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FORMULATION AND EVALUATION OF NANOSUSPENSION OF CLOZAPINE BY EMULSIFICATION SOLVENT EVAPORATION METHOD

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Abstract:
Clozapine is an atypical antipsychotic drug primarily used in the management of treatment-resistant schizophrenia. However, its poor aqueous solubility may limit its dissolution and absorption. The present study aimed to formulate and evaluate clozapine nanosuspensions using the emulsion–solvent evaporation method to improve dissolution characteristics and achieve controlled drug release. Twelve formulations were prepared using PVP K30, PVA, and Pluronic F127 as stabilizers, with SLS as surfactant. Preformulation studies including physical appearance, solubility, melting point, FTIR, and UV spectrophotometric analysis were performed to establish the suitability of the drug and excipients. The prepared nanosuspensions were evaluated for drug content, entrapment efficiency, particle characteristics, surface morphology, and in-vitro drug release. Among the prepared formulations, NS12 exhibited satisfactory drug content and entrapment efficiency along with a controlled drug-release profile and was selected as the optimized formulation. Release kinetic analysis indicated that the drug release followed the Higuchi model, while the Korsmeyer–Peppas model indicated anomalous (non-Fickian) release behavior. Overall, the study demonstrated that nanosuspension technology is a promising approach for improving the dissolution characteristics and achieving controlled release of clozapine.

Keywords: *Clozapine, Nanosuspension, Emulsion–solvent evaporation, Pluronic F127, SLS, Higuchi model, Korsmeyer–Peppas model.*

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INTRODUCTION:

Drug delivery systems are becoming increasingly sophisticated as pharmaceutical scientists acquire a better understanding of the physicochemical and biological parameters pertinent to their performance. Despite tremendous advantages in drug delivery, the oral route remains the most preferred route for the administration of therapeutic agents because of the low cost of therapy and ease of administration leads to high-level of patient compliance. On the other hand, this high-throughput screening process has done little to address the issue of poor bioavailability of orally administered drug candidates. During the past two decades, numerous oral delivery systems have been developed to act as drug reservoirs from which the active substance can be released over a defined period of time at a predetermined and controlled rate.[1]

Oral drug delivery system (oral DDS) has been known for decades as the most widely utilized route for drug administration among all the routes that have been explored for the systemic delivery of drugs via various pharmaceutical products of different dosage forms. More than 50% of drug delivery systems available in the market are oral DDS.[2]

Ideal drug delivery systems that possess two main properties:

- It should be a single dose for the whole duration of treatment.
- It should deliver the active drug directly at the site of action.

Such ideal systems are difficult to design. Scientists try to develop systems that can be as close to ideal systems as possible. Attempts to develop a single dose therapy for the whole duration of treatment has focused attention on controlled or sustained

Novel drug delivery system

The design of oral controlled DDS should be primarily aimed to achieve more predictable and increased bioavailability. Now-a-days most of the pharmaceutical scientists are involved in developing the ideal DDS. This ideal system should have advantage of single dose for the whole duration of treatment and it should deliver the active drug directly at the specific site. Controlled release implies the predictability and reproducibility to control the drug release, drug concentration in target tissue and optimization of the therapeutic effect of a drug by controlling its release in the body with lower and less frequent dose. However, this approach is be filled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the GIT due to variable gastric emptying and motility. Furthermore, the relatively brief GET in humans which normally average 2-3 hrs through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose. Therefore, control of placement of a DDS in a specific region of the GI tract offers advantages for a variety of important drugs characterized by a narrow absorption window in the GIT or drugs with a stability problem.

Many of the new chemical entities (approximately 40% or more) being developed through drug discovery programmers are poorly water soluble. The formulation of poorly water soluble drugs has been always a challenging problem faced by pharmaceutical scientists. The low saturated solubility and dissolution velocity leads to poor bioavailability. The problem is more severe for drugs belonging to BCS class II, such as Itraconazole and carbamazepine as they are poorly soluble in both aqueous and organic media. The performance of these drugs is dissolution-rate-limited and is affected by fed/fasted state of the patient. Dissolution rates of sparingly soluble drugs are associated to the shape as well as the particle size. Hence decrease in particle size results in increase in dissolution rate. There are number of formulation approaches that can be used to solve the problems associated with the low solubility and low bioavailability of class II drugs. Some of the approaches to increase solubility include micronization, solubilisation using cosolvents, use of permeation enhancers, surfactant dispersions, salt formation and precipitation techniques. Most of these techniques for solubility enhancement have advantages as well as some limitations and hence have limited utility in solubility enhancement. Other techniques used for solubility enhancement like microspheres, emulsions, micro emulsions, Liposome's, supercritical processing, solid dispersions and inclusion complexes using Cyclodextrins show reasonable success but they lack in universal applicability to all drugs, which are not soluble in both aqueous and organic media. Nanosuspensions have revealed their potential to undertake the problems associated with delivery of poorly water-soluble and lipid soluble drugs and are unique because of their simplicity and the advantages they confer over other strategies.

Nanotechnology opens up new vistas of research in the development of novel drug delivery systems. "Nano" word comes from the Greek word 'nanos" which means dwarf, Nano means it is the factor of 10^{-9} or one billionth. Nanosuspension is submicron colloidal dispersion of drug particles. A pharmaceutical nanosuspension is defined as very

finely colloid, biphasic, dispersed solid drug particles in an aqueous vehicle, size below 1 µm stabilized by surfactants and polymers prepared by suitable methods for drug delivery applications. Nanosuspension has revealed their potential to solve the problem associated with the delivery of poorly water soluble and poorly water and lipid soluble drugs. It enhances the absorption and bioavailability and help to reduce the dose of conventional oral dosage forms. For a long duration of time micronization of poorly soluble drugs by colloid mills or jet mills was preferred. The overall particle size distribution ranges from 0.1µm to approximately 25µm, only negligible amount being below 1µm in the nanometer range.

When to go for Nanosuspensions Approach

- Preparing nano suspensions is preferred for the compounds that are insoluble in water (but are soluble in oil) with high log P value.
- Conventionally the drugs that are insoluble in water but soluble in oil phase system are formulated in liposome, emulsion systems but these lipidic formulation approaches are not applicable to all drugs.
- In these cases nanosuspensions are preferred. In case of drugs that are insoluble in both water and in organic media instead of using lipidic systems Nanosuspensions are used as a formulation approach.
- Nanosuspension formulation approach is most suitable for the compounds with high log P value, high melting point and high dose.

Depending upon the production technique applied changes in the crystalline structure of the drug particle may occur. An increase amount of amorphous drug fraction could induce higher saturation solubility. Nanosuspension not only solves the problem of poor solubility and poor bioavailability but also alters the pharmacokinetics of the drug and improves the drug safety and efficacy.

Advantages:

- **Improved Solubility and Dissolution Rate**

Reduction of drug particle size to the nanometer scale increases surface area, enhancing solubility and dissolution rate of poorly water-soluble drugs.

- **Enhanced Bioavailability**

Faster dissolution improves drug absorption across biological membranes, leading to increased bioavailability and improved therapeutic performance.

- **Suitable for BCS Class II and IV Drugs**

Ideal for drugs with low solubility and erratic absorption, overcoming formulation challenges seen with conventional dosage forms.

- **Versatility in Administration Routes**

Can be used for oral, parenteral, ophthalmic, pulmonary, topical, and transdermal delivery, increasing formulation flexibility.

- **High Drug Loading**

Consists mainly of pure drug particles with minimal excipients or carriers, allowing high drug loading and reducing formulation complexity.

Major Advantages of Nanosuspensions

- Its general applicability to most drugs and its simplicity.
- Can be applied for the poorly water soluble drugs.
- Can be given by any route.
- Reduced tissue irritation in case of subcutaneous/intramuscular administration.
- Rapid dissolution and tissue targeting can be achieved by IV route of administration.
- Oral administration of Nanosuspensions provide rapid onset, reduced fed/fasted ratio and improved bioavailability.
- The absorption from absorption window of the drugs can be increased, due to reduction in the particle size.
- Higher bioavailability and more consistent dosing in case of ocular administration and inhalation delivery.
- Drugs with high log P value can be formulated as nanosuspensions to increase the bioavailability of such drugs.
- Improvement in biological performance due to high dissolution rate and saturation solubility of the drug.
- Ease of manufacture and little batch-to batch variation.
- Long term physical stability (Due to absence of Ostwald ripening).
- Nanosuspensions can be incorporated in tablets, pellets, hydrogel and suppositories are suitable for various routes of administration.
- Increasing the amorphous fraction in the particles, leading to a potential change in the crystalline structure and higher solubility.
- Possibility of surface-modification of nanosuspension for site specific delivery.
- Possibility of large-scale production, the pre-requisite for the introduction of a delivery system to the market.

Emulsion-solvent evaporation technique

An emulsion is formed by first dissolving drug in an organic solvent or cosolvents and subsequently dispersing in an aqueous phase containing a surfactant, which act as a stabilizer. Rapid evaporation of solvent under reduced pressure

instantaneously produce nanosuspension. Key factors that should be considered during emulsification method are globule size and concentration of stabilizer.

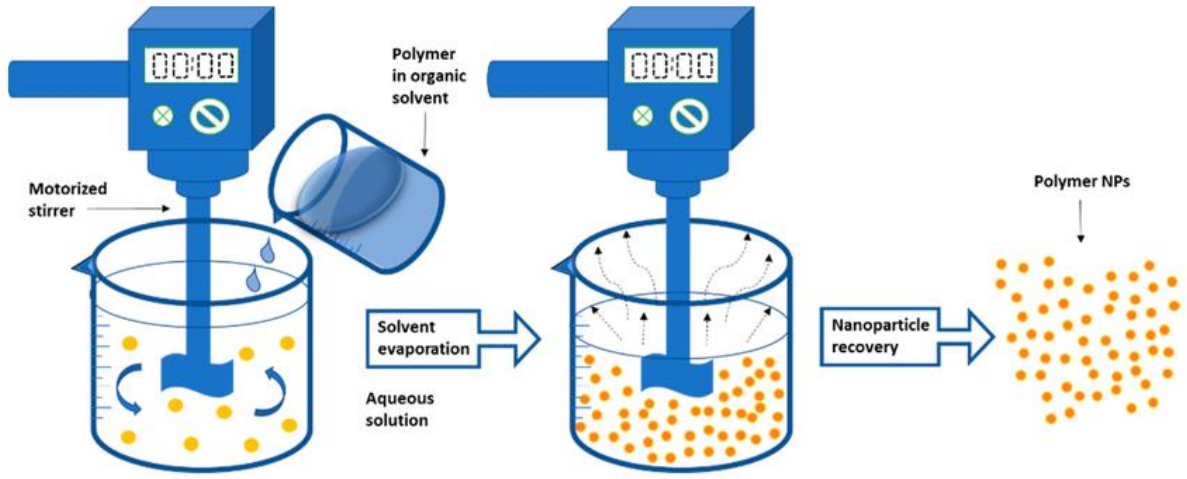


Fig 1: Emulsion-solvent evaporation technique

APPLICATIONS OF NANOSUSPENSIONS

Drug Solubility Enhancement

Nanosuspensions significantly improve the aqueous solubility of poorly water-soluble drugs, enhancing dissolution rate and allowing drugs in BCS Class II and IV categories to achieve better therapeutic performance.

Oral Drug Delivery:

Because of the numerous advantages oral route is the most preferable route for many of the drugs (figure) especially in the case of orally administering antibiotics such asatovaquone and bupravaquone.



Fig 2: Nanosuspensions for Oral Drug Delivery

By making it in nanosize, its solubility and bioavailability will increase. The oral administration of naproxen nanoparticles leads to an area under the curve (AUC) (0-24 h) of 97.5 mg-h/l compared with naproxen nanosuspension and naproxen tablets. In the case of danazole (gonadotrophin inhibitor) nanosuspension has absolute bioavailability of 82.3 and the conventional dispersion only 5.2%.

Parentral Drug Delivery:

Nanotechnology is also used in the parentral drug delivery System. The advantage of this method is it needs only less amount of toxic cosolvent for poorly soluble drugs. This will uplift the therapeutic effect of the drug compared with the conventional oral formulation and targeting the drug to the macrophages. The drug clofazimine is given as i v the concentration in the liver, spleen and lungs reached a high level i.e; greater than minimum inhibitory concentration, for most of the mycobacterium avium strains. Tarazepide is formulated as Nanosuspensions in order to overcome the use of surfactants and cyclodextrins to improve the bioavailability.

Pulmonary Drug Delivery:

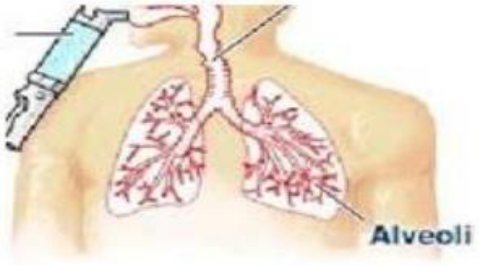


Fig 3: Nanosuspension for Pulmonary Drug Delivery

In pulmonary drug delivery we are using nanopreparations for the drugs which have poor solubility in pulmonary secretions (figure 7). For the lung delivery it is nebulised by mechanical or ultrasonic nebulizer. Uniform distribution of drug is possible and each droplet contain at least single drug particle. Nanosizing improves the diffusion and dissolution of the drug. It enhances the adhesiveness of drug to the mucosal surface and increased residence time at the site of absorption. Nanosuspensions has fast onset of action initially and then controlled release of active moiety occur which is required for most of the pulmonary Nanosuspensions has fast onset of action initially and then controlled release of active moiety occur which is required for most of the pulmonary disease. E.g: budenoside

Occular Drug Delivery:
Certain drugs have poor solubility in lachrymal fluid. If it is formulated as nanoparticles its saturation solubility and bioavailability will increase. Mainly applied for hydrophobic drugs. It increases the residence time in cul de sac. The best example of Nanosuspensions is ibuprofen. The anti-inflammatory activity of ibuprofen increased compared with the aqueous preparation.

Targeted Drug Delivery:
Nanosuspensions also used for targeting their surface properties and changing of the stabilizer can easily alter the *in vivo* behavior. The drug will be up take by the mononuclear phagocytic system to allow regional specific drug delivery. This can be used for targeting anti-mycobacterial, fungal drugs to the macrophages. Atovaquone is used as targeting Nanosuspensions to the brain.

Muco adhesion of Nanoparticle:
If the nanosuspension is orally administered it diffuse in to the liquid medium and adhere to the mucosal surface before absorption.it improve the bioavailability and targeting to the parasite persisting the git.eg; bupravaquone against cryptosporidium parvum.

Stability Improvement of Labile Drugs
Nanosuspensions protect chemically or physically unstable drugs by reducing degradation and preventing crystal growth during storage.

Use in Veterinary Medicine
They are widely beneficial for improving solubility and compliance in animals, particularly for poorly soluble antiparasitic and antibiotic drugs.

MATERIALS & METHODOLOGY:

LIST OF MATERIALS AND EQUIPMENTS:

TABLE 1: Materials used in Formulation

Excipient Used	Supplier
Clozapine	Xenon Pharma Pvt Ltd
PVP K30	Lobachemie, Mumbai
PVA	Lobachemie, Mumbai
Pluronic F127	Lobachemie, Mumbai
Ethyl acetate	Lobachemie, Mumbai
SLS	Narmada chemicals

TABLE 2: Equipment's used in Design Formulation

S.No.	Instruments	Sources
1	Electronic Weighing Balance	Shimadzu Corporation Tokyo, Japan
2	UV-Vis Spectrophotometer (T60)	Single Beam Spectrophotometer (YIS-294)
3	FTIR Spectrophotometer	Shimadzu Corporation Tokyo, Japan
4	Dissolution Apparatus	LAB India
5	Magnetic stirrer	Remi industries, Kerala

Pre-formulation studies:
Prior to the development of nanosuspension dosage form, it is essential that certain fundamental physical and chemical properties of the drug molecule alone and when combined with excipients are determined. This first learning phase is known as pre-formulation. The overall objective of the pre-formulation is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced.

The goals of pre-formulation studies are:

- To evaluate the drug substance analytically and determine its necessary characteristics, and
- To establish its compatibility with different excipients.

Solubility studies:
Solubility of Clozapine was carried out in different solvents like- 0.1 N HCl, 7.4 phosphate pH buffer and 6.8 phosphate pH buffer, and also in organic solvents like Ethanol, Methanol. Solubility studies were performed by taking excess amount of drug in different beakers containing the solvents.The mixtures were shaken for 24hrs at regular intervals. The solutions were filtered by using whattmann's filter paper grade no.41. The filtered solutions were analyzed spectrophotometrically.

Determination of Melting Point:

Melting point of Clozapine was determined by capillary method. Fine powder of Clozapine was filled in glass capillary tube (previously sealed at one end). The capillary tube was tied to thermo meter and the thermometer was placed in the Thais tube and this tube was placed on fire. The powder at what temperature it melted was noticed.

Determination of absorption maximum (λ_{max})

The wavelength at which maximum absorption of radiation takes place is called as λ_{max}. This λ_{max} is characteristic or unique for every substance and useful in identifying the substance. For accurate analytical work, it is important to determine the absorption maxima of the substance under study. Most drugs absorb radiation in ultraviolet region, as they are aromatic or contain double bonds.

Accurately weighed 10mg Clozapine separately was dissolved in 10ml volumetric flask. The volume was made up to 10ml with the same which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 6.8 phosphate pH buffer to obtain stock solution-II with a concentration 100µg/ml. From stock solution-II, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 6.8 phosphate pH buffer to get a concentration of 10µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max).

Construction of calibration curve using 6.8 phosphate pH buffer:
Accurately weighed 10mg Clozapine was dissolved in 6.8 phosphate pH buffer which gives a concentration of 1000µg/ml. From this standard solution, 1ml was pipette out in 10ml volumetric flask and volume was made up to 10ml using 6.8 phosphate pH buffer to obtain a concentration of 100µg/ml. From the above stock solution, aliquots of 0.25, 0.50,0.75, 1.0, 1.25 and 1.50 ml each was transferred to a separate 10ml volumetric flask and solution was made up to 10ml using 6.8 phosphate pH buffer to obtain a concentration of 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 µg/ml respectively. The absorbance of each solution was measured at 237 nm.

Drug excipient compatibility study:
The drug and excipient compatibility was observed using Fourier Transform – Infra Red spectroscopy (FT-IR). The FT-IR spectra obtained from Bruker FT-IR Germany (Alpha T) was utilized in determining any possible interaction between the pure drug and the excipients in the solid state. The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about 8t/in^2. The spectra were recorded over the wave number of 4000 to 400cm^{-1}.

Preparation of Nanosuspensions:

Preparation of Clozapine Nanosuspension by Emulsification solvent evaporation method:

Clozapine (25 mg) was accurately weighed and dissolved/dispersed in 2 mL ethyl acetate along with the selected stabilizer—PVP K30, PVA, or Pluronic F127 (12.5, 25, 37.5, or 50 mg)—to form the organic phase. Separately, SLS (10 mg) was dissolved in 100 mL distilled water to form the aqueous phase. The organic phase was slowly added to the aqueous phase under continuous stirring at 1000 rpm to form an emulsion. The emulsion was sonicated for 15 min and then continuously stirred to facilitate evaporation of ethyl acetate, resulting in the formation of clozapine nanoparticles. The prepared nanosuspension was collected and subjected to further evaluation.

Table 3: Formulation of Nanosuspension of Clozapine

Ingredients	NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8	NS9	NS10	NS11	NS12
Clozapine (mg)	25	25	25	25	25	25	25	25	25	25	25	**25**
PVP K30(mg)	12.5	25	37.5	50	--	--	--	--	--	--	--	--
PVA (mg)	--	--	--	--	12.5	25	37.5	50	--	--	--	--
Pluronic F127 (mg)	--	--	--	--	--	--	--	--	12.5	25	37.5	**50**
SLS(mg)	10	10	10	10	10	10	10	10	10	10	10	**10**
Ethyl acetate (ml)	2	2	2	2	2	2	2	2	2	2	2	**2**
Water(ml)	100	100	100	100	100	100	100	100	100	100	100	**100**
Stirring RPM	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**
Sonication time (mins)	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**

Evaluation parameters of Nanosuspensions:

The Nanosuspensions was evaluated for various parameters:-

- Entrapment efficiency
- Scanning electron microscopy
- Particles size and shape
- Zeta Potential
- In-vitro drug release studies
- Drug release kinetics studies

Entrapment efficiency

The 25mg of the Clozapine weight equivalent Nanosuspensions was analysed by dissolving the sample in 10ml of methanol. After the drug was dissolved 10ml of clear layer of dissolved drug is taken. There after the amount of drug in the water phase was detected by a UV-Spectrophotometric method at 237 nm (U.V Spectrophotometer). The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase from the total amount of the drug in the Nanosuspensions. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\%\ \textbf{of Drug entrapment} = \frac{\textbf{Mass of drug in Nanosuspensions}}{\textbf{Mass of drug used in formulation}} * \mathbf{100}$$

Particle size measurement:

The particle size was determined using the particle size analyzer (Zeta sizer Nano series, UK). The formulations were diluted with an appropriate volume of 6.8 phosphate pH buffer. The measurements were carried out three times where the mean value was used.

Scanning electron microscopy:

The morphological features of prepared Nano suspensions are observed by scanning electron microscopy at different magnifications.

Dissolution study:

Diffusion Parameters

Medium : 6.8 phosphate pH buffer
Apparatus : Basket (USP-I)
RPM : 50
Temperature : 37° C±0.5
Time Points : 5,10,15,20, 30, 45 & 60 minutes

Procedure:

The dialysis membrane diffusion technique was used. Five millilitre of the nanosuspension was placed in the dialysis membrane (Mw cutoff 12,000–14,000 Hi-media), fixed in a Franz

diffusion cell with the receptor volume of 20 ml. The entire system was kept at 37 °C with continuous magnetic stirring. Sample of 1 ml was withdrawn from the receptor compartment at predetermined time intervals and replaced by fresh medium. The amount of drug dissolved was determined using UV spectrophotometer.

Modelling of Dissolution Profile

In the present study, data of the in vitro release were fitted to different equations and kinetic models to explain the release kinetics of Clozapine from the matrix tablets. The kinetic models used were Zero order equation, First order, Higuchi release and Korsmeyer-Peppas models.

Kinetic Studies: Mathematical models:

Different release kinetic equations (zero-order, first-order, Higuchi's equation and Korsmeyer-peppas equation) were applied to interpret the release rate of the drug from matrix systems for the optimized formulation. The best fit with higher correlation (r2) was calculated.

Zero-order model:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation

$$Qt = Q0 + K0t$$

Where Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

Application: It is used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as tablets with low soluble drugs in coated forms, osmotic systems, etc.

First Order Model:

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

Release behavior generally follows the following first order equation:

$$\text{Log } C = \text{Log } C_o - kt/2.303$$

Where C is the amount of drug dissolved at time t, C_o is the amount of drug dissolved at t=0 and k is the first order rate constant.

A graph of log cumulative of % drug remaining vs time yields a straight line.

The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release the drugs in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminishes.

Higuchi model: The first example of a mathematical model aimed to describe drug release from a system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then sustained to different geometrics and porous systems. This model is based on the hypothesis that

- initial drug concentration in the is much higher than drug solubility;
- drug diffusion takes place only in one dimension (edge effect must be negligible);
- drug particles are much smaller than system thickness;
- swelling and dissolution are negligible;
- drug diffusivity is constant; and
- Perfect sink conditions are always attained in the release environment.

In a general way the Higuchi model is simply expressed by following equation

$$Q = K_H - t^{1/2}$$

Where, K_H is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.

Application: This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and tablets with water soluble drugs.

Korsmeyer-Peppas model:

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first60% drug release data were fitted in Korsmeyer-Peppas model,

$$Mt / M\infty = Kt^n$$

Where Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices.

In this model, the value of n characterizes the release mechanism of drug as described in the following table.

Table No 4: Drug transport mechanisms suggested based on 'n' value.

S. No	Release exponent	Drug transport mechanism	Rate as a function of time
1	0.5	Fickian diffusion	$t^{-0.5}$
2	0.45 < n = 0.89	Non -Fickian transport	t^{n-1}
3	0.89	Case II transport	Zero order release
4	Higher than 0.89	Super case II transport	t^{n-1}

RESULTS AND DISCUSSION:

Preformulation studies:

Physical Appearance

Clozapine was visually examined for colour, odour, texture and physical nature under normal illumination.

Table 5: Physical Appearance of Clozapine

Parameter	Observation
Appearance	White to pale-yellow crystalline powder
Odour	Odourless
Nature	Crystalline

Discussion: Clozapine was observed as a white to pale-yellow, crystalline and odourless powder, which was consistent with its reported physical characteristics. The absence of visible discoloration, foreign particles, or abnormal odour indicated that the drug was suitable for further preformulation and formulation studies.

Solubility Study

An excess amount of clozapine was added separately to different solvents and shaken until equilibrium was achieved. The solubility was assessed visually and/or quantitatively to select a suitable solvent for formulation.

Table 6: Solubility studies of Clozapine

Solvent	Solubility (mg/mL)
Distilled water	0.08
6.8 Phosphate Buffer	0.65
7.4 Phosphate Buffer	0.25
Ethyl acetate	1.8

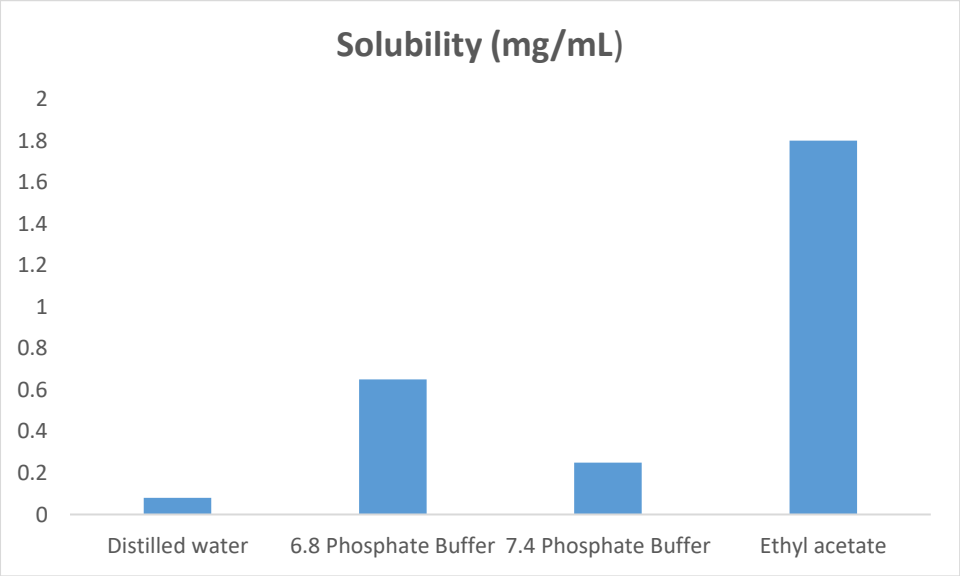


Fig 4: Solubility studies of Clozapine

Discussion: Clozapine is a weakly basic drug, so its solubility is very poor in water, in near-neutral phosphate buffers, the solubility was improved, supporting the need for a nanoparticle/Nano suspension approach to improve its dissolution behavior**.**

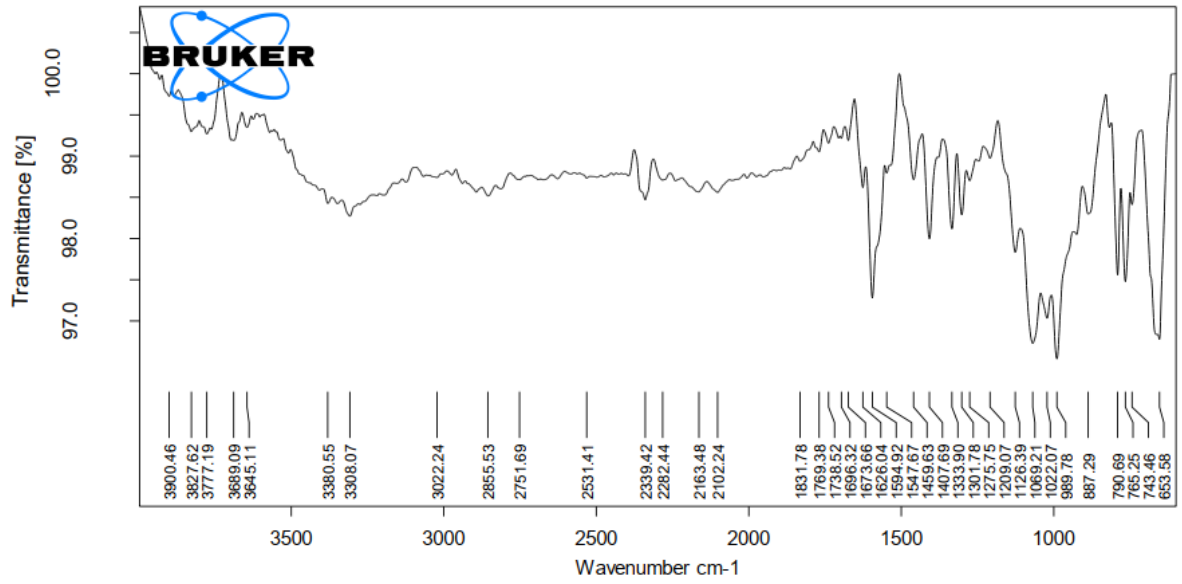
Melting Point

A small quantity of clozapine was filled into a capillary tube and analyzed using a melting-point apparatus. The melting point of clozapine was found to be 184 ± 1.2°C.

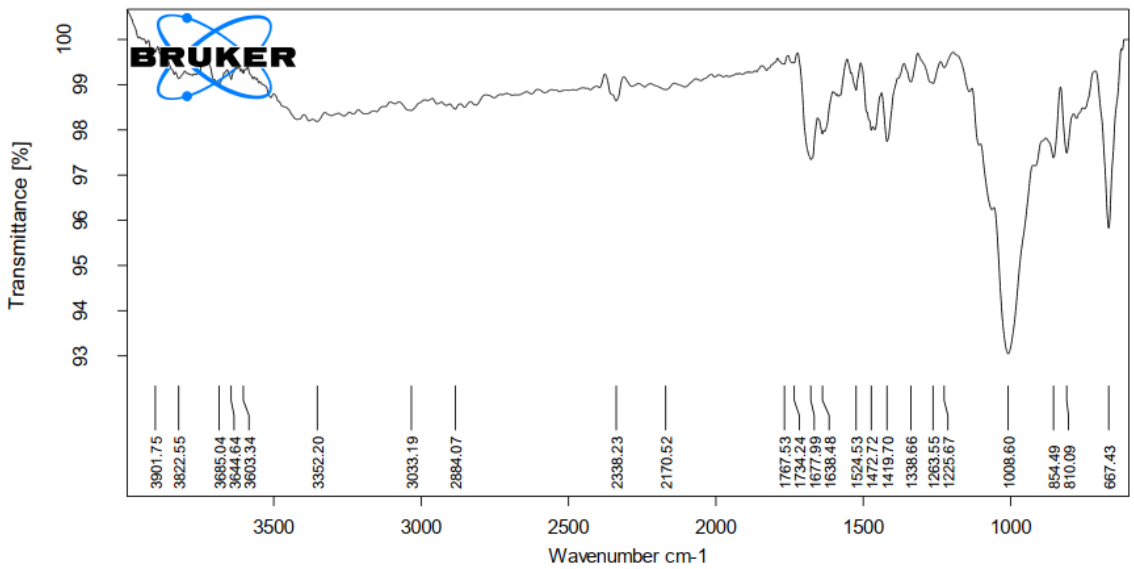
FTIR Study

FTIR spectra of pure clozapine and physical mixtures of clozapine with the selected excipients were recorded over the appropriate wavenumber range. The characteristic peaks were compared to assess drug–excipient compatibility.

Pure Drug



Optimized formulation



Discussion: The FTIR spectrum of pure clozapine showed its characteristic functional-group peaks, confirming the identity of the drug. The spectra of clozapine with excipients showed no significant disappearance or formation of major peaks, indicating the absence of significant drug–excipient interactions.

Determination of absorption maximum (λmax):

Determination of Clozapine λ-max was done in pH 6.8 phosphate buffer medium for accurate quantitative assessment of drug Diffusion rate.

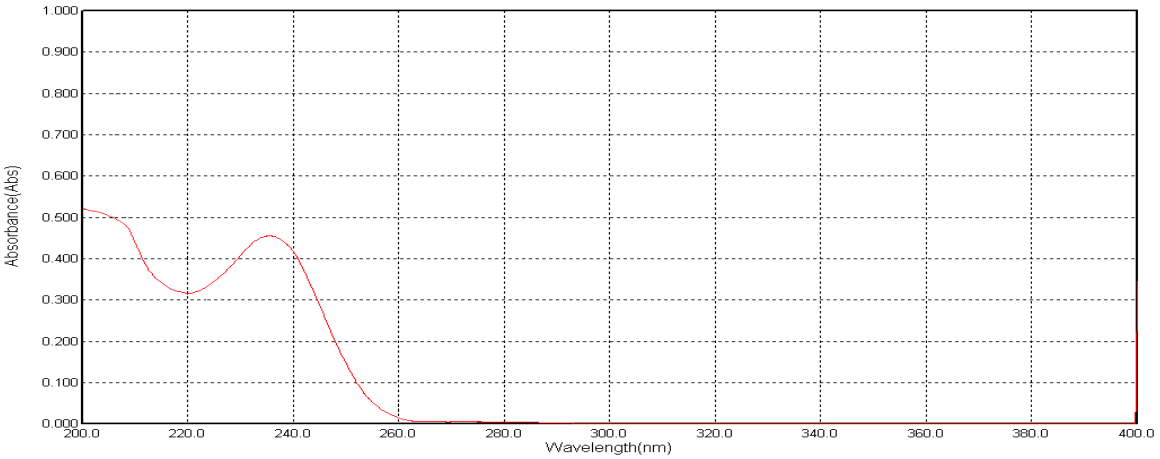


Fig 5: UV spectrum of Clozapine

Discussion: The λ-max of Clozapine of 100% solution i.e 10ppm (µg/ml) by using Single Beam Spectrophotometer (YIS-294) was found to be at 237 nm by using pH 6.8 phosphate buffer.

Calibration curve of pure Drug:

Table.No.7: Standard graph of Clozapine in pH 6.8 phosphate buffer (λmax 237 nm)

Concentration (µg/ml)	Absorbance
0	0
2.5	0.107
5	0.227
7.5	0.350
10	0.459
12.5	0.568
15	0.675

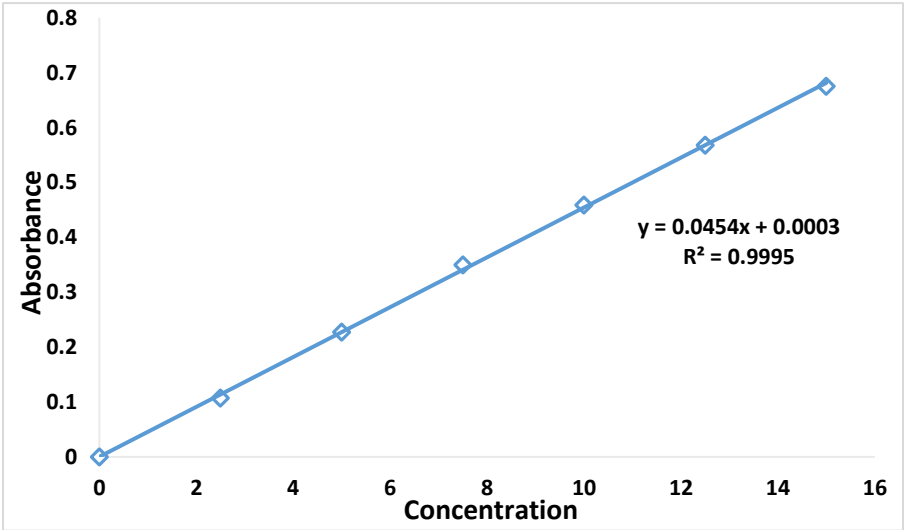


Fig 6: Standard calibration curve of Clozapine in pH 6.8 phosphate buffer

Discussion: The linearity was found to be in the range of 2.5-15.0 µg/ml in pH 6.8 phosphate buffer. Regression analysis was selected because it minimizes the deviation and correct the variance heterogeneity. The regression line was defined by its slope (m) and its intercept (C) for normal regression analysis was found as 0.0454 and 0.0003, with regression coefficient of 0.9995 respectively. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law.

Formulation of Clozapine Nanosuspension by Emulsion Solvent evaporation method:
Clozapine (25 mg) was dissolved/dispersed in 2 mL ethyl acetate with the selected stabilizer (PVP K30, PVA, or Pluronic F127) at 12.5–50 mg. Separately, SLS (10 mg) was dissolved in 100 mL distilled water. The organic phase was added slowly to the aqueous phase under stirring at 1000 rpm, followed by 15 min sonication and continued stirring for evaporation of ethyl acetate. The resulting clozapine nanosuspension was collected and evaluated for physicochemical properties.

Evaluation Tests of Clozapine Nanosuspension:

Particle Size and Polydispersity Index (PDI):
Particle size was determined using dynamic light scattering (DLS). The average particle size of the formulations was recorded in nanometers. PDI was also measured along with particle size using DLS. It was used to assess the uniformity of particle-size distribution.

Measurement Results

Date	:	16, August, 2024 9:14:54 AM
Measurement Type	:	Particle Size
Sample Name	:	4
Scattering Angle	:	90
Temperature of the holder	:	25.1 °C
T% before meas.	:	30810
Viscosity of the dispersion medium	:	0.893 mPa·s
Form Of Distribution	:	Standard
Representation of result	:	Scattering Light Intensity
Count rate	:	429 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.05	10.6 nm	3.3 nm	10.9 nm
2	0.95	114.3 nm	72.1 nm	87.5 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	109.3 nm	73.8 nm	87.5 nm

Cumulant Operations

Z-Average	:	86.4 nm
PI	:	0.263

Molecular weight measurement

Molecular weight	:	---
Mark-Houwink-Sakurada parameters	:	---

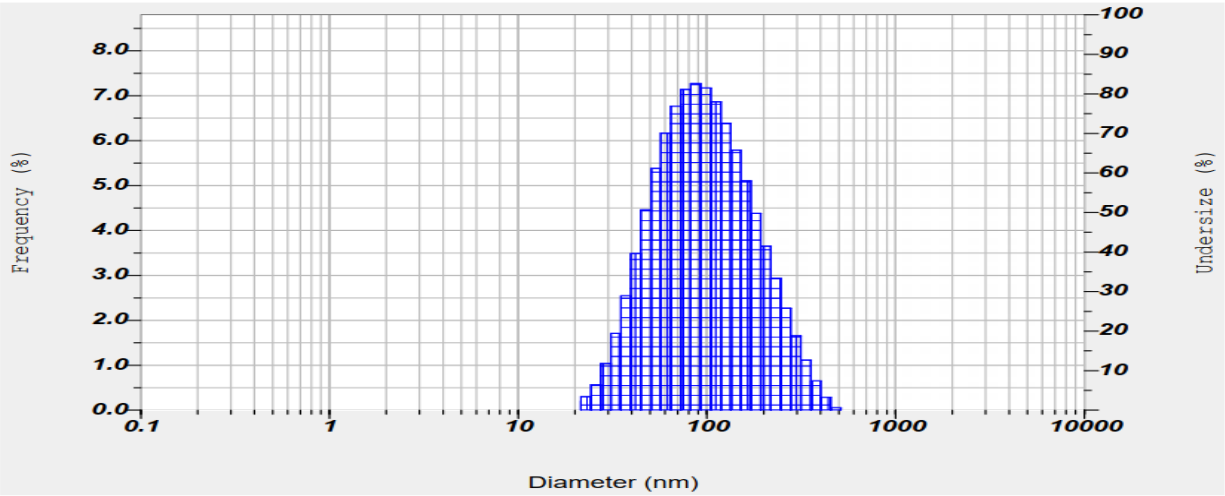
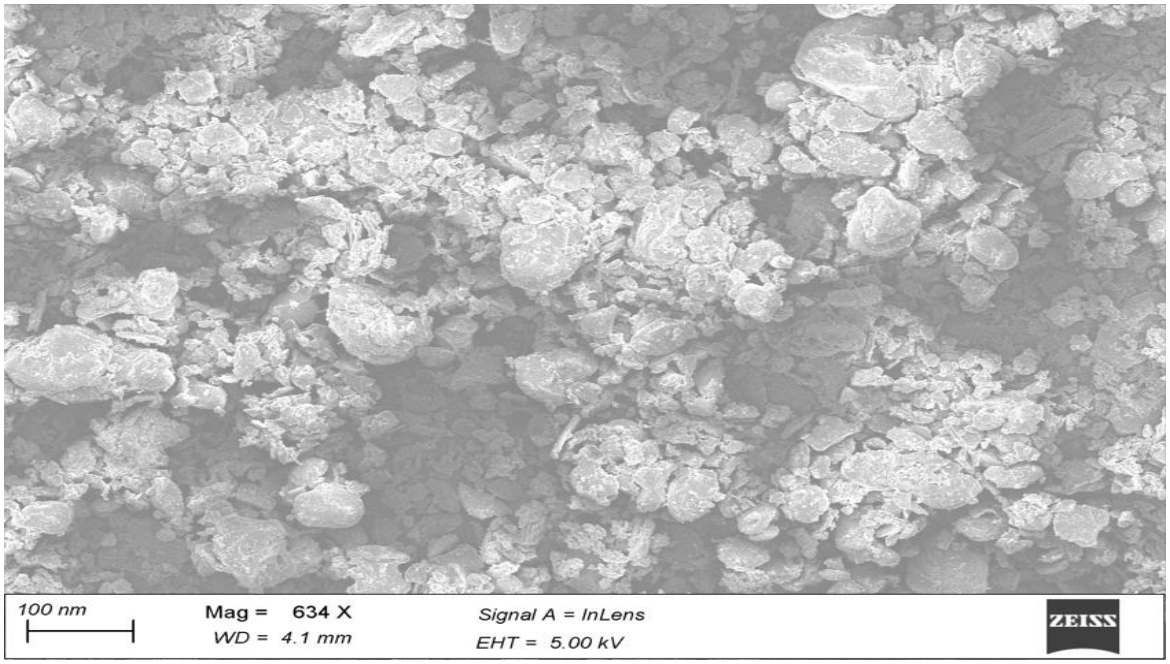


Figure No 7: Particle Size Analysis Of Optimized Formulation

Discussion: Average particle size of nanosuspension of optimized formulations (NS12) was found to be having maximum particles at a range of 86.4 nm.

Surface Morphology



Discussion: The morphology of the nanoparticles was examined using **Scanning Electron Microscopy (SEM)**. The images were used to determine particle shape and surface characteristics. In this SEM, the nanosuspension particles are appeared within slightly spherical in shape and particle size was reduced up to 100 nm.

Drug Content and Entrapment efficiency

An accurately weighed quantity of nanoparticles was dissolved in a suitable solvent and analyzed spectrophotometrically. The drug content was calculated as the percentage of clozapine present in the formulation. Entrapment efficiency was calculated as the percentage of drug associated with the nanoparticle **system**.

Table 8: Drug content & Entrapment efficiency of clozapine nanosuspensions

Formulation	Drug Content (%)	Entrapment Efficiency (%)
NS1	91.2 ± 1.4	78.5 ± 1.8
NS2	92.8 ± 1.3	82.3 ± 1.6
NS3	94.6 ± 1.1	86.7 ± 1.4
NS4	96.1 ± 1.0	89.2 ± 1.3
NS5	90.8 ± 1.5	77.6 ± 1.9
NS6	93.2 ± 1.2	82.8 ± 1.7
NS7	95.0 ± 1.1	87.5 ± 1.4
NS8	96.8 ± 0.9	90.4 ± 1.2
NS9	91.5 ± 1.4	80.1 ± 1.7
NS10	94.1 ± 1.2	84.6 ± 1.5
NS11	96.0 ± 1.0	88.9 ± 1.3
NS12	97.2 ± 0.8	**91.2 ± 1.1**

Discussion: The prepared clozapine nanoparticles showed satisfactory drug content (90.8–97.4%), indicating good drug recovery within the formulations. The entrapment efficiency (77.6–91.2%) increased with increasing stabilizer concentration, which may be due to improved stabilization and reduced drug loss during emulsification and solvent evaporation. NS12 showed the highest drug content (97.2 ± 0.8%) and entrapment efficiency (91.2 ± 1.1%), and was therefore considered a promising optimized formulation.

In-vitro Drug Release

The release of clozapine from the nanoparticles was studied using a suitable dissolution apparatus and release medium. Samples were withdrawn at predetermined intervals and analysed spectrophotometrically.

Table 9: Drug release profiles (NS1-NS12)

Time (h)	% Drug Release											
	NS1	**NS2**	**NS3**	**NS4**	**NS5**	**NS6**	**NS7**	**NS8**	**NS9**	**NS10**	**NS11**	**NS12**
0.5	29	25	19	13	20	9	14	12	17	15	11	**18**
1	45	42	29	25	34	17	27	22	31	28	20	**30**
2	65	56	47	42	53	29	44	36	50	45	33	**49**
4	84	79	65	59	72	44	62	51	69	63	48	**67**
6	96	94	86	78	82	56	73	63	80	74	60	**78**
8			98	97	96	67	80	73	87	81	70	**85**
10						79	95	94	94	90	82	**97**

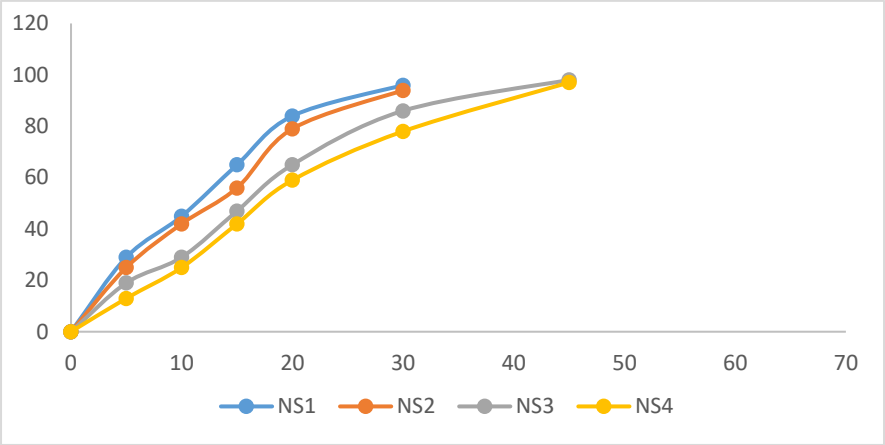


Fig: 8 % Drug Release of Clozapine Nanosuspension (NS1-NS4)

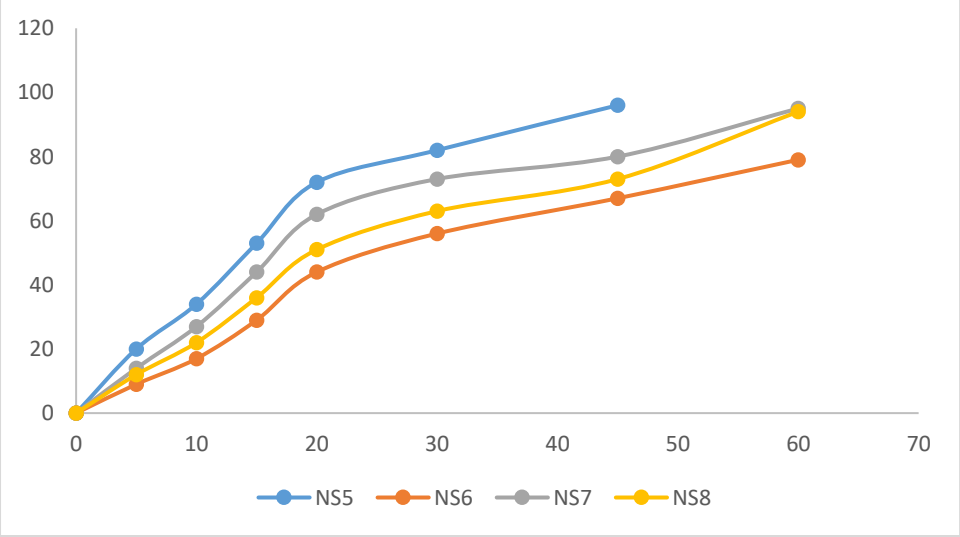


Fig: 9 % Drug Release of Clozapine Nanosuspension (NS5-NS8)

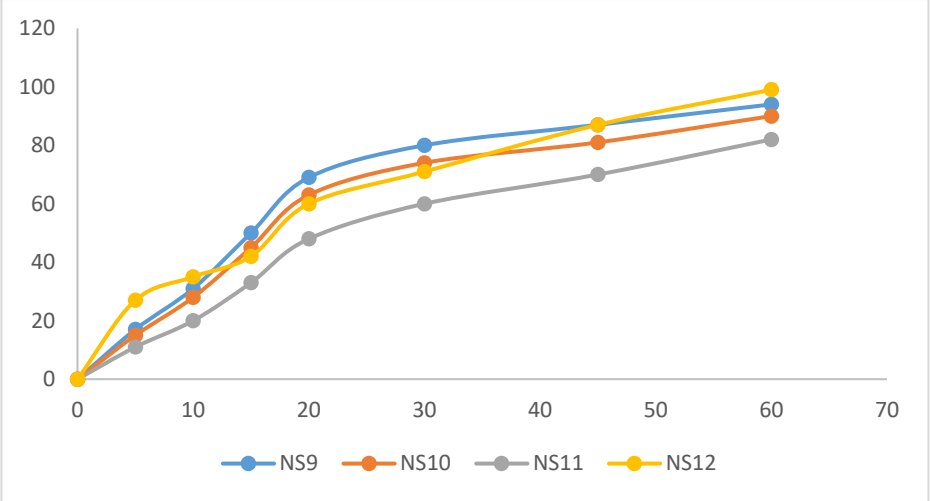


Fig: 10 % Drug Release of Clozapine Nanosuspension (NS9-NS12)

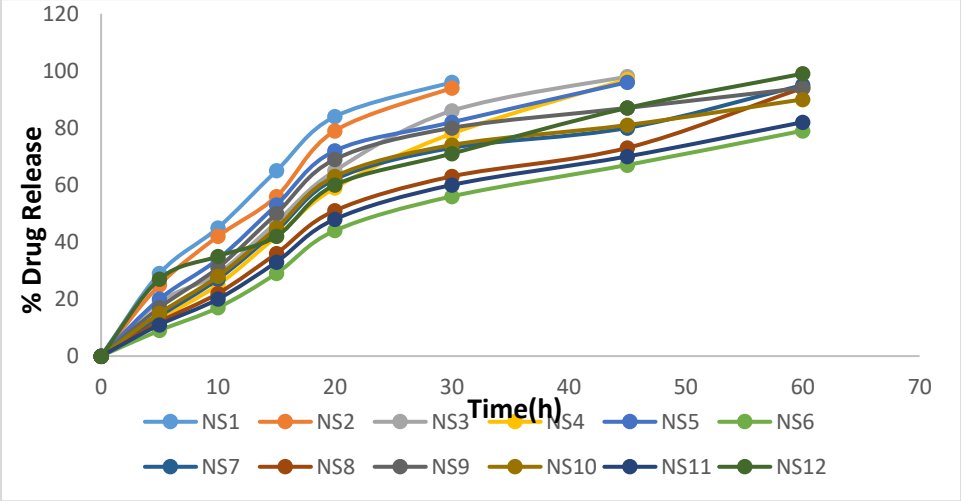


Fig:11 % Drug Release of Clozapine Nanosuspension (NS1-NS12)

Discussion: The in-vitro drug release study showed that the increasing the concentration of stabilizer resulted in a decrease in the drug-release rate, which may be attributed to better stabilization of drug particles and reduced dissolution of clozapine. Among all formulations, NS12 showed the most controlled release profile. Thus, NS12 demonstrated a desirable controlled-release pattern and was considered a promising formulation for further evaluation.

Drug Release kinetics of optimised formulation NS12

Zero Order Kinetics

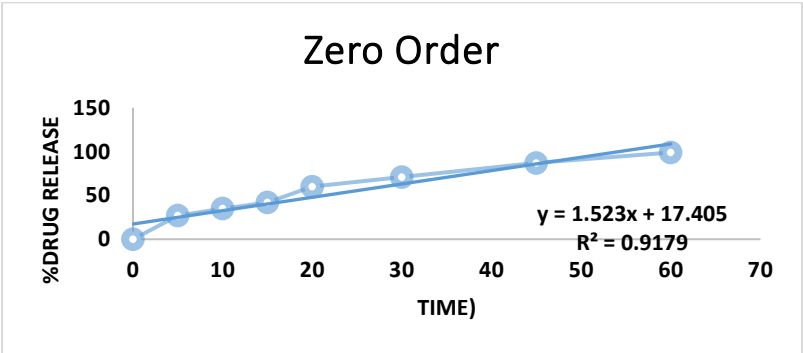


Fig:12 Zero order Plot of Optimised Formulation NS12

First order Kinetics



Fig:13 First order Plot of Optimised Formulation NS12

Higuchi Plot

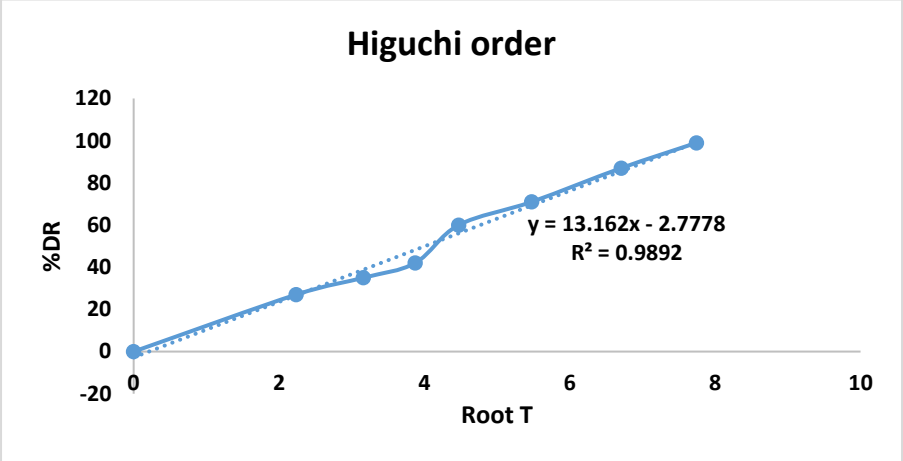


Fig:14 Higuchi Plot of Optimised Formulation NS12

Peppas Plot

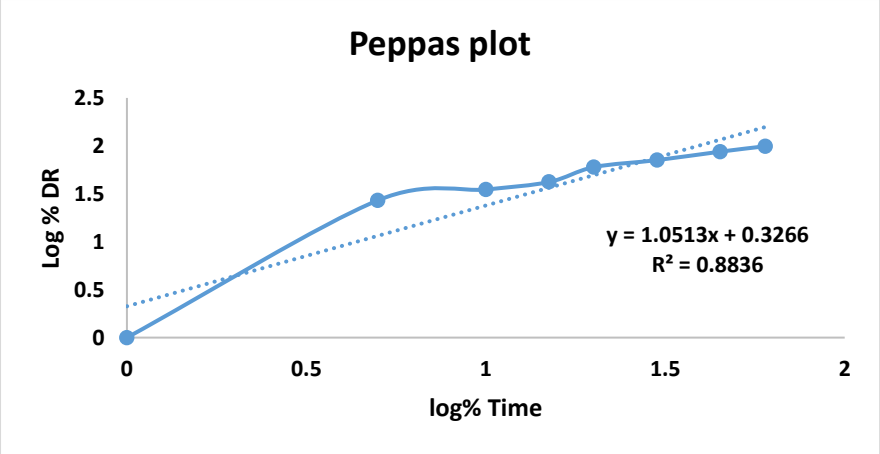


Fig:15 Peppas Plot of Optimised Formulation NS12

Table 10: Order of Kinetics and Regression values

ORDER OF KINETICS	REGRESSION
Zero order	0.917
First Order	0.893
Higuchi	0.982
Korsmeyer–Peppas model	0.883
n value	1.051

Discussion: The release kinetics of optimized formulation NS12 were evaluated using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. Among the models studied, the Higuchi model showed the highest correlation coefficient (R^2 = 0.982), indicating that drug release was predominantly diffusion-controlled. The Korsmeyer–Peppas model showed an n-value of 1.051, suggesting anomalous (non-Fickian) transport, indicating that drug release may involve a combination of diffusion and polymer relaxation/erosion mechanisms.

CONCLUSION:

The present study successfully developed clozapine nanosuspensions using the emulsion–solvent evaporation method. Preformulation studies confirmed the suitability and compatibility of clozapine with the selected excipients, while the UV method demonstrated good linearity with an R^2 of 0.9995. Among the twelve formulations, NS12, containing 50 mg Pluronic F127, showed the highest drug content (97.2 ± 0.8%) and entrapment efficiency (91.2 ± 1.1%) and exhibited a controlled drug-release profile up to 10 h. The release data followed the Higuchi model (R^2 = 0.998) with an n-value of 0.703, suggesting predominantly diffusion-controlled and anomalous drug-release behavior. Overall, NS12 was identified as a promising clozapine nanosuspension formulation for further optimization and detailed stability and performance studies.

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