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Preparation, In Vitro Evaluation and Characterization studies of Clozapine Nanosuspension

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Abstract

Clozapine (CLZ) is a tricyclic dibenzodiazepine, classified as an atypical antipsychotic agent. It belongs to the Biopharmaceutical Classification System class II drug (High Permeability, Low Solubility), with a high first-pass effect. The current study used a technique called the solvent-antisolvent method to prepare a nanosuspension of clozapine with different stabilizers and solvents in an effort to increase the drug's solubility and dissolution. Evaluations were done for particle size, polydispersity index, zeta potential, and in vitro dissolution. All developed clozapine formulations had particle size values in the nano-size range. The best formula, A7, had a particle size of 80.43 nm and contained soluplus as stabilizer at a ratio of 1:3 (drug: soluplus). Methanol was used as a solvent in a 1:3 ratio, with water acting as an anti-solvent, and a stirring speed of 1000 rpm was employed. Characterization studies included FTIR, XRD, and FESEM, which revealed smooth, uniform particles within the nano size. The location of the clozapine nanosuspension functional group was not altered by FTIR, while Powder X-ray diffraction studies revealed that clozapine had changed from a crystalline to an amorphous form. In conclusion, the solubility and dissolution rate of clozapine were significantly increased when clozapine was prepared as a nanosuspension.

Keywords: Clozapine, Nanosuspension, Particle Size, Solubility, Solvent-antisolvent Method.

Introduction

Drug effectiveness is significantly impacted by solubility. One fundamental aspect to be considered is drug solubility in water. Orally administered medications with low solubility will have low bioavailability in systemic circulation [(1)]. Since it is generally known that the rate-limiting step for gastrointestinal absorption of a drug from solid dosage forms is often dissolution, the water solubility is an essential factor affecting formulation, biological activity, and in vitro and in vivo biopharmaceutical properties. As a result, several attempts have been undertaken to alter these poorly water-soluble drugs' dissolving properties to achieve quick and full absorption performance [(2)]. Many strategies are available to overcome the solubility issue of poorly soluble drugs, by co-crystallization, the use of a co-solvent, self-emulsification, solid dispersion, salt formation, solubilization by surfactant, micronization, and nanosization [(3)].

Nanosuspension (NS) is a colloidal dispersion of nano-sized drug particles stabilized by surfactants. They can also be defined as a biphasic system consisting of pure drug particles with a diameter of less than 1µm in size suspended in an aqueous vehicle [(4)]. With their advantages over conventional approaches (rapid dissolution rates, high saturation solubility, reduced usage of potentially hazardous solvents, and quick absorption rates in vivo), drug nanosuspensions have gained acceptance as a formulation technique to improve low solubility[(5)]. By decreasing particle size, increasing surface area, and enhancing solubility, nanosuspension may increase the bioavailability of poorly soluble drugs. The main distinction between nanosuspensions and other nano-formulations is that the latter does not require a carrier part, allowing for the highest potential drug loading [(6)]. Additional approaches are vesicular systems like liposomes, dispersion of solids, emulsion, and microemulsion methods, and inclusion complexes with cyclodextrins, which show the beneficial effects as drug delivery systems but major problems of these techniques lack universal applicability to all drugs [(7)]. The major advantages of nanosuspension technology are its general applicability to most drugs, its simplicity, high drug loading, avoiding toxic solvents, and its being the universal formulation approach for most drugs[(8)].Clozapine (CLZ) is an atypical antipsychotic drug that is a member of the tricyclic dibenzodiazepine class. Of all the antipsychotic medications, CLZ has the best effect.

Furthermore, CLZ has been shown to lower the suicide death rate in people with schizophrenia. Being a mild antagonist of the dopamine (D2) receptor and serotonin (5-HT) receptor antagonist gives CLZ a distinct pharmacological profile[9]. It is classified as a class II medication with limited solubility and high permeability according to BCS. Following oral administration, it showed a significant first-pass metabolism with low bioavailability (27-50%) [10]. In 2021, Hetal P. *et al*. developed CLZ as a nose-to-brain nanosuspension by the high-speed homogenization method using (+)- alpha-tocopherol polyethylene glycol 1000 succinate (TPGS) stabilizer to improve the drug bioavailability. The optimized nanosuspension of clozapine generates a particle size of 281 nm. The drug permeability results were 96.15% and 41.12% for clozapine nanosuspension and conventional suspension, respectively after 24 hrs [11]. In 2023, Rosamaria L. *et al*. encapsulated CLZ in n Eudragit® RS100 and RL100 polymeric nanoparticles for intranasal administration to enhance the drug's bioavailability. Sustained release nanoparticles were obtained with particle sizes ranging between 400-500 nm. The release profile of CLZ from EUD-NPs exhibited complete release within 8 hr [12].

Materials and Methods

Materials

Clozapine (99.5%) was purchased from (China), Soluplus and Polyvinyl alcohol (PVA) were purchased from (Basf SE, Germany), Poloxamer 407 and Poloxamer 188 (PMX) were obtained from (Eastman Chemical Company, USA), Polyvinyl pyrrolidone K30 and Polyvinyl pyrrolidone K90 (PVP) were obtained from (Fluka Chemi AG, Switzerland), Methanol was obtained from (Thomas Baker, India), Tween 40 was supplied by (Himedia, India), Sodium lauryl sulfate was supplied by (Didactic, Spain). Disodium hydrogen phosphate and Potassium Dihydrogen Phosphate were supplied by (BDH Laboratory Supplies (England) and SPINE- CHEM. Limited) respectively.

Methods

Preparation of clozapine nanosuspension

The nanosuspension precipitation method is used to prepare CLZ nanosuspension. In brief, 12.5 mg of CLZ was dissolved in an organic solvent of 3 ml of methanol (acetone or ethanol). The aqueous solution (10ml of water) containing the selected stabilizers (soluplus, poloxamer 188, poloxamer 407, PVA, HPMC E15, PVP k90, and PVP k30) at different ratios, with different surfactants (tween 40 and Sodium lauryl sulfate (SLS)) which acts as the antisolvent system as illustrated in Table 1. This was followed by the addition of the organic solution into stabilizer/surfactant aqueous solution at a rate (0.5ml /min) with the help of a syringe, under different stirring speeds using a magnetic stirrer for 1 hour at 25±1°C to allow the organic solvent to evaporate and get the desired nanosuspension [13].

Table 1. The compositions of the prepared formulations of clozapine nanosuspension

F. code	Stabilizer	Surfactant	Ratio (Drug: stabilizer)	Stirring Speed (rpm)
A1	Soluplus	Not used	1:1	500
A2	Soluplus	Not used	1:2	500
A3	Soluplus	Not used	1:3	500
A4	Soluplus	Not used	1:4	500
A5	Soluplus	Not used	1:5	500
A6	Soluplus	Not used	1:3	750
A7	Soluplus	Not used	1:3	1000
A8	Soluplus	Not used	1:3	1500
A9	Soluplus	Not used	1:3	1000
A10	Soluplus	Not used	1:3	1000
A11	Soluplus	Not used	1:3	1000
A12	Soluplus	SLS	1:3:1	1000
A13	Soluplus	Tween 40	1:3:1	1000
A14	Soluplus	Tween 40	1:3:1	1500
A15	PVP 30	Tween 40	1:3:1	1500
A16	PVP 90	Tween 40	1:3:1	1500
A17	HPMC E15	Tween 40	1:3:1	1500
A18	PVA	Tween 40	1:3:1	1500
A19	Poloxamer 188	Tween 40	1:3:1	1500
A20	Poloxamer 407	Tween 40	1:3:1	1500

Evaluation of clozapine nanosuspension

Measurement of the particle size and polydispersity index of clozapine nanosuspension

The particle size analyzer (Malvern zeta sizer, Spectris Company, United Kingdom) was used to analyze all CLZ nanosuspension formulations. This dynamic light scattering apparatus measures the amount of light molecules scatter as a time function at a constant temperature of 25°C and a scattering angle of 90° [(14)].

Zeta potential evaluation of clozapine nanosuspension

Zetasizer (Zetasizer Nano ZS, Malvern instrument, Worcestershire, UK) was used to evaluate zeta potential. The surface charge properties were examined to assess the created nanosuspension's stability [(15)].

In vitro dissolution behavior of clozapine nanosuspension formulas

A USP Type II dissolution equipment with a dialysis membrane (molecular weight cutoff of 12000–14000 Da) was used to study dissolution. At 37 °C and 100 rpm, the membrane was clamped to the paddle and submerged in 900 mL of pH 6.8 phosphate buffer containing 1% Brij 35. To maintain sink conditions, 5 mL of the sample was collected at regular intervals of 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 115, and 120 minutes, and an equal volume of new buffer solution was then added to maintain a constant volume. The amount of CLZ released in the buffer was calculated using spectrophotometry at the wavelength at which CLZ had the highest absorbance. A plot of the drug's cumulative % release against time was determined [(16,17)].

Factors affecting particle size and polydispersity index of selected clozapine nanosuspension formulas

A list of factors that can exert an influence on the particle size and PDI of clozapine nanosuspensions is shown below:

1- Stabilizer type effect

To determine their impact of stabilizer4 type on the particle size of CLZ nanosuspensions, different stabilizer types, namely Soluplus, PXM 407, PXM 188, PVP K 30, PVP K 90, HPMC E15, and PVA were used at distinct ratios.

2- Stabilizer ratio effect

Five different CLZ: stabilizer ratios (1:1) to (1:5) were prepared to determine the influence of varying stabilizer ratios on the particle size of CLZ nanosuspensions.

3- Stirring rate effect

Stirring rates (500, 750, 1000, and 1500 rpm) were employed to examine the influence of stirring rate on the properties of CLZ nanosuspensions.

4- Solvent type effect

Three different organic solvents were used to evaluate the effect of solvent type on the particle size of CLZ nanosuspensions.

5- Surfactant type effect

Two surfactants (tween 40 and sodium lauryl sulfate) were used to examine the effect of surfactant type on the particle size of CLZ nanosuspension.

6-Prob- Sonication effect

One formula was treated with an ultrasonic probe sonicator at an ultrasonic power of 50W for 10 minutes to assess the influence of probe sonication on the particle size of CLZ nanosuspension.

Lyophilization of selected clozapine nanosuspension formula

The formula was freeze-dried using a vacuum freeze dryer to produce CLZ powder for further analysis. The nanosuspension was put in regular flasks to be inserted into the freeze-dryer channels. These flasks were frozen for 12 h. at -20 °C in a deep freezer, and then sublimation of solvent from frozen samples took 48 -72 hrs [(18)].

Characterization of CLZ lyophilized nanosuspension

Saturation solubility determination

Studies of the saturation solubility of the lyophilized formula and the pure CLZ were conducted in distilled water containing 1.5% w/v Brij 35 and phosphate buffer pH 6.8 containing 1% w/v Brij35. An extra sample was added to 10 milliliters of solvents and shaken for 72 h. at 25 ºC for water and 37 ºC for buffer in a test tube shaker. Following equilibration, samples were passed through 0.45 µm Millipore filters, appropriately diluted with the corresponding solutions, and the amount of drug dissolved was measured by measuring the absorbance at the designated wavelength [(19)].

Self-dispersibility of freeze-dried powder in aqueous medium

To ascertain the re-dispersibility of lyophilized powder, the freeze-dried powder containing 12.5 mg of CLZ was re-dispersed with vigorous shaking in 10 mL of water to get the same drug concentration in NS prior to lyophilization. Particle size was then measured, and by using equation 1 the redispersibility index (RDI) value was determined.

$$RDI = (D/D0) * 100\% \quad \text{…. (Equation 1)}$$

The D represents the P.S. of re-dispersed NS, and D_0 represents the P.S. of the pre-freeze-dried NS. The lyophilized CLZ powder was well re-dispersed when the RDI value was close to 100% [(20)].

Fourier transform infrared spectroscopy (FTIR)

To find out if CLZ and other ingredients in the formulation of CLZ nanosuspensions were

interacted or complexed, FTIR (Shimadzu 8300 Japan) was employed. Furthermore, the selected formula and untreated CLZ were compressed using potassium bromide. Spectra were obtained within a scanning range of 4000-400 cm^{-1} [21].

Powder X-ray diffraction (PXRD)

The purpose of PXRD is to determine the drug's crystalline pattern and to identify any physical transformation that happened concurrently with formulation; as a result, the test was done for the pure CLZ and the optimized lyophilized formula. The study used a powder x-ray diffractometer with an operating voltage of 30 kV, a current of 20 mA, and a continuous scan range of $2\theta = 10 - 50$ [22].

Field emission scanning electron microscope (FESEM)

Using FESEM, the morphology of the selected CLZ nanosuspension formula liquid was assessed. The FESEM (Inspect F50, FEI company) was used to analyze the morphology of the nanosuspension material. After the samples were examined at various magnifications, computers were uploaded with the resulting photos [23].

Statistical analysis

The data of the study were demonstrated as a mean±SD sample of three readings by using the one-way analysis of variance (ANOVA). The results were considered statistically significant when $p \leq 0.05$ and termed non-significant at a level of ($p > 0.05$).

The statistical analysis for the dissolution study was done using the model-independent method (using similarity factor *f2*) by DDsolver to compare the in vitro release profiles of pure CLZ and CLZ NSs. The pure CLZ was considered to be the reference, while the CLZ nanosuspensions were supposed to be the test[24].

Results and Discussion

Measurement of the particle size and polydispersity index of clozapine nanosuspension

The resulting particle size and distribution for all formulations were documented in Table (2). Formulas (A14-A20) prepared by distinct stabilizers (Soluplus, PVP K30, PVP K90, HPMC E15, PVA, PXM 188, and PXM 407) with tween 40 as a surfactant at a ratio of 1:3:1 (drug: stabilizer: surfactant) using methanol as a solvent and at a speed of 1500 rpm. A significant difference ($p<0.05$) was noted in the particle size among the seven types of stabilizers. This means that the affinity of stabilizers for clozapine particles differs. As shown in Figure 1, formula A14, which is stabilized by soluplus, exhibited the smallest particle size (82.67) nm; this may be attributed to the unique interaction between soluplus and clozapine.

Soluplus is an amphiphilic graft copolymer with a hydrophilic part (polyethylene glycol backbone) and a lipophilic part (vinyl caprolactam/vinyl acetate side chains). The hydrophobic part of the polymer will be adsorbed onto the hydrophobic CLZ surface. In contrast, the hydrophilic portion may be extended outward into the aqueous phase, so the interfacial tension formed between the newly formed nanoparticles can be reduced, providing full surface coverage and steric stabilization to NS, and this resulted in the inhibition of particle growth and agglomeration[25]. All the other stabilizers resulted in a larger particle size, as shown in Table 2 and Figure 1. This could happen if there is no affinity between the CLZ molecule and these stabilizers. When the stabilizer and particle surface lack affinity, electrostatic forces between the particles increase as the stabilizer between them decreases, leading to a depletion of repulsive forces. As a result, the capacity of various stabilizers to bind to a pharmacological molecule has resulted in variations in particle size [26].

Table 2. The particle size and PDI of clozapine nanosuspensions [27]

F. code	Particle size (nm)	PDI	F. code	Particle size (nm)	PDI	F. code	Particle size (nm)	PDI
A1	113.1±1.48	0.09±0.016	**A8**	104.2±8.47	0.21±0.005	**A15**	1217±8.98	0.47±0.21
A2	100.9±2.25	0.14±0.015	**A9**	111.4±5.87	0.12±0.009	**A16**	796.4±11.3	1.2±1.29
A3	94.56±0.706	0.03±0.01	**A10**	85.2±4.98	0.08±0.15	**A17**	305.7±7.92	0.5±0.33
A4	98.1±3.01	0.14±0.018	**A11**	100.9±2.25	0.18±0.08	**A18**	1283±8.32	0.61±0.52
A5	98.2±1.6	0.09±0.02	**A12**	283.7±8.13	0.4±0.18	**A19**	850±6.83	0.33±0.18
A6	87.32±0.972	0.01±0.005	**A13**	137.9±6.24	0.2±0.12	**A20**	465.7±8.43	0.45±0.22
A7	80.43±0.851	0.01±0.002	**A14**	82.67±3.23	0.13±0.81			

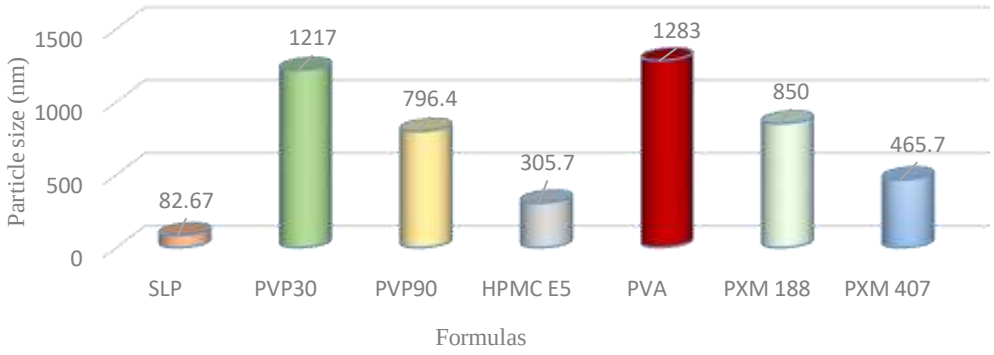

Figure 1. Stabilizer-type effect on the particle size of clozapine nanosuspension

To study the effect of different drug: stabilizer ratios, formulas (A1-A5) prepared in a ratio of 1:1 to 1:5. As shown in Table 2 and Figure 2, as a ratio of stabilizer increases, the particle size decreased significantly, this indicates that there was sufficient polymer concentration for covering the produced nanoparticles [28]. Particle size began to increase when a ratio of 1:4 was reached. This could be attributed to an increase in the anti-solvent solution's viscosity, which might interfere with particle movement and result in more drug particle coating. These results were in agreement with Dora *et al* [29].

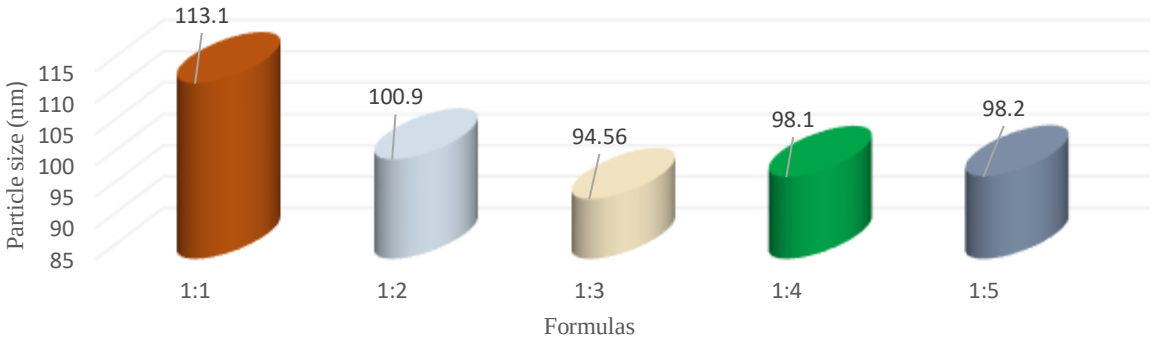

Figure 2. Stabilizer ratio effect on the particle size of clozapine nanosuspensions

The impact of stirring speed on the particle size of CLZ nanosuspension was studied by formulas (A3 and A6-A8). As shown in Figure 3, by increasing the stirring speed from 500 rpm to 1000 rpm, the particle size decreased from 94.56nm to 80.43nm. Further increase in the speed to 1500 rpm leads to an increase in the particle size to 104.2nm. This can be explained by the fact that shear mixing occurs more effectively, and the organic solvent diffuses into the water phase more quickly when a high stirring rate (1000 rpm) is used. It will cause drug particles to nucleate quickly and form tiny drug particles [28]. While a high mixing speed will reduce the particle size, it also increases the chance of aggregation, which will cause the particle size to rise and the suspension's sedimentation rate to speed up, as it happened in the case of 1500 rpm [30].

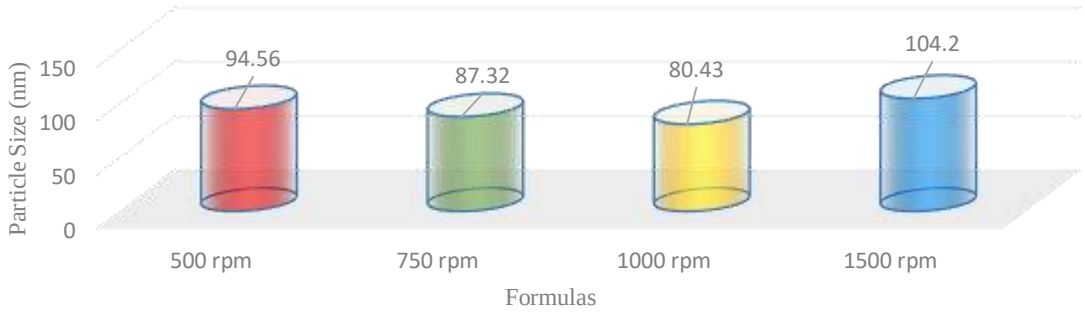

Figure 3. Stirring rate effect on the particle size of clozapine nanosuspension

Different solvents (methanol, ethanol, and acetone) were used in the formulas (A7, A9, and A10); the smallest particle size was obtained by methanol (80.43 nm) while the bigger particle size was obtained by ethanol (111.4 nm) and acetone (85.2 nm). This may be attributed to the higher solubility of clozapine in methanol [31].

The dynamics of methanol and water molecules resemble each other, these similarities in the dynamics of both molecules are consistent with the similarity in structure and this will lead to a good miscibility degree of the methanol with water compared to others (ethanol and acetone) which facilitate the diffusion of the CLZ particles when mixed with antisolvent media leading to the formation of smallest particle size and stabilizes the nanosuspensions by regulating the number of crystal nuclei forming, so methanol was the best solvent for CLZ nanosuspension preparation [32,33].

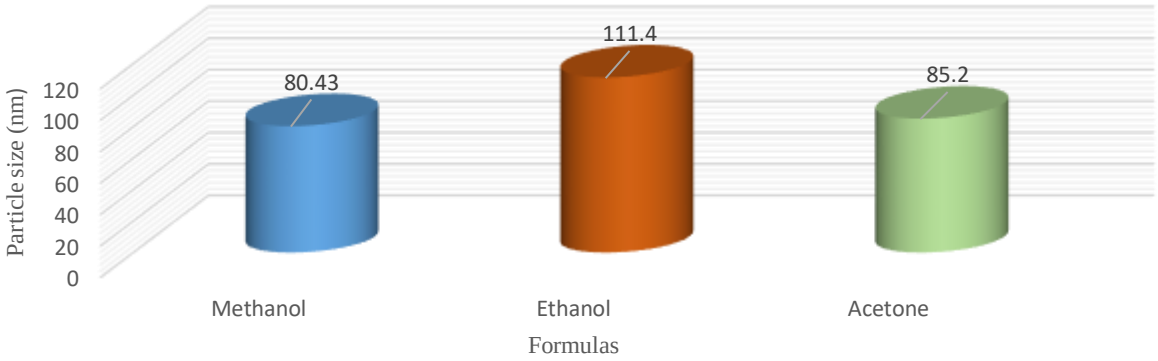

Figure 4. Solvent-type effect on the particle size of clozapine nanosuspension

The effect of surfactant (tween 40 and SLS) was studied in the formulas (A12 and A13). As illustrated by the outcome in Table 2 and Figure 5, there was a significant difference in particle size (p<0.05) with a change in the surfactant type. With tween 40, a smaller particle size (137.9nm) was obtained due to the higher solubility of the clozapine in tween 40 [34]. While in the case of SLS, the particle size was larger, which may be due to a high HLB value for SLS, also generally non-ionic surfactants were more efficient than anionic ones in nanosuspension production [35]. These findings are in agreement with Rashid AM, *et al* [36]

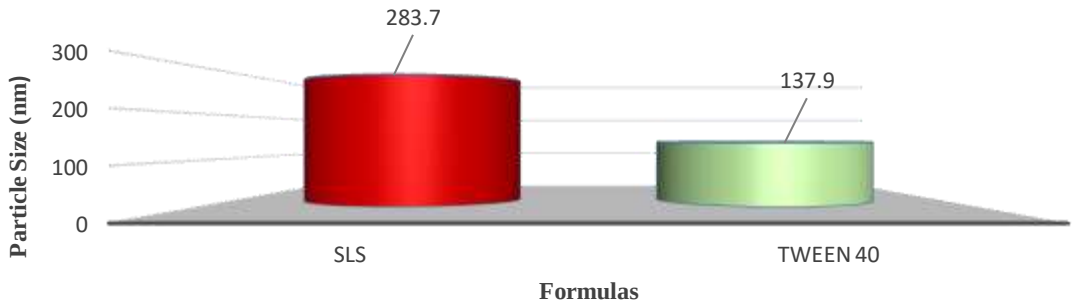

Figure 5. Surfactant type effect on the particle size of clozapine nanosuspension

The formulas (A7 and A11) studied the probe-sonication effect, and the results are shown in Figure 6; using the probe sonicator resulted in a larger particle production. The explanation may be due to higher thermal motion that increases the likelihood of accelerated particle collision, which influences interparticle attraction and leads to particle agglomeration [37].

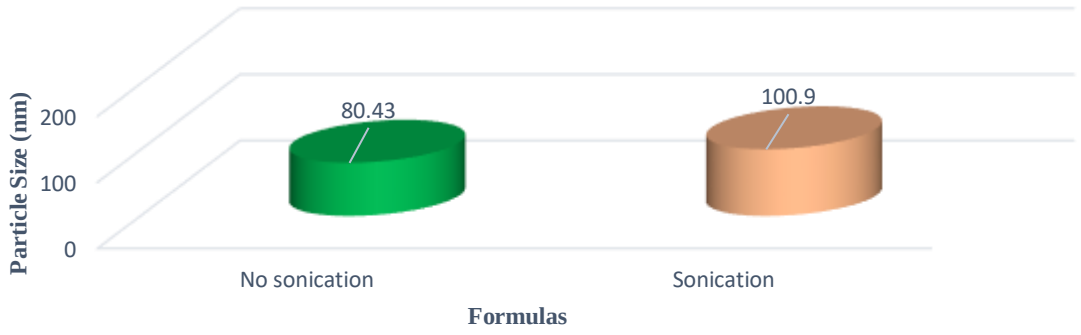

Figure 6. Probe-sonication effect on the particle size of clozapine nanosuspension

The range of values for formulations' polydispersity index (PDI) typically was (0.01-1.2). This is because, as PDI is closely correlated with particle size, the greater particle size mainly generates a higher PDI [38].

Zeta potential evaluation of clozapine nanosuspension

The zeta potential for the selected formula A7 was determined and measured at -2.486 mV. Zeta potential is important because it reflects the stability of the nanosuspension. Lower zeta potential is expected to be accompanied by a decrease in stability. However, this is not always true because of the lack of correlation between two parameters according to DELVO theory. Although the repulsive forces were low (proportional to the zeta potential), they may still be larger than attractive forces [39].

In vitro dissolution behavior of clozapine nanosuspension formulas

The release of CLZ nanosuspension formulas (A7, A10, and A14) and clozapine's pure powder was measured using the USP dissolution test apparatus type II, as shown in Figure 7. It was found that there was a significant (p<0.05) difference in the dissolution profile of optimized nanosuspension formulas compared to pure CLZ. The reason for such a result is mainly attributed to the Noyes-Whitney equation that states the dissolution rate enhanced as the particle size decreased as a result of the large surface area and consequently enhanced the wettability and contact of nanoparticles with the dissolution media. This validates the superiority of the developed CLZ nanoparticle compositions compared to the pure medication. The *in-vitro* release of the optimized formulas (A7 and A14) exhibited an interesting, faster release. A7 was 100% in 50 minutes., with a primarily burst effect drug release within 10 min, while A14 reached 99% in 60 min and A10 released 98.2% in 90 min compared with pure CLZ, which released only up to 43% after 2 hours. The similarity factor (f_2), an indicator used in pharmaceutical sciences to evaluate the similarities between two dissolution profiles, was utilized to compare the two. The two dissolution profiles were equivalent when the *f2*

value was more than 50, which ranges from 50 to 100, under FDA criteria [40].

As shown in Table 3, the values of the resulting similarity factors were lower than 50. This indicates a distinct difference in the dissolution behavior between the prepared clozapine NS and the pure clozapine powder.

Table 3. Similarity factor test of A7, A10, and A14 formulas versus pure clozapine

Formula code compared with pure clozapine	Similarity Factor (f_2) value
A7	9.17±0.239
A10	13.5±0.055
A14	10.64±0.285

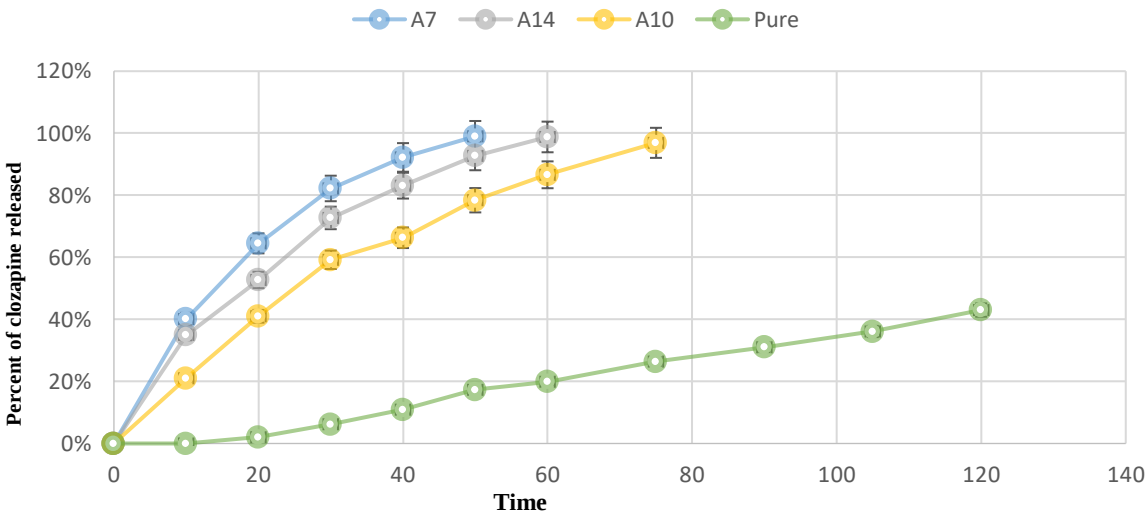

Figure 7. The *in-vitro* dissolution profile of the prepared clozapine nanoparticles (A7, A10, and A14) compared to the pure clozapine in phosphate buffer pH 6.8

Saturation solubility determination

Solubility studies for the pure CLZ and lyophilized formula were achieved in distilled water with 1.5% w/v Brij35 and phosphate buffer pH 6.8 with 1% w/v Brij35. The results illustrated in Figure (8) showed clozapine solubility has significantly (p<0.05) increased in both media when compared to the pure CLZ.

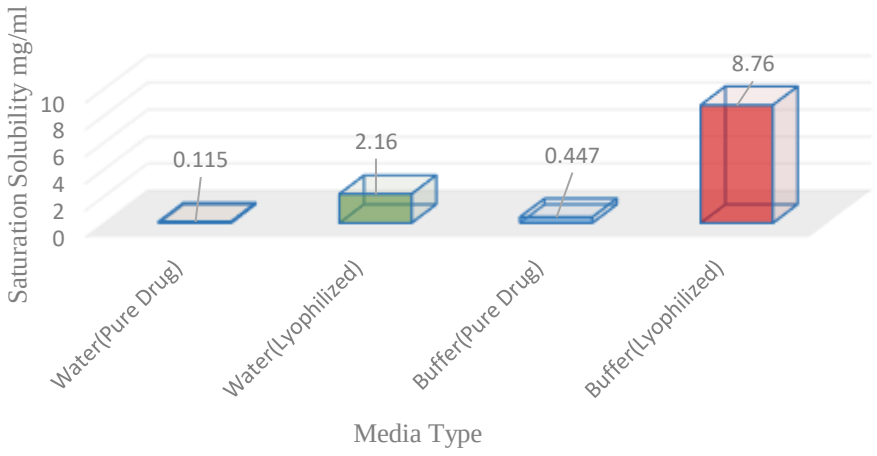

Figure 8. Saturation solubility of the pure clozapine and lyophilized formula in water and buffer pH 6.8

Self-dispersibility of freeze-dried powder in aqueous medium

The freeze-dried A7 formula was examined for self-dispersibility in aqueous solutions. In a matter of seconds, the powder in the aqueous medium was fully distributed, creating a homogeneous solution. The particle size of the re-dispersed lyophilized sample was determined (92.98±0.682), which was greater than before

lyophilization (80.43). The RDI value of particle size was determined (115.6±0.888%).

Fourier transform infrared spectroscopy (FTIR)

Both lyophilized formula and pure clozapine were subjected to FTIR analysis. The FTIR data indicates that the fingerprint region's peaks in the spectra of the lyophilized clozapine and pure medication clozapine do not vary from one another or exhibit any chemical interaction [41][9]. This indicates that the drug and the polymer used were chemically compatible, and there was no detectable interaction as shown in Figures (9) and Table (4).

Table 4. FTIR peaks of pure clozapine and lyophilized clozapine

Functional group	Reference (cm^{-1})	Pure drug (cm^{-1})	Clozapine NPs (cm^{-1})
C-H stretch aliphatic	2931 and 2845 cm^{-1}	2929 and 2845 cm^{-1}	2926 and 2856 cm^{-1}
C-H stretch aromatic	3291 cm^{-1}	3290 cm^{-1}	3259 cm^{-1}
C = N stretch	1597 and 1552 cm^{-1}	1593 and 1550 cm^{-1}	1558 and 1510 cm^{-1}
C-N stretch	1293 cm^{-1}	1292 cm^{-1}	1288 cm^{-1}
N-O stretch	1255 cm-1	1249 cm^{-1}	1240 cm^{-1}
N-H stretch	3290 cm^{-1}	3290 cm^{-1}	3259 cm^{-1}
C-CL stretch	816 cm^{-1}	813 cm^{-1}	813 cm^{-1}

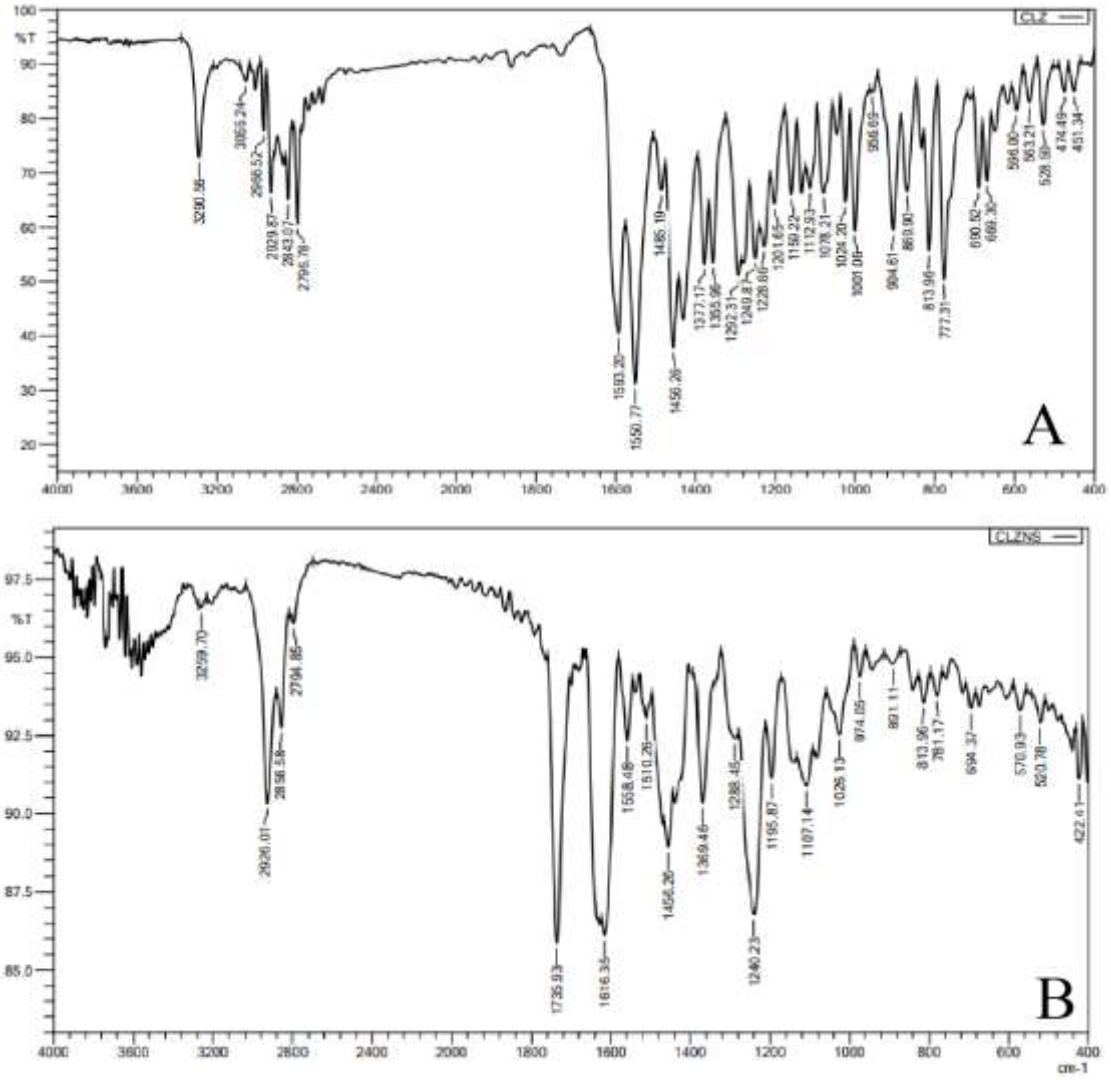

Figure 9. FTIR spectrum (A: pure clozapine and B: lyophilized formula)

Powder X-ray diffraction (PXRD)

An X-ray diffraction (XRD) analysis was carried out on pure clozapine powder and lyophilized clozapine powder, illustrated in Figure (10). X-ray diffraction peak analysis may be used to confirm the crystalline structure of a substance. This data is employed to determine if a product is crystalline or amorphous in nature.

Clozapine's XRD pattern revealed distinct and significant crystallinity reflection peaks (strongest peak occurring at 2θ = 17.32◦ and intensity = 5692). The optimized clozapine

nanosuspension's lack of XRD peaks suggested the presence of an amorphous stabilizer (soluplus) and the processing parameters' capacity to convert the crystalline drug into an amorphous form [11].

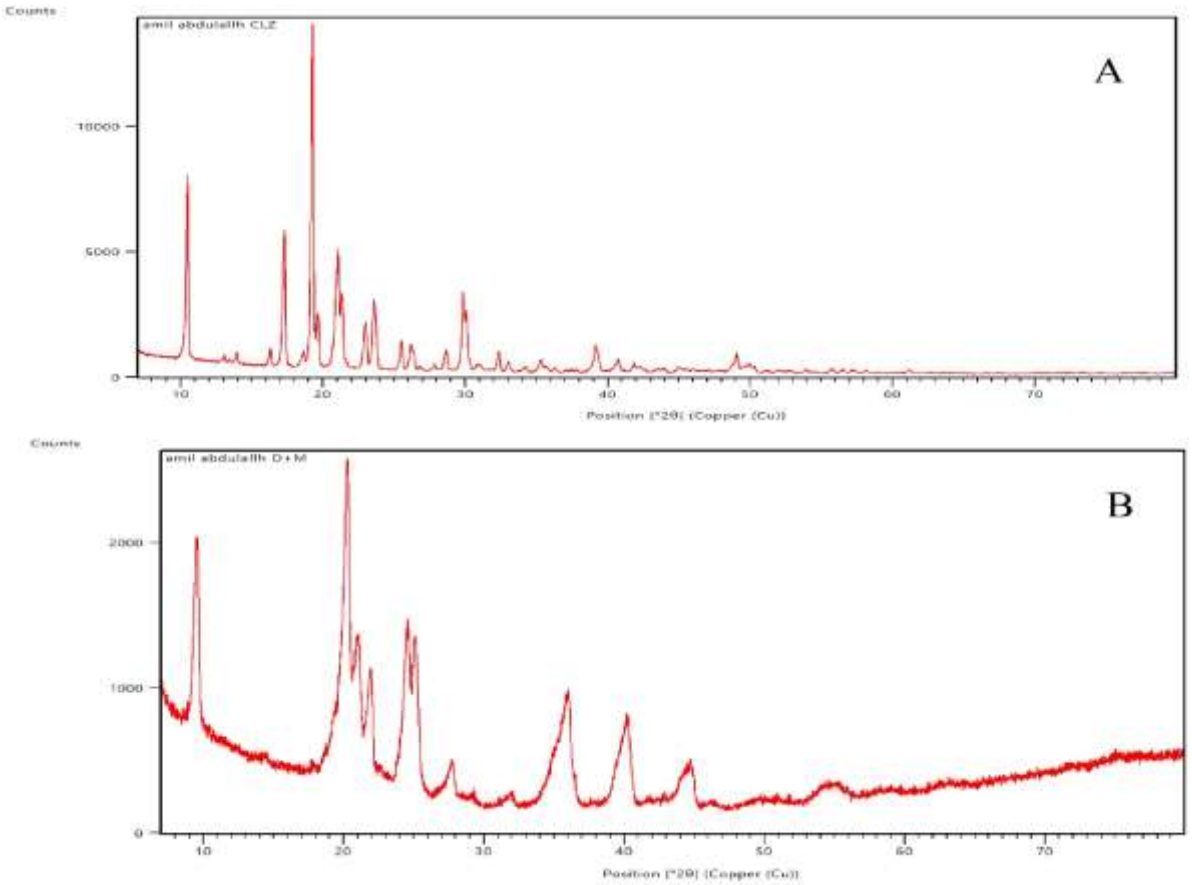

Figure 10. XRPD spectrum (A: pure clozapine and B: lyophilized formula)

Field emission scanning electron microscope (FESEM)

The lyophilized A7 formula, as seen in the FESEM Figure (11), had a uniform dispersion of tiny flake smooth irregular particles within a nanosized range, which was facilitated by the stabilizer that prevented the particles from re-agglomeration.

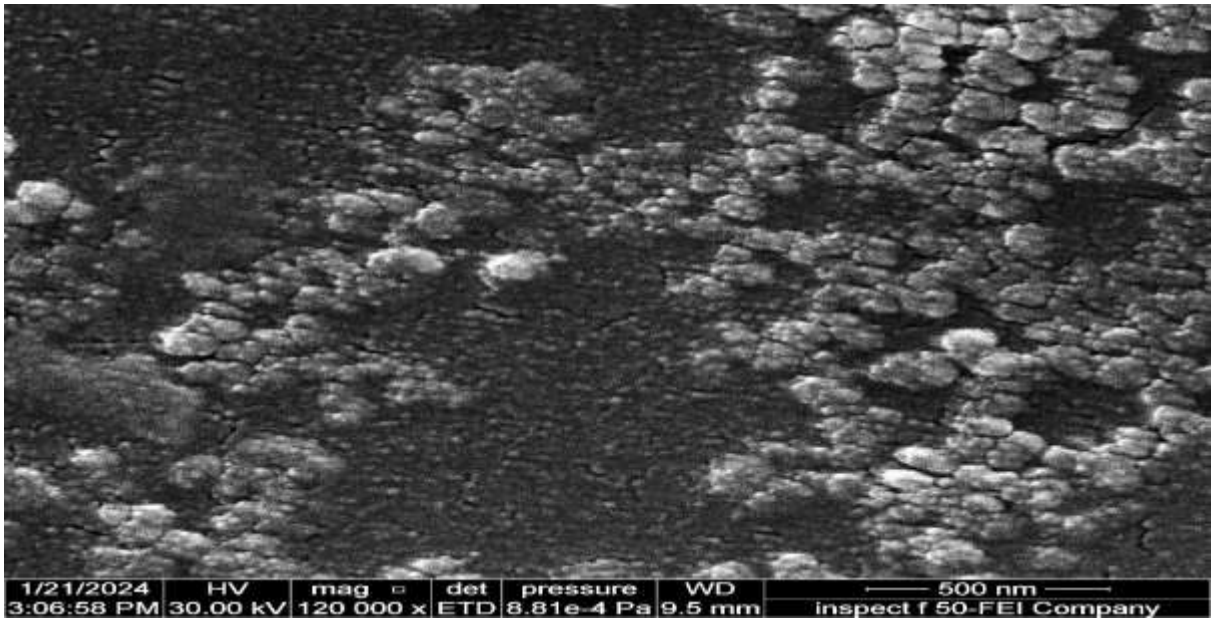

Figure 11. FESEM of the lyophilized formula

Conclusion

By using soluplus as a stabilizer, clozapine nanosuspension was prepared successfully. The optimized formula that produced generated nanoparticles with a size of 80.43 nm and a polydispersity index of 0.01 when it was used with methanol as the solvent at an agitation rate of 1000 rpm with soluplus in a drug: stabilizer ratio of 1:3. In comparison with the pure CLZ, the improved formulation significantly increased the rate of clozapine dissolution, according to in vitro dissolution experiments. For this reason, producing clozapine nanoparticles using the solvent anti-solvent technique enhances the clozapine's solubility rate.

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Conflicts of Interest

We (authors) hereby declare that there are no conflict-of-interest issues

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Ethics Statements

No human subjects nor living animals were used in this study thus no consents were required.

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تحضير وتقييم المختبري لعقار الكلوزابين كمعلق نانوي

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الخلاصة

عقار كلوزابين هو دايبنزوديازبين ثلاثي الحلقات ويصنف على أنه أحد مضادات الذهان الغير تقليدي. يصنف الكلوزابين ضمن الصنف الثاني حسب نظام تصنيف الصيدلانيات البايولوجية والذي يتميز بالذوبانية القليلة مع النفاذية العالية.

الهدف من البحث هو زيادة أذابة ومعدل الذوبانية لعقار كلوزابين من خلال تحضيره كمعلق نانوي بأستخدام مثبتات ومذيبات مختلفة بطريقة المذيب والمضاد المذيب. تم تقييم الحجم الحبيبي, جهد الزيتا وتحلل المادة خارج الجسم لتلك التركيبات. الحجم الحبيبي لجميع التركيبات المحضرة كان ضمن الحجم النانوي.تم أختيار تركيبة(أ٧) كأفضل تركيبة بالحجم الحبيبي (٨٠,٤٣) نانوميتر والتي أحتوت سولوبلس كمثبت بنسبه ١:٣ مع الدواء الخام بإستخدام االمثانول كمذيب بنسبة ١:٣ مع الماء كمضاد للمذيب وبسرعة دوران ١٠٠٠ دورة بالدقيقة. خضعت الصيغة المختارة لتقييمات مختلفة لغرض فحص الحالة البلورية للمسحوق النانوي المجفف وكانت النتائج تشير الى كونها جزيئات ذات حجم نانوي وذات سطح ناعم. أظهرت التحليل الطيفي للأشعة تحت الحمراء عدم تغيير في مواقع المجموعات الوظيفية في حالة المعلق النانوي, بينما نتائج الحيود الأشعة السينية أثبتت تحول الشكل البلوري للكلوزابين الى غير متبلور.

نستنتج ان تحضير الكلوزابين كمعلق نانوي أدى الى تحسن كبير في حل الدواء وعزز قابليته على الذوبان .

الكلمات المفتاحية: كلوزابين، معلق نانوي، حجم الجسيم، ذوبانية، طريقة المذيب والمضاد الذيب.