in the melting point²⁸.

Drug loading and drug encapsulation efficiency of the drug loaded pellets are given in the Table 3. Drug content in all the formulations were in the range of 16.78 to 18.56 % w/w. Drug content was least in formulation A1 (16.78 % w/w) and high for formulation A5 (18.56 % w/w). Drug encapsulation efficiency was found to be more in formulation A5 (98.12 % w/w) and less in formulation A1 (95.34% w/w). It was observed that drug content and drug encapsulation efficiency increases with increased pellets sizes (1024 to 1212 µm). This might be due to increased relative surface area of the pellets, leads to more drug content and drug encapsulation efficiency.

Loose surface crystal (LSC) study is an important parameter giving indications of the amount of drug on the surface of the pellets. Physical state of clozapine in all formulations with different drug loading was investigated by polarized light microscopy. The microscopic study indicated that crystalline clozapine was observed in all formulation and more clear in formulation A5 (Drug content was 18.56 % w/w) .

Drug release from clozapine loaded pellets in a biphasic manner, consisting of initial fast release followed by a slow release. Surface accumulated drug showed fast releases and rapid penetration of dissolution media from the matrix structure. More amount of clozapine released from formulation A5 (97.12 %) and Syclop® 25 mg tablet (98.34 %) as compared to all other formulations, A1 (86.43 %), A2 (87.98 %), A3 (89.27%) & A4 (91.78 %). This result clearly indicates that lowered drug release was noticed for the systems containing higher content of HPC. Because HPC particles are high water swellable forms leads to higher viscosity, retards the penetration of dissolution media into pellets, thus limiting the drug release from pellets. This typical behavior was commonly observed in diffusion controlled drug delivery systems³⁰.

Interestingly drug release profile obtained for formulation A5 indicated that it is an ideal formulation for administration for every 24 hour, as it released 97 % of the embedded drug in 24 hour. In this investigation,

author made an attempt to prepare the pellets with lower levels of HPC pellets exhibited initial burst release of drug. However, the formulations exhibited little burst effect at higher levels of HPC. Further increased HPC amount, formed thicker gel around the pellets, strongly inhibiting the dissolution media penetration, resulting in significant reduction in the drug release. This finding indicated a considerable release retarding potential of the drug from pellets by varying ratios of HPC / GPS / MCC and pore former.

The effect of curing of pellets at different temperature clozapine release from HPC / GPS/ MCC pellets was studied. Interestingly pellets cured at 40 °C for 24 hour showed controlled drug release. Drug release upon curing at 40 °C (24 hour) might be due to residual moisture content present in the pellets. This result indicates that the moisture present in the pellets reduces the cohesive force, which facilitates the wetting of pellets and increased the pellets disintegration (confirmed visually). Pellets cured above 45 °C for 24 hour, showed the least drug release, due to least amount of residual moisture content present in the pellets responsible for low wettability. Drug contain pellets are softened and produced a denser structure, less permeable for dissolution media, delayed the disintegration of pellets (confirmed by visual observation).

The rate of drug release followed first order kinetics and numerical data fitted into Peppas' equation. Statistically estimated values of n of drug from pellets at 95 % confidence limit, is in the range 0.36 to 0.42 for formulation A1 to A5 studied and 0.41 for Syclop® 25 mg tablet, indicated that the drug release from the formulations A1 to A5 and Syclop® 25 mg tablet was Fickian diffusion. In our experiments the result of 'n' clearly indicates that the diffusion is the dominant mechanism of drug release from these formulations. Diffusion is helps to transport the drug from dosage matrix into the *invitro* study fluid depending on the concentrations of the HPC. As gradient varies, the drug is released, and the distance for diffusion increases. From this, it was noticed that drug diffuses at a slower rate as the distance for diffusion increases. This is a good agreement with literature findings. The obtained correlation coefficient, R² for the clozapine loaded pellets lies in the range of 0.986 – 0.998. The same result was noticed for Syclop® 25 mg tablet (0.999).

The drug release profiles of the optimized formulation A5 was compared with oral formulation Syclop® 25 mg tablet. The plot of the cumulative percent drug release as a function of time for formulation A5 and Syclop® 25 mg tablet is shown in Fig. 6. From the figure, it is evident that the drug release was controlled from formulation A5 pellets than the commercially available product Syclop® 25 mg tablet.

Differential factor (f_1) and similarity (f_2) factor was calculated from dissolution profile and the results were compared to the formulation, A5 and Syclop® 25 mg tablet. The differential factor (f_1) and similarity factor (f_2) obtained from dissolution profile indicates that the formulation A5 (9.97, 10.56) and Syclop® 25 mg tablet (76.12, 78.54) were similar.

The optimized formulation A5 was subjected for accelerated stability studies. Stability studies were carried out $40^{\circ} \pm 1^{\circ} \text{C}$ and $75\% \pm 5\%$ relative humidity for a period of 90 days (Table 4). It was observed that, no significant change in the drug content from the pellets was observed. It is evident from the table that, formulations A5 exhibited good stability during investigation period, which indicates the drug was in stable form.

CONCLUSION

The objective of the study was to prepare and evaluate clozapine loaded pellets by extrusion/ spheronization for controlled release. The method employed was simple, rapid and economical and does not imply the use of toxic solvents. Pellets containing a pore forming agent, aqueous SLS, which forms micropores on the surface of the pellets. The results of micromeritic properties, hausner ratio and friability of the pellets were well within the limits, which indicates good flow potential for the prepared pellets. Drug loaded pellets exhibited spherical in nature as evidenced by SEM photomicrographs and sphericity studies. From the FTIR and DSC studies, it was observed that there was no chemical interaction between the drug and polymers used indicating that drug was in stable form. X-ray diffraction patterns revealed the crystalline nature of pure clozapine. The drug content study revealed uniform distribution of the drug in the pellets. The drug release rate was found vary among the formulations depending on the compositions of polymers used. The obtained dissolution data indicated that the drug release through the microporous polymeric membrane follows fickian diffusion. Formulation A5 is an ideal formulation for once daily administration. From the present work, it can be concluded that the prepared matrix pellets demonstrate the potential use of hydroxypropyl cellulose/ glyceryl palmito stearate/ methyl crystalline cellulose blend for the development of controlled drug delivery systems for many water insoluble drugs.

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