PRONIOSOMAL CLOZAPINE TABLET: FORMULATION, EVALUATION OF PRONIOSOMAL TABLET AND RELEASE STUDY OF TABLETS

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Abstract:
Novel drug delivery system have emerged various route of administration, among all the drug delivery system oral drug delivery system is most favourable drug delivery system. A lipid base drug delivery system is one of positive approach for Poorly water soluble drugs. Based on Lipid vesicular system like liposomes, proniosomes and niosomes have been developed. Aim of present study to developed Clozapine based proniosomes to enhance solubility and bioavaibility of the drug. Clozapine loaded Proniosomes prepared by the slurry method using different ration of Cholesterol: span 60 and Mannitol as a carrier. Respectively were continuously compressed into tablet using direct compression method. Proniosomes was evaluating for Particles size, Micromertic properties, entrapment efficiency, and dissolution. Proniosomal tablets showed improve dissolution characteristics over the plain tablets which were improve dissolution behaviour. The Transformation of crystalline form to the amorphous was represented by the solid state characteristics.

KeyWords: Clozapine, Proniosomes, Tablets, Cholesterol, slurry method, Mannitol, Dissolution

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INTRODUCTION

Novel Drug delivery system using colloidal particulate carriers such as liposomes of niosomes have distinct advantage over the conventional dosage form because the particles can act as containing reservoir and modification of the particle composition of surface can adjust the drug release rate on targeted site[1].Niosmoes demonstrated that the alternative to liposomes, they are chemically stable and cost effective as compare to liposomes. Aggregation, fusion leakage of included drugs, or hydrolysis of encapsulated drugs can lead to a shorter shelf life of aqueous niosomal dispersions, similar to liposomes. To address the stability issues associated with niosomes, proniosomes were developed. Proniosomes are a granular product that is dried, free-flowing, and can be conveniently administered orally after reconstitution with water. Unlike conventional niosomes, the reconstituted niosomes formed from proniosomes have a uniform size.

Various nonionic surfactants with different hydrophilic-lipophilic balance (HLB) values were utilized in the preparation of proniosomes, including polyoxyethylene sorbitan esters (Tweens), polyoxyethylene alkyl ethers (Brijs), and sorbitan esters (spans). The HLB range of spans, which is from 1 to 8, was found to be the optimal range for achieving smaller particle size and higher entrapment efficiency of the proniosomes formulations. The incorporation of cholesterol in the hydration of proniosomes enhances the rigidity of niosomes and reduces drug leakage, resulting in improved entrapment efficiency. Moreover, cholesterol exerts a steric effect by stabilizing the niosomal structure, thereby inhibiting aggregate formation[2].

[3]Mental and behavioural disorders are brain disorders that are chronic, severe, and disabling, and affect individuals across their lifespan. In India, it is estimated that 6-7% of the population suffers from common mental disorders, while 1-2% have severe mental disorders. The economic burden associated with schizophrenia is particularly significant, with treatment costs totaling approximately $63 billion per year.

Clozapine is an atypical antipsychotic drug that is used as a prototype treatment for schizophrenia patients who are unresponsive or intolerant to typical antipsychotics. Clozapine exerts its therapeutic effect through a combination of antagonistic effects on D2 receptors in the mesolimbic pathway and 5-HT2A receptors in the frontal cortex. The D2 receptor antagonism provides relief from positive symptoms, whereas 5-HT2A receptor antagonism alleviates negative symptoms. Additionally, clozapine has been found to be more effective in treating resistant schizophrenia and reduces suicidal behavior in patients, which is a common adverse effect of older antipsychotics. Clozapine is an atypical antipsychotic agent with a dibenzodiazepine structure that is indicated for the management of severely ill schizophrenic patients who are refractory or intolerant to standard treatments Although it is considered the gold standard for treating schizophrenia, noncompliance with treatment is a significant issue during clozapine administration, particularly in patients with severe symptoms and possibly poor insight. Additionally, abrupt discontinuation of clozapine may result in a rapid exacerbation of psychosis, as reported in previous studies [4].

The aim of present study to improve therapeutic efficacy of antipsychotic drug through proniosomal encapsulation. Clozapine is synthetic drug commonly used in treatment of schizophrenia it having poor bioavailability due to first pass metabolism, it is difficult to cross Blood brain barrier due to having poor aqueous solubility, to develop Proniosomes for Clozapine oral tablet for better drug release as compare to conventional tablets . Proniosomes prepared by slurry method using Mannitol, Span 60 and cholesterol as Carrier, Surfactant and membrane stabilizer respectively to enhance Aqueous solubility of Clozapine.

MATERIAL AND METHOD

Material for Proniosomes:

Clozapine was procured form CTX Lifescience, Mannitol from Roquette ferres and Cholesterol obtained from Loba chemie , Span 60, Methanol obtained from SD fie chem. Mumbai India. Spray dried Anhydrous Lactose from DFE Pharma and Magnesium stearate gifted as free sample form Peter grvens.

Preparation of Proniosomes

Clozapine Proniosomes were prepared by slurry method using Mannitol as a carrier. Weigh required amount of lipid mixture contains Cholesterol and span 60 were dissolved in 20 ml methanol: Chloroform (1:1), further add Clozapine above mixture. The solution was transferred in round bottom flask and Mannitol added to form slurry. The flask was rotated at 60-70 rpm by using roatatory evaporator to evaporate organic solvent at a temperature 45 °C± 2°C and reduce pressure to 600 mm Hg. A resultant powder further dried in a vacuum oven overnight at room temperature to obtain free flowing powder. Stored Proniosomal powder at 4°C in tightly closed container.

Preparation of Niosomes from Proniosomes

The niosomal suspensions were prepared by hydrating the proniosomal powder using 25 mL of phosphate buffered solution (PBS) with a pH of 7.4 at a temperature of 80°C ± 1°C. The hydration was carried out using a Probe sonication for a duration of 5 minutes. The resulting niosomal dispersion was used for the determination of entrapment efficiency and morphological study**.**

Table 1: Composition of clozapine Proniosomal formula

Batch No	Drug (mg)	Span 60 (mg)	Cholesterol (mg)	Mannitol (mg)	Solvent Methanol : Chloroform (ml)
PNC 1	25	40.0	36.6	500	20
PNC 2	25	36.0	36.6	500	20
PNC 3	25	60.0	36.6	500	20
PNC 4	25	64.5	38.6	500	20
PNC 5	25	36.0	36.6	500	20
PNC 6	25	73.2	30.9	500	20
PNC 7	25	36.0	40.0	250	20
PNC 8	25	36.0	36.6	250	20
PNC 9	25	36.0	20.0	250	20
PNC 10	25	20.0	20.0	1000	20
PNC 11	25	20.0	36.6	1000	20
PNC 12	25	20.0	36.6	1000	20

Entrapment efficiency of clozapine Proniosomes derived niosomes

Entrapment efficacy of clozapine loaded niosomes can be performed by Freez throing/centrifugation method. Frozen samples niosomes prepared from proniosomes were let stable at room temperature. The niosomal sample were further centrifuged at 14,000 RPM for 40 min at 4ºC. Niosmal deposits were isolated and reconstituted with Phosphate buffer pH 7.4 and centrifuge again to wash out untrapped free drug. The supernatants where collected and analysed assay of free drug using UV spectrophotometrically at 292 nm using Phosphate buffer pH 7.4 as blank. The entrapment efficacy was designated as the proportionate percentage of the concentration of the drug that was captured within the confinement of the niosomes in relation to the total amount of drug concentration. The calculation was performed by means of the subsequent equation:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount of Drug entrapped X100}}{\text{Total amount of drug}}$$

Particle size and Particle sized distribution:

The mean particle size distribution was performed using Malvern instrument. The sample was analysed at 25ºC, utilizing a 45 mm centre focal point and bar length 1.05 mm. Each examination was resolved in triplicate.

DSC Thermogram of Clozapine

Differential scanning colorimetry (DSC) of pure drug Clozapine was analysis using DSC instrument. Clozapine was sealed in standard DSC aluminium pans, crimped it and then scanned over a temperature from 50°C to 300 °C at heating rate 10 °C/min.

Scanning Electron Microscope

Vesicle shape analysis out by Scanning Electron Microscope. Clean brass specimen studs were used for taking the sample. Wet solvent point was added on these studs and while the paint was wet the proniosomes powder was put on each stude and allowed to dry. Then photomicrographs were selected

Micromeritic study: The proniosomal powder blend was evaluated by measuring the subsequent parameters: Bulk density, tapped density carrs index and Hausners ratio.

Bulk Density: Bulk density (V_D) was determined by pouring determined weight (W) of the powder into graduated cylinder for bulk volume (V) determination. Bulk density was calculated using following equation

$V_D = W/V$

Where W = weight of powder

V= Bulk Volume of powder

Tapped Density

The cylinder contain Predetermined powder weight of powder mix (W) was tapped for specific no of time in USP Bulk density apparatus. The minimum tapped volume (t) of cylinder was measured. Tapped density (V_T) was calculated using following equation.

$(V_T) = W/T$

Where W = weight of powder

T= Tapped Volume of powder

Carr's Index

The Carrs index is as display of the comprasibility of a powder. The higher the carr's index is more compressible powder. Carr's index measure with following equation

$$\text{Carr's Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \quad X\ 100$$

Hauser Ratio

Hausner ratio is an indirect estimate of flowability of a powder. It is the proportion between tapped density and bulk density

Hauser Ratio: $\frac{\text{Tapped Density}}{\text{Bulk Density}}$

Proniosomal tablets Manufacturing

Proniosomal tablets Prepared from Proniosomal powder of clozapine according to the to the following formula used to prepare 100 tablets each uncoated tablet contain 25 mg/tab clozapine and average weight of each tablets 360 mg using direct compassion method were Mixture of Proniosomal powder and Lactose anhydrous mixed for 15 min in octagonal blender and Magnesium stearate for 5 min. Blend was compressed in double rotatory tablet punching machine equipped with 9.5 mm Flat Faced Punch.

Proniosomal Powder: 34.76 gm
Spray dried Anhydrous Lactose: 1.20 gm
Magnesium Stearate: 0.04 gm

EVALUATION OF TABLETS

Weight Variation

A sample of 20 tablets was taken from each prepared proniosomal formula in a random manner. The tablets were weighed to determine their average weight. Each tablet was then individually weighed and compared to the calculated average weight. The mean value and standard deviation (SD) were calculated based on the collected data.

Thickness and Diameters

To measure the thickness and diameter of the tablets, a digital Mitutoyo caliper from Japan was used. This was performed on ten tablets from each batch, and the measurements were expressed in millimeters. The mean value and standard deviation (SD) were then calculated based on the measured values.

Hardness:

The hardness of the tablets was determined using a Campbell tablet hardness tester from India, and the values were expressed in units of kilopascal (KP). Ten tablets were randomly selected from each batch for this test, the measured hardness values.

Disintegration time:

For the disintegration test, tablets were immersed in a disintegration apparatus containing Water, and kept at a temperature of 37°C. The time taken for the tablets to disintegrate was recorded. This test was performed on six tablets, the recorded disintegration times.

In Vitro Release of Clozapine Proniosomal Tablets

Tablet Dissolution was conducted through USP Type I (Basket) Dissolution apparatus at revolution speed at 100 rpm.The dissolution medium included 900 ml of pH 4.0 Acetate Buffer kept up at 37 ±0.5˚ C which was maintained till the end of the experiment. Withdrawn 20 ml of sample at predetermined time intervals 0, 5, 10, 15, 20, 30, 45, and 60 min and the volume was repaid the volume by replacing with fresh dissolution medium after each sampling. The sample were filtered from 0.45 µm Nylon syringe filter and analysed with UV visible spectrophotometer with UV detector at 290 nm. The trial was done in triplicate and the data of In vitro dissolution were presented as mean ±SD.

RESULT AND DISCUSSION

DSC Thermogram of Clozapine

The DSC thermogram of Clozapine showed a sharp endothermic peak at 197.58 °C which correspond to the melting point of Clozapine which indicate crystalline and purity of drug.

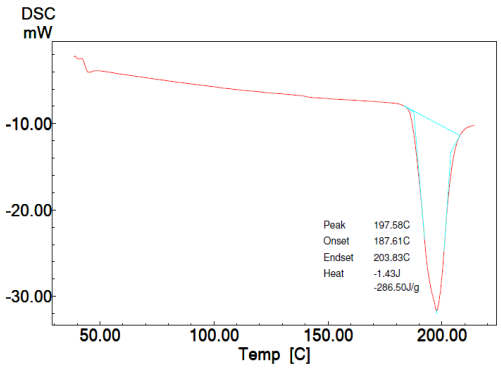

Fig 1: DSC Thermogram of pure drug.

Particle size and Particle sized distribution:

The particle size distribution of the Proniosomal formulation showed symmetrical frequency distribution pattern in fig 2. The mean vescicle size of hydrated Clozapine proniosomes was 410.5 nm. The polydispersity index PDI is an important parameters that describe the vescicle size distribution and it was varies from 0.0 to 1.0.

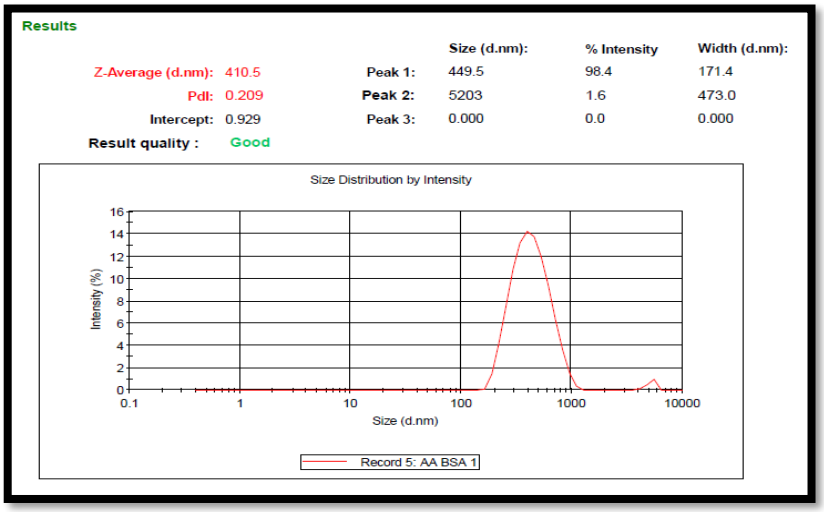

Fig 2: Particle size Distribution of Optimized batch

Entrapment efficiency

The entrapment efficiency of Clozapine proniosomes was in the range of 83.04±1.99 to 90.14±0.30, table 2. High values of encapsulation efficiency may be attributed to the poor water solubility of Clozapine and its dissolving in the lipid bilayers of niosomal vesicles.

F4 showed the highest encapsulation efficiency of 90.14±0.30%. As the ratio of cholesterol to Span 60 increased, the entrapment efficiency increased. That may be explained by the ability of cholesterol to increase the bilayer rigidity by intercalation between the bilayers of non-ionic surfactants. However, very high cholesterol concentration was found to be of a lowering effect on the entrapment efficiency of the drug. This could be due to the fact that cholesterol beyond a certain level has the ability to disrupt the regular bilayered structure leading to decreasing drug entrapment. Moreover, that may be attributed to competition between clopidogrel and cholesterol for packing in the limited sites in the bilayered structure of proniosomal vesicles thus excluding Clozapine and disrupting the well-ordered bilayered structure.

Table 2: Entrapment efficiency of Proniosomal batch

Sr. No	Batch No:	% Entrapment efficiency
1	PNC 1	88.52 ±1.525
2	PNC 2	70.36 ±0.955
3	PNC 3	62.41 ±2.521
4	PNC 4	85.15 ±0.564
5	PNC 5	79.85 ±0.152
6	PNC 6	92.41 ±0.253
7	PNC 7	62.41 ±0.255
8	PNC 8	90.52 ±0.525
9	PNC 9	86.25 ±1.250
10	PNC 10	80.52 ±0.625
11	PNC 11	86.251 ±0.362

Micrometric Properties

The Values Obtained bulk density of the prepared Clozapine Powder blend range from 0.452 gm/ml to 0.562 gm/ml, while the value obtained for the tapped density of the prepared Clozapine Powder blend range 0.645 gm/ml to 0.702 gm/ml.

Table 3: Bulk density, tapped density, Hauser ration and carrs index

Batch No	Bulk Density	Tapped Density	Hauser Ratio	Carr's Index
PNC 1	0.488	0.689	1.411	29.27
PNC 4	0.452	0.645	1.42	29.92
PNC 6	0.551	0.692	1.25	20.37
PNC 8	0.562	0.685	1.21	17.95
PNC 11	0.515	0.702	1.36	26.63

Tablet Physical Evaluation

The Tablets were prepared by direct compression method using 9.5 mm Flat bevelled edges punch.

The Prepared Tablets Further Evaluating for Weight variation, Thickness, Hardness, friability and Disintegration time.

All formulation successfully shows in tables. The Clozapine Proniosomal tablets thickness found between 3.16 mm to 3.24 mm respectively Hardness found to be 8.25 kp to 10.89 kp. In all formulation the friability was less than 1 % w/w.

The average weight of compressed tablets was found between 352 mg to 365 mg and the disintegration time was found to 2.40 min to 4.25 min.

Table 4: Average weight, thickness, diameters, friability, Hardness and disintegration time

B. No	Average Weight (mg)	Thickness (mm)	Diameters (mm)	Friability (%)	Hardness (KP)	Disintegration time (min)
PNC 1	362.5	3.16	9.51	0.04	10.89	4min 12 sec
PNC 4	365.4	3.20	9.50	0.12	8.25	3 min 45 sec
PNC 6	361.5	3.21	9.52	0.05	9.65	2 min 52 sec
PNC 8	358.4	3.24	9.54	0.07	9.45	3 min 10 sec
PNC 11	352.8	3.22	9.50	0.03	10.28	4 min 25 sec

Scanning Electron Microscopy (SEM)

Scanning electron microscopy technique used to evaluate the surface morphology of the pure drug and optimized formulation. the surface Maltodextrin carrier particle was coated with surfactant solution during Proniosomal formation . SEM illustrated smooth surface of the optimized formulation, this indicate homogeneous layer of span 60 on the surface of Maltodextrin during Proniosomes preparation. The porous surface of Maltodextrin particle in the formulation make it effective carrier and provides more surface area which could helpful coating by the surfactant mixture.[11]SEM images shown in figure.

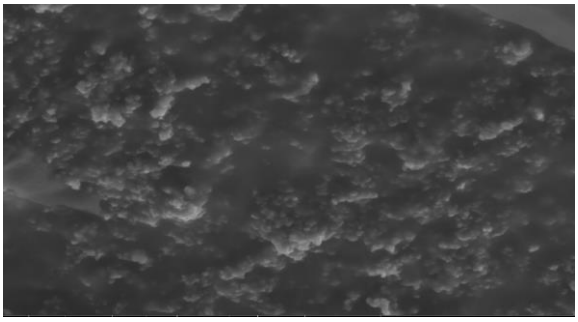

Fig 3: SEM images of Optimized formulation

In Vitro Release of Clozapine Proniosomal Tablets

The release of Clozapine from Proniosoems PNC6 dosage form was evaluated in Different buffer PH 4.0 acetate buffer, pH 1.2, and 6.8 phosphate buffer. The data showed that release of clozapine was faster in 4.0 phosphate Buffer than other Media. The dissolution efficiency of insoluble drug clozapine has been significantly improved when encapsulation in proniosomes. This might be due to enhanced solubility of clozapine by span 60 molecules. However the Proniosomal formulation promoted higher dissolution of clozapine compared to other formulation. The improved dissolution of the drug from proniosomes tablet might be due to the alters physical state of the drug entrapped within the niosomes bilayer and enhanced effective surface area available for dissolution medium. From above results PNC6 Formulation selected as optimized batch. The dissolution profile and cumulative dissolution of clozapine from proniosomes tablets formulations and control in PH 1.2, PH 4.0 and PH 6.8 Dissolution media are shown.

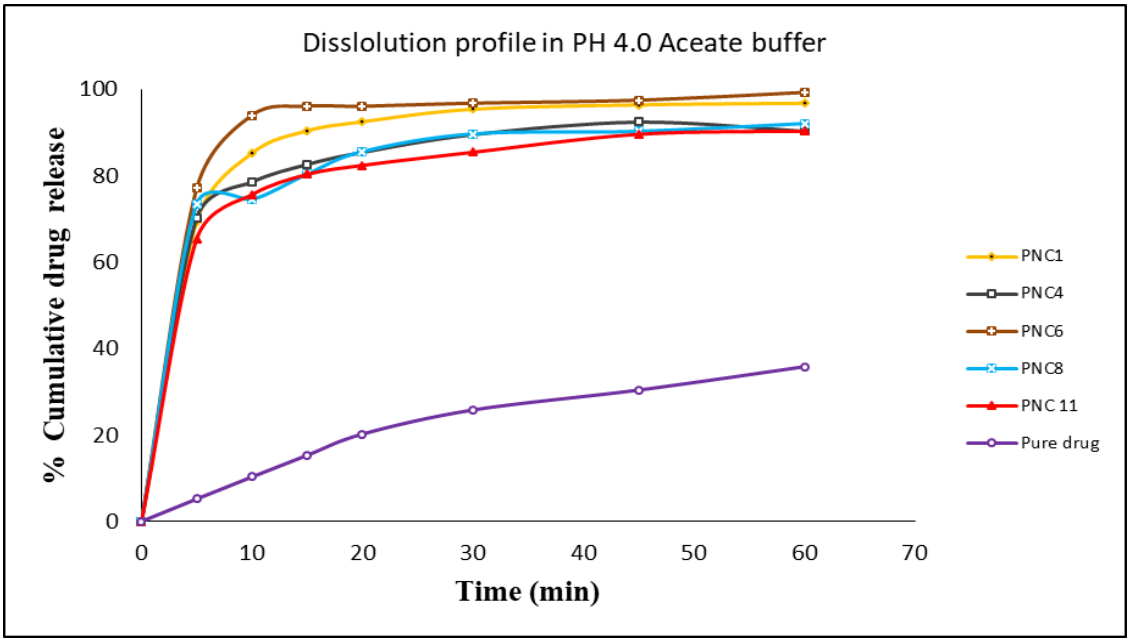

Fig 5: % Cumulative drug release in pH 4.0 Acetate Buffer

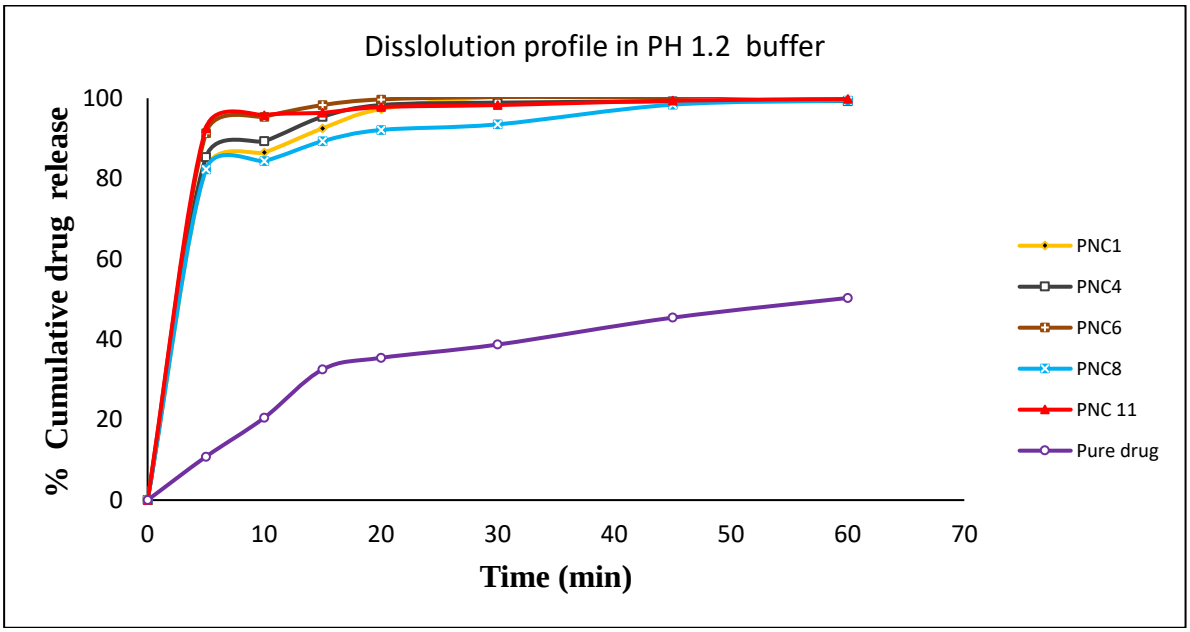

Fig 6: % Cumulative drug release in pH 1.2 Buffer

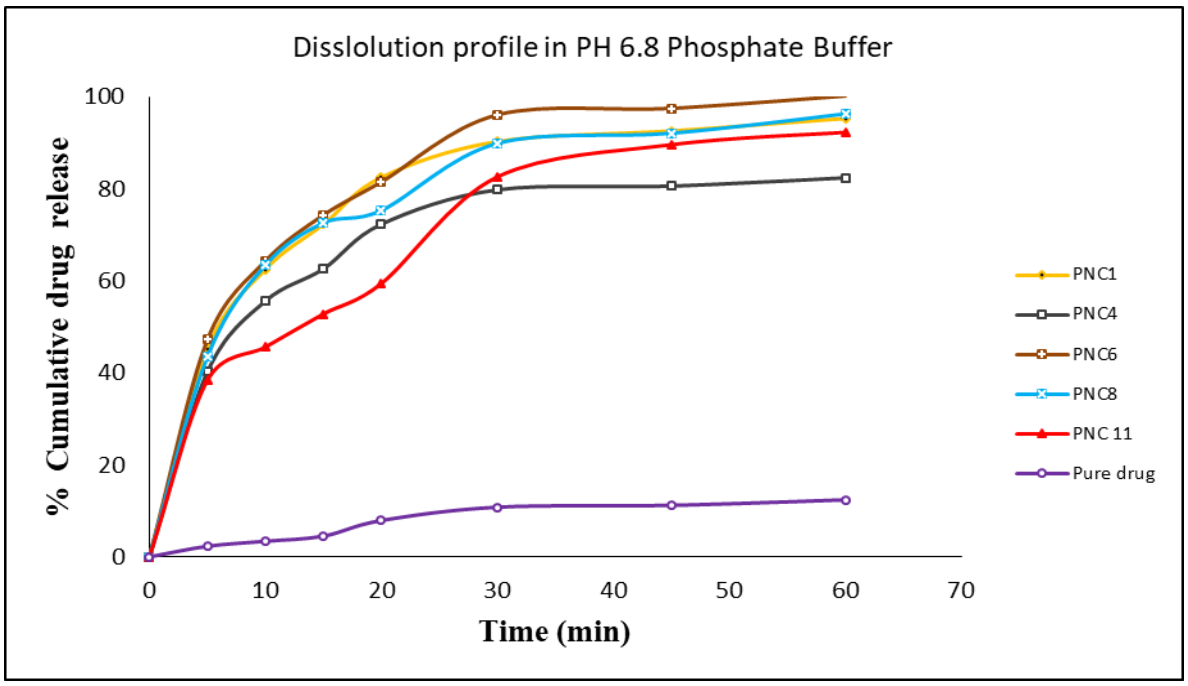

Fig 7: % Cumulative drug release in pH 6.8 Phosphate Buffer

CONCLUSION

In our study, the dry free flowing Proniosomal formulae overcome the physicochemical issue connected with niosomes might have been perfectly prepared for oral route. That Proniosomal powder might have been smoothly transferred into tablets as most favourable dosage form. The proniosomes derived suspension exhibited and effective entrapment efficiency from the homogenous nano-sized vesicle. Thus, the developed proniosomal tablet formulation may help to improve the dissolution rate and may enhance the bioavailability of clozapine.

ABBREVIATIONS

HLB	Hydrophilic–lipophilic balance
ML	Millilitre
MG	Milligram
^{0}C	Degree Celsius
mm	Millimetre
gm	Gram
pH	Potential of Hydrogen
SD	Standard Deviation
KP	Kilopascal
USP	United State Pharmacopeia

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