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Optimization and development of ondansetron HCl nanocrystals by using D-optimal design expert® and *in-vitro* characterization

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Abstract: **Background**: Over the last few years, nanocrystal technology has expanded to improve the bioavailability of poorly water-soluble drugs, which is challenging. Nanocrystals (NCs) exhibit many properties, such as enhancing drug solubility, dissolution, oral absorption, and high drug loading. Ondansetron hydrochloride (ONH) is an antiemetic drug that antagonizes a serotonin 5-HT3 receptor in the peripheral and central nervous system and is widely prescribed for the management of chemotherapy and radiotherapy-induced vomiting and nausea, as well as for postoperative nausea. ONH exhibits approximately 60% bioavailability due to its poor solubility and first-pass metabolism in the liver. Its solubility is pH-dependent, its precipitates above pH 6. **Objective**: Preparation of ONH-NCs to increase its dissolution rate as a preliminary study to be prepared as a sublingual film to avoid first-pass metabolism. **Method**: NCs were prepared using the nanoprecipitation method and optimized via a D-optimal surface design considering drug to stabilizer ratio, solvent to anti-solvent ratio, stirring rate, and stabilizer type. **Results**: The process yielded particle sizes (PS) ranging from 99-409 nm and a polydispersity index (PDI) range of 0.08-0.44 using stabilizers such as Soluplus®, Brij 35®, and Kollidon VA 64® (Koli 64). Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) demonstrated showed reduced crystallinity, while Fourier transform infrared (FTIR) spectroscopy confirmed drug-stabilizer compatibility, and Field Emission Scanning Electron Microscopy (FESEM) depicted its morphological characterization. **Conclusion**: The results revealed that nanocrystallization of ONH may enable faster drug release at pH 6.8.

Keywords: Design expert software; Kollidon VA64®; Nanocrystals; Ondansetron HCL; Solubility.

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INTRODUCTION

One of the biggest factors influencing the quality of the patient's life is chemotherapy-induced nausea and vomiting (CINV). Up to 40% of cancer patients experience severe physical and psychological symptoms of CINV, which is a major cause for their refusal to continue receiving chemotherapy. (Gupta *et al*., 2021) To address this, various antiemetic medicines with various mechanisms of action have been developed for CINV; most of these are usually administered as preventive drugs. Nowadays, 5-hydroxytryptamine (5-HT3) receptor antagonists, NK1 receptor antagonists and corticosteroids are the most commonly used drugs with anti-emetic and anti-nausea effects. Ondansetron HCl (ONH) is the first in a class of (5-HT3) receptor antagonists used to prevent nausea and vomiting related to initial and subsequent rounds of emetogenic cancer chemotherapy, radiation therapy, anesthesia and after surgery. (Aapro *et al*., 2018) The mechanism of action involves blocking serotonin by inhibiting its binding to certain nerves in the peripheral and central nervous system, which can cause nausea and vomiting. (Zhong *et al*., 2021) ONH has a poor bioavailability of around 60% in healthy persons after oral administration of a single 8-mg tablet since it is poorly soluble and undergoes a first-pass metabolism in the liver (Fakhfouri *et al*., 2019). The nanosizing method can be utilized to enhance the oral bioavailability of poorly soluble drugs, as the reduction in particle size leads to enhancement of the saturated solubility of the drug, since below the critical size (1-2 μm) the saturated solubility is a function of the particle size. The dissolution rate also increased due to surface area enlargement (Nugroho *et al*., 2023). Drug NCs are colloidal dispersions of tiny drug particles with a crystalline structure that are smaller than 1 μm, consisting of pure active pharmaceutical ingredients (APIs) with no carriers and are stabilized by a surfactant or polymer. (Pelikh O *et al*., 2018) One of the many benefits of using NCs is reducing a variety of undesirable effects of some excipients by using less stabilizer amount in the formulation and reducing drug administration doses (Lhaglham P *et al.,* 2024). This work aims to prepare ONH-NCs for increasing its dissolution rate as a preliminary study to be prepared as a sublingual film to avoid first-pass metabolism, a nanoprecipitation process was used for the preparation of such NCs with the aid of a D-optimal Design Expert software.

MATERIALS AND METHODS

Materials

Ondansetron HCl was purchased from Hangzhou Hyperchemical Limited, China was used. Additionally, the

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following chemicals were used: Soluplus® (D. BASF, Germany), Kollidon VA 64® (Baoji Guokang Bio technology Co. Ldt, China), Brij 35® (Loba Chemie Pvt. Ltd.). All the reagents were of analytical grade.

Optimization of ONH-NCs

It is essential to identify probable material property and process parameters and to understand how they affect the intended quality of the final product. In this research, D-optimal response surface design expert software version 13.0 (Stat-Ease Inc., Minneapolis, MN, USA) was used to optimize ONH-NCs. Three discrete numerical factors with one categorical factor were selected to examine their effects on the final product. The four parameters in question were the stabilizer to drug ratios (X_1), solvent to anti-solvent ratios (X_2), stirring rate (X_3), and the stabilizer type (X_4) as displayed in table.1

The levels of independent variables were selected to provide suitable practical NCs processing. Two responses, PS represented by (Y_1) and PDI represented by (Y_2) were chosen to be monitored closely for the optimization of the components under study. To attain the best overall desirability value, ONH-NCs formulations were tuned for the two responses to obtain the lowest PDI and smallest PS. The optimal ONH-NCs were then prepared using the best independent variables for further evaluation.

Table. 2 displays 31 formulations of experimental runs produced by the design-expert program, five of which were replicated. All data were fitted to response surface models that were quadratic, two-factor interaction (2FI) and linear. Statistical metrics, including *p-value*, lack-of-fit *p-value*, adjusted multiple correlation coefficient (Adjusted R^2), predicted multiple correlation coefficient (predicted-R^2) and multiple correlation coefficient (R^2) were evaluated to confirm the significance of the chosen model by automatically using analysis of variance (ANOVA) as shown in table 3. The model with the highest Adjusted-R^2 and Predicted-R^2, along with the least variation between the two parameters and a negligible lack of fit, was considered.

Table 1: The independent and dependent variables (responses) were used to optimize ONH-NCs

Numerical factors	Applied levels
X_1 Stabilizer: drug ratio(mg/mg)	0.25 0.5 0.75
X_2 solvent: antisolvent ratio (ml/ml)	1:2.5 1:5 1:7.5
X_3 Stirring rate (rpm)	700 1000 1300
Categorical Factors:	Applied Levels:
X_4 Type of stabilizers	Soluplus, Brij 35, Koli 64
Response:	Optimization Goals:
Y1 Particle size (nm)	Minimize
Y2 Poly dispersity index	Minimize

Choosing the optimal formulation

Significance analysis was conducted on the selected response prediction model and 3D plots for each response were produced. The ideal values for the variables that are dependent were determined by applying the criteria of lowest values of PS and PDI. PDI depicts the PS distribution width and values nearer 0 denote monodisperse NC distributions. The desirability function was used to conduct graphical and numerical evaluations for optimization. According to reports, responses that are closer to 1 on the desirability scale represent the most desired reaction. (Bakhaidar *et al*., 2022) Following the selection of the best ONH-NCs preparation, a physical mixture (PM) was prepared from its constituents, and then additional investigations were done.

Preparation of optimized ONH-NCs suspensions

ONH-NCs were prepared, as per the experimental design, via the nanoprecipitation process in a bottom-up manner. The organic phase was prepared by dissolving 5 mg of ONH (equivalent to 4 mg ondansetron) in 2 ml methanol and then dropped utilizing a syringe pump (PG 907s, Proetti, Germany) at a rate of 1 ml/3min into an aqueous phase that was previously prepared by dissolving different amounts (1.25mg, 2.5mg and 3.75 mg) of biocompatible and FDA approved stabilizers such as Soluplus®, Brij 35® and Kollidon VA 64® (Koli 64®) in different volume of distilled water (5ml, 10ml, 15ml) with continuous stirring rates of either 700, 1000, or 1300 rpm on a hotplate magnetic stirrer (Joanlab®, China) set to 25º C (room temperature).

Preparation of freeze-dried ONH-NCs

The desirable ONH-NCs suspension obtained by the experimental design was frozen by nitrogen for 10 minutes at -70◦C. After that, the frozen nanosuspension was lyophilized for further characterizations using a lyophilizer (Martin Christ Alpha 1-2 LDplus freeze dryer, Germany) set to - 50◦C and 0.021 mbar vacuum for 12 hours as main drying, which was followed by the final drying at 0◦C and 6.2 mbar vacuum for 6 hr. to get lyophilized ONH-NCs.

Characterization of ONH-NCs

Zeta potential, PDI and crystals size

Zeta potential (ZP), (PDI) and PS, of 1ml ONH-NCs suspensions ascertained by utilizing the Zetasizer Instrument (Malvern Instruments, spectris company, UK). Triplicate measurements were made at 25°C with a 90° light scattering angle. The three replicates' mean value ± SD was determined. (Alhagiesa *et al*., 2020)

Dissolution studies

The *in-vitro* drug release profiles of the pure ONH powder, optimized ONH-NCs and its PM were carried out using a USP paddle dissolution apparatus (Copley 8000, UK). The samples were placed in dialysis bags prepared by previously soaked dialysis membrane (MYM company,

USA) in dissolution media for 24 hr. Dissolution investigations were conducted at 37◦C in 500 mL of phosphate buffer solution at pH 6.8 (pH of the oral cavity). Aliquots of 5 mL were withdrawn at different time intervals (5, 10, 15, 30, 45, 60, 75 and 90 min) and replaced with equal volumes of fresh medium. A UV spectrophotometer was used to examine the samples for drug release at 310 nm wavelength. The drug release profiles of the studied samples were compared using the similarity factor (*f2*) and all the obtained measurements were performed in triplicate (n = 3). (Noor *et al*., 2020).

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) of pure ONH, Koli 64, the optimal lyophilized NCs and their PM was determined by FTIR-7600 Emission spectrometer (Lmda Scientific System, Inc, Australia Dry potassium bromide was used to grind with the sample (3-5 mg). The prepared sample was scanned with a wave number ranging from 4000 to 400 cm^{-1}.

Differential scanning calorimetry

The differential scanning calorimetry (DSC) of pure ONH, Koli 64, lyophilized ONH-NCs and their PM was examined by DSC (DSC-60plus, Shimadzu, Japan) Approximately (3-5) mg of each sample was placed in a pierced aluminum pan and the thermal profiles were documented at a scanning rate of 10°C/min from 0 to 300°C.

X-ray diffraction

X-ray diffraction (XRD) of ONH powder, Koli 64, lyophilized ONH-NCs and their PM was determined by X-ray diffractometer (AERIS, Malvern PANalytical, Netherlands) with a current of 7.5 mA and voltage of 40 kV was used to acquire XRD profiles.

Morphological characterization

Structural morphology analysis of the selected ONH-NCs was observed by Field Emission Scanning Electron Microscopy (FESEM) operated at a voltage of 30 kV (1-2 mg) of ONH-NCs suspension dropped on a carbon tab and coated with gold by sputter coater. After that, the holder was positioned in the vacuum chamber of the FESEM (INSPWCTTM F50, FEI, Netherlands) and images were acquired at various magnification powers.

Statistical analysis

All the *in-vitro* studies were conducted in triplicate and the results were reported as mean ± standard deviation. To conduct multiple comparisons, a one-way analysis of variance (ANOVA) was used. A *p-value* less than 0.05 was deemed to be statistically significant.

RESULTS

Analysis of PS and PDI

The PS and PDI of 31 ONH-NCs prepared formulations, summarized in table 2, were evaluated by a design expert with a *p-value* of (< 0.0001), and the lack of fit *p-value* was (0.210), implying the model is significant. The evaluation of the effect of stabilizer-to-drug ratio (X_1), solvent-to-antisolvent ratio (X_2), stirring rate (X_3), stabilizer type (X_4) and their linear interactions on the resultant ONH-NCs yielded a PS ranging from 99 to 409 nm and PDI ranged from 0.08 to 0.44 for all 31 experimental runs, as shown in Figs. 1, Figs. 2 and Table 3.

Table 3 shows the three parameters that had the greatest significant impact on PS and PDI ($p < 0.0001$for all 3 factors) were the stabilizer amount in the aqueous phase (X_1), ($p < 0.0001$, 0.039 respectively), solvent-to-antisolvent ratio (X_2) (*p-value* = 0.006 and 0.0003 respectively). and the stabilizer type (X_4) ($p = 0.001$ and 0.012, respectively). Experimental runs prepared with Koli 64 as the stabilizer showed smaller PS and PDI values than Soluplus and Brij 35. However, neither PS nor PDI was affected by the stirring rate (X_3) since the *p-value*s appear to be more than 0.05 (0.94 and 0.21, respectively).

Selection of the optimal ONH-NCs formulation

The optimal ONH-NCs formulation with the required criteria was achieved by using the analysis of the D-optimal response surface design. Based on the analysis results, the most desirable ONH-NCs formulation, which exhibits a desirability value of 0.953 in (Fig. 3), was prepared using Koli 64 as a stabilizer in a 0.75:1 Koli 64 to ONH ratio in 15 ml of distilled water and the formulation was stirred at 700 rpm. Validation experiments confirmed the model's accuracy, with no significant differences ($p > 0.05$) observed between the predicted values from the D-optimal Design-Expert and the actual experimental results for the optimized formulation (Table 4). After preparing this formula, it was subjected to lyophilization. Additional characterization of the lyophilized powder was carried out afterward.

Zeta potential analysis (ZP)

The surface charge of the optimal ONH-NCs by measuring the zeta potential. The ONH-NCs nanosuspension showed a ZP of -8.45±1.4mV.

Dissolution study

As illustrated in Fig. 4, the release profiles of ONH-NC formulations showed a superior improvement. After 90 minutes, ONH-NCs exhibited a release of 93.3±2.8%. In contrast, the pure drug and PM released only 40.1±1.5% and 46.6±0.9 %, respectively, within the same media and time. A comparison was conducted between the release patterns of ONH-NCs, PM, and ONH pure powder, which is used as a reference, by using the similarity factor *f2*. The resulting similarity factor *f2* value was 19.9 between pure ONH and ONH-NCs, while *f2* was 64.5 between pure ONH and the PM.

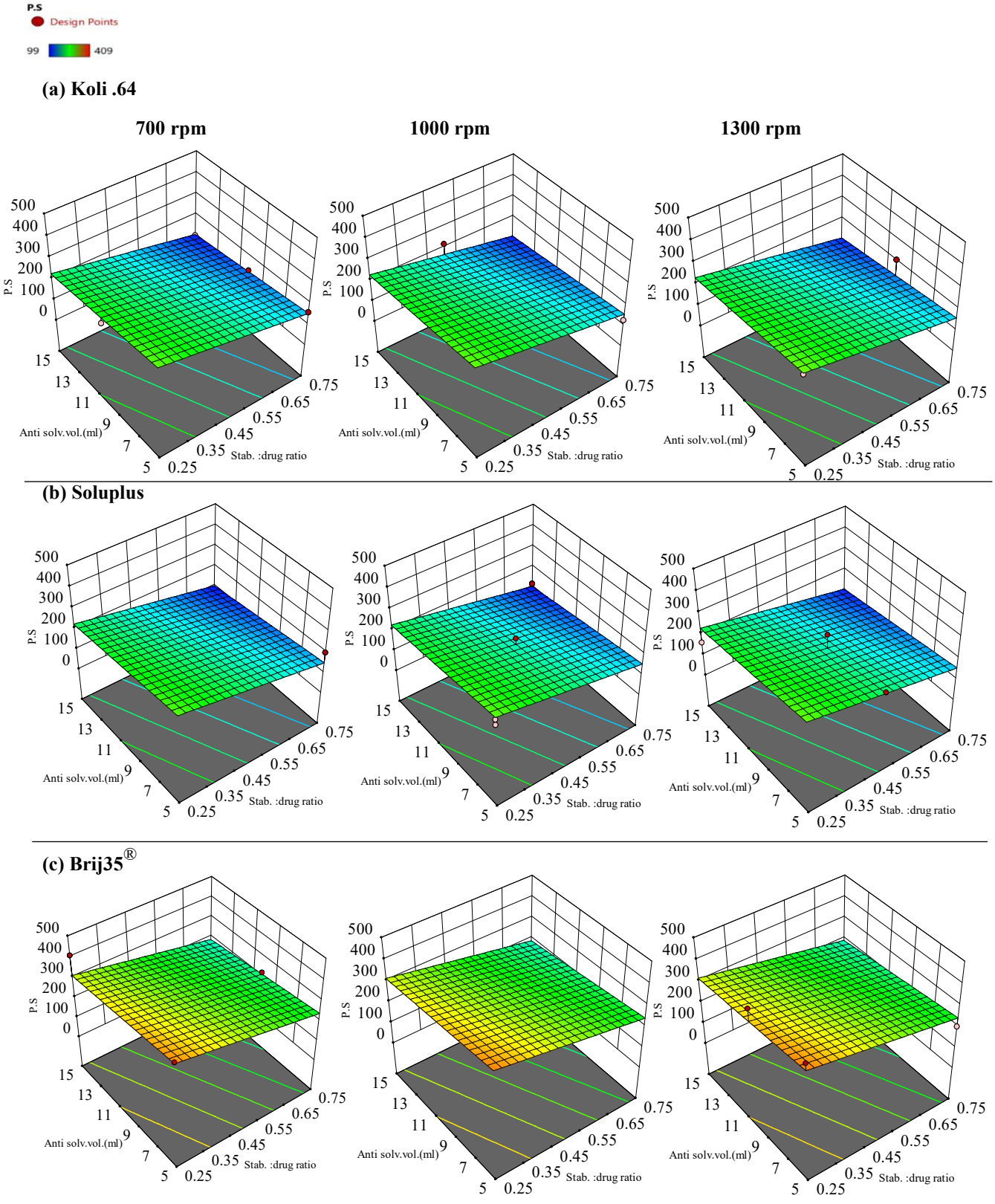


Fig. 1: 3D graph shows the effect of the independent variables: stabilizer: drug ratio and antisolvent volume on PS at different stirring rates using different stabilizers. (a) Koli 64, (b) Soluplus®, and (c) Brij35®.

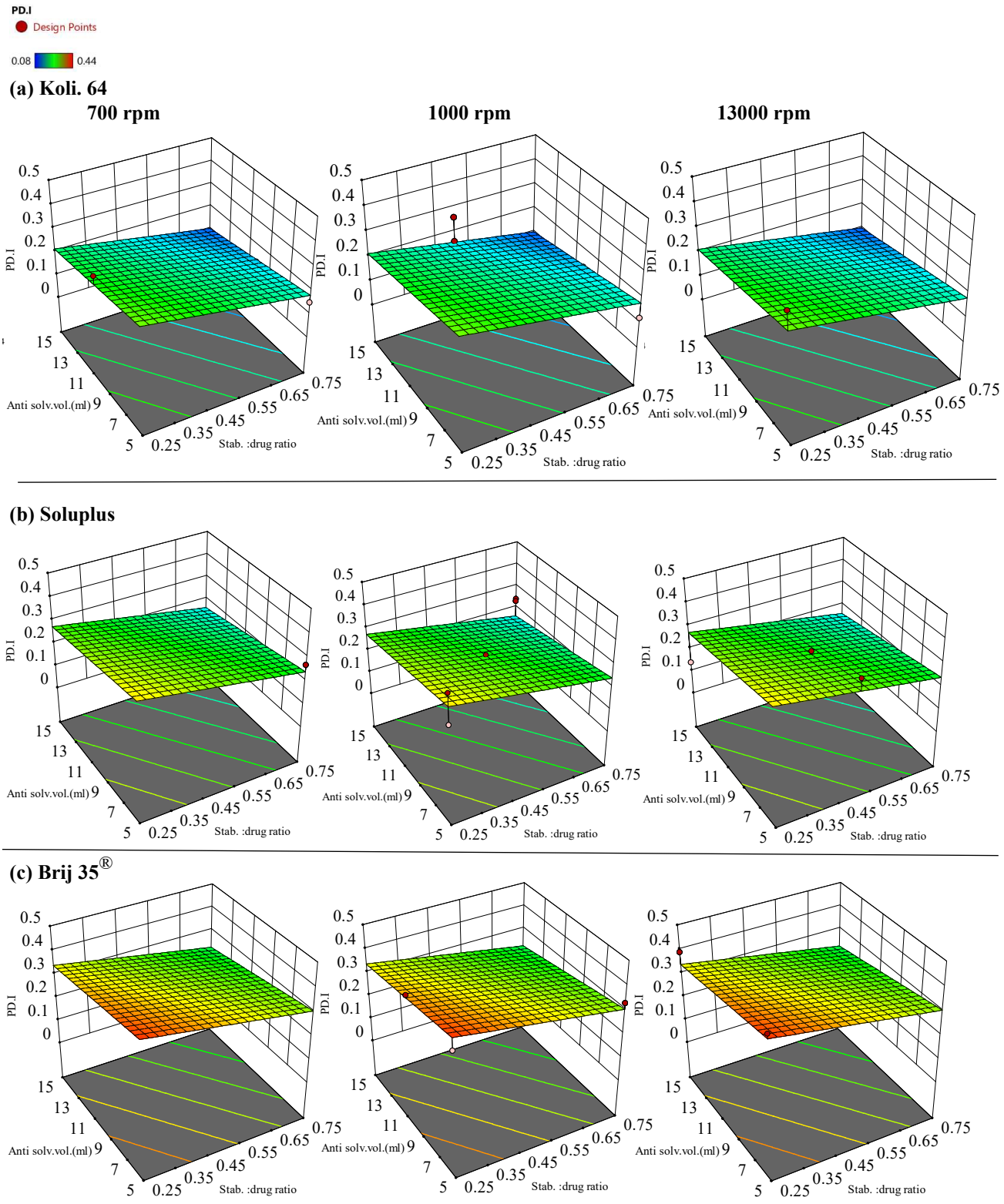


Fig. 2: 3D graphs show the effect of the independent variables: stabilizer: drug ratio and antisolvent volume on PDI at different stirring rates using different stabilizers. (a) Koli 64, (b) Soluplus®, and (c) Brij35®

Table 2: Design runs and the dependent responses for the optimization of ONH-NCs.

F	X_1(mg/mg)	X_2 (ml/ml)	X_3 (rpm)	X_4	Y_1 (nm)	Y_2
F_1	0.25	1:5	700	Koli 64	229±3	0.31±0.02
F_2	0.75	1:2.5	1000	Koli 64	234±12	0.33±0.06
F_3	0.75	1:5	700	Brij 35	222±7	0.24±0.02
F_4	0.75	1:2.5	1300	Brij 35	203±4	0.33±0.05
F_5	0.5	1:7.5	1000	Koli 64	135±6	0.16±0.06
F_6	0.25	1:5	1300	Brij 35	401±37	0.39±0.05
F_7	0.75	1:7.5	1000	Soluplus	115±8	0.20±0.11
F_8	0.25	1:7.5	700	Brij 35	409±43	0.39±0.07
F_9	0.75	1:5	1000	Koli 64	115±7	0.23±0.04
F_{10}	0.75	1:5	1300	Koli 64	142±3	0.12±0.04
F_{11}	0.5	1:7.5	700	Soluplus	134±2	0.18±0.01
F_{12}	0.5	1:5	1000	Brij 35	210±7	0.36±0.01
F_{13}	0.5	1:5	1000	Soluplus	236±7	0.30±0.01
F_{14}	0.75	1:7.5	1300	Brij 35	154±9	0.21±0.03
F_{15}	0.25	1:2.5	1000	Soluplus	208±49	0.31±0.04
F_{16}	0.5	1:7.5	1000	Koli 64	224±56	0.21±0.11
F_{17}	0.25	1:7.5	1300	Soluplus	169±21	0.27±0.01
F_{18}	0.25	1:2.5	700	Brij 35	401±91	0.37±0.06
F_{19}	0.25	1:2.5	1300	Koli 64	280±4	0.34±0.07
F_{20}	0.5	1:7.5	700	Soluplus	130±14	0.15±0.07
F_{21}	0.75	1:5	700	Koli 64	134±8	0.17±0.10
F_{22}	0.5	1:2.5	1300	Soluplus	228±9	0.29±0.03
F_{23}	0.25	1:2.5	1300	Brij 35	372±66	0.44±0.02
F_{24}	0.5	1:5	1300	Soluplus	256±22	0.35±0.01
F_{25}	0.75	1:2.5	700	Soluplus	202±13	0.25±0.11
F_{26}	0.5	1:5	1000	Koli 64	169±9	0.16±0.04
F_{27}	0.75	1:2.5	700	Koli 64	159±5	0.15±0.04
F_{28}	0.25	1:2.5	1000	Soluplus	245±12	0.25±0.03
F_{29}	0.75	1:7.5	700	Koli 64	99± 10	0.08±0.02
F_{30}	0.75	1:7.5	1000	Soluplus	116±4	0.24±0.02
F_{31}	0.5	1:5	1000	Brij 35	226±18	0.31±0.05

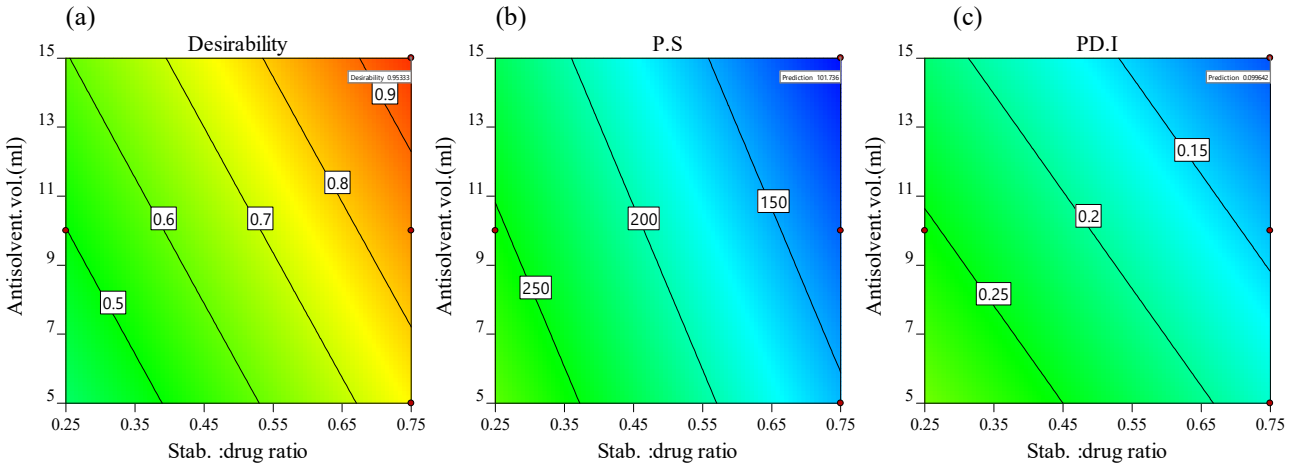


Fig. 3: Contour graph showing (a) the desirability values, (b) the predicted PS, and (c) predicted PDI of the studied factors used in the optimization of ONH-NCs using Koli 64 as a stabilizer at 0.75:1 stabilizer: drug ratio, 1:7.5 solvent : anti solvent ratio, and 700 rpm as a stirring rate.

Table 3: Outcomes of the designed formulations.

Responses	Model	R^2	Adjusted R^2	Predicted R^2	Model *p-value*	Lack of fit *p-value*	*p-value* of ind. factors
P S (nm)	Linear	0. 718	0.661	0.544	< 0.0001	0.100	X_1 < 0.0001
							X_2 0.006
							X_3 0.948
							X_4 0.001
PDI	Linear	0.701	0.581	0.434	< 0.0001	0.135	X_1 0.039
							X_2 0.0003
							X_3 0.215
							X_4 0.012

Table 4: Responses of the predicted and optimized ONH-NCs.

Variable	Optimized values	responses	Predicted values	Observed values
X_1	Stabilizer: drug ratio 0.75:1	PS	101.7	99 ± 10
X_2	Solvent: antisolvent ratio 1:7.5	PDI	0.099	0.08±0.02
X_3	Stirring rate 700 rpm			
X_4	Stabilizer type Koli 64			

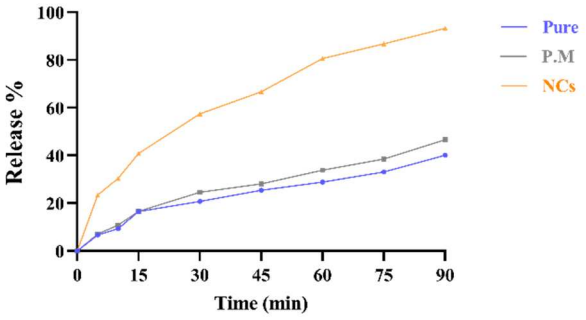


Fig. 4: The dissolution profile of Pure ONH, PM, and ONH-NCs.

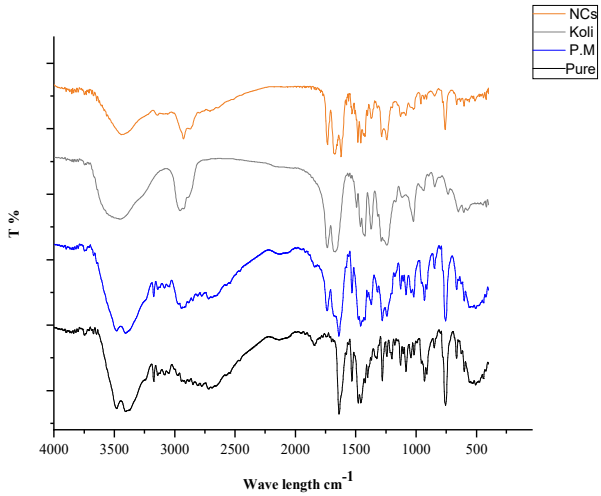


Fig. 5 FTIR of pure ONH, PM, Koli 64, and ONH-NCs.

Fourier transform infrared spectroscopy

Fig. 5 displays the FTIR spectra of pure ONH, Koli 64, ONH-NCs and its PM. The FTIR of ONH reveals characteristic bands at 3482.9 cm^{-1} of (NH) group and 1622 cm^{-1} of carbonyl (C=O) group, which are in accordance with previous results (Noor *et al*., 2020). The FTIR of PM of the drug and Koli 64 showed no significant shift or reduction in the intensity of ONH peaks. However, the FTIR of ONH-NCs showed a broadening of the OH peak at 3482.9 cm^{-1}

Differential scanning calorimetry

The DSC of pure ONH exhibited sharp endothermic peaks at 122.17°C and 176.35°C, as shown in fig.6.a, which are in agreement with previous results (Hasian *et al*., 2023). The thermogram of PM showed endothermic peaks within the same range as that of the pure drug with decreased intensity fig.6.b. On the other hand, the thermogram of lyophilized ONH-NCs in fig.6.c showed an endothermic peak at 183.25°C. with decreased intensity.

X-ray diffraction

The findings of XRD analysis are depicted in fig.7. The XRD pattern of ONH showed a characteristic peak at 2θ at 12°, 17°, 23°, 28°, and 30°, as previously reported (Noor *et al*., 2020). While a hollow pattern for Koli 64 was obtained. The XRD of PM. exhibited the same peaks as that of ONH but with less intensity. The diffraction pattern of lyophilized ONH-NCs showed the appearance of the major peaks of ONH, but with a further reduction in the intensity of the major peaks.

Morphological characterization

In the FESEM monograph, particles appear as spherical shapes with a narrow distribution size. As shown in fig.8.a. In contrast, the FESEM of pure ONH exhibits an irregular crystalline shape with PS ranging from 24 µm to 59 µm, Fig. 8b.

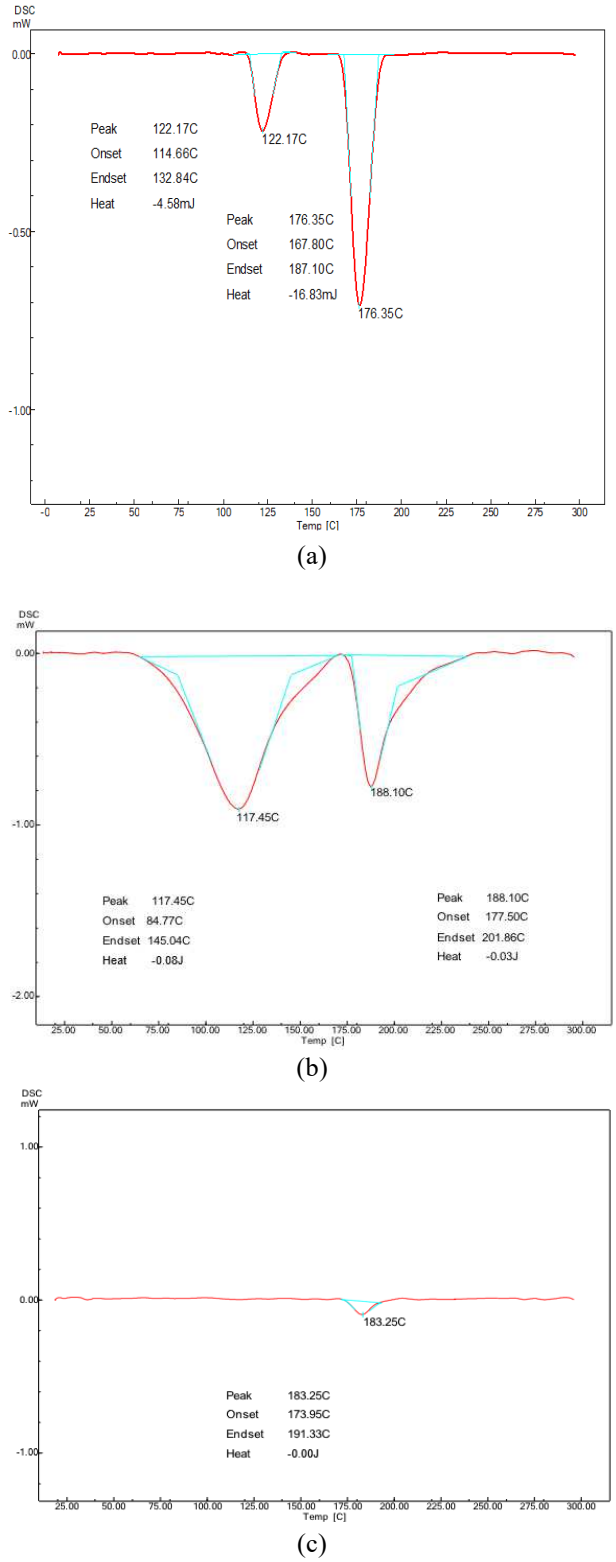


Fig. 6: DSC of (a) pure ONH, (b) PM, and (c) ONH-NCs.

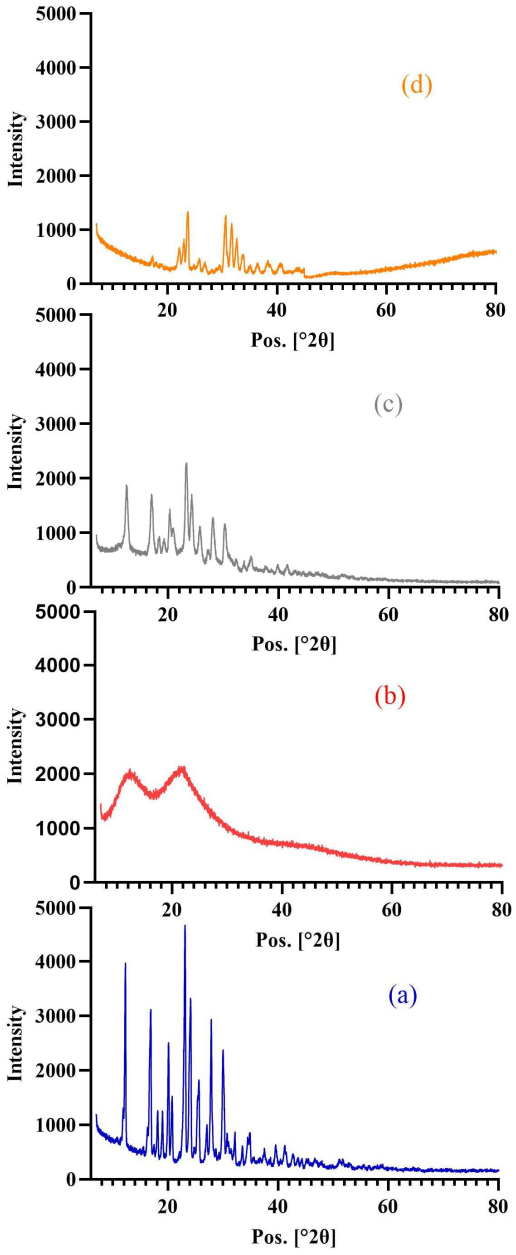
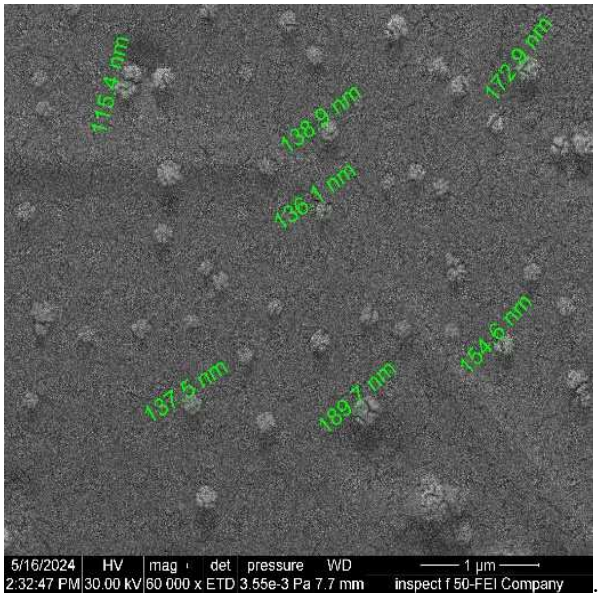


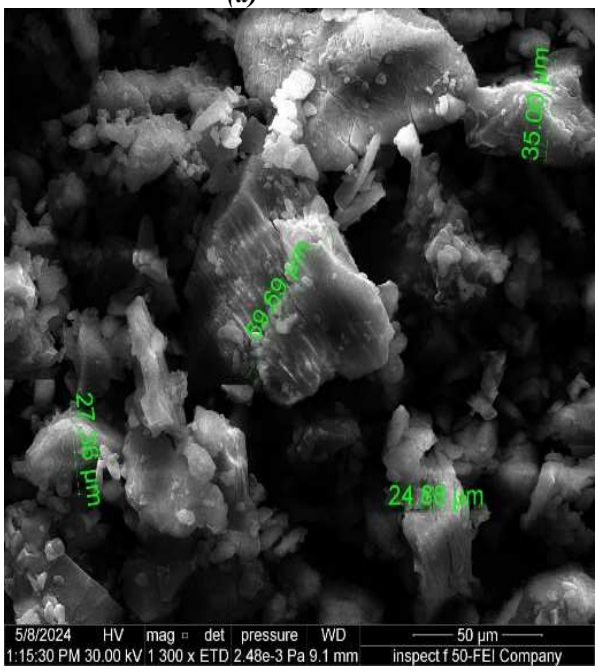
Fig. 7: XRD of (a) pure ONH, (b) Koli 64, (c) PM, and (d) ONH-NCs.

DISCUSSION

The formulation variables showed distinct effects on the characteristics of ONH-NCs. The stabilizer-to-drug ratio (X1) played a crucial role in reducing PS and narrowing the PDI. Increasing the stabilizer concentration likely enhanced the surface coating of drug particles, thereby inducing steric repulsion and preventing particle aggregation. This effect supports the improved uniformity observed across the formulations. (Pelikh O *et al*., 2018, Muhesen *et al*., 2023)



(a)



(b)

Fig. 8: FESEM of (a) ONH-NCs and (b) pure ONH

The solvent-to-antisolvent ratio (X2) also significantly influenced PS and PDI. Higher antisolvent volumes promoted rapid supersaturation and nucleation, producing smaller NCs, whereas lower antisolvent volumes allowed crystal growth, yielding larger particles (Wu *et al*., 2022). This balance between nucleation and growth kinetics highlights the importance of precise solvent–antisolvent ratios for controlled PS reduction.

In contrast, stirring rate (X3) did not significantly affect PS or PDI; the results agreed with a prior study (Tanaka *et al*., 2023). The type of stabilizer (X4) used in the preparation of the NCs had a significant impact on PS and PDI since different stabilizers have varying affinities for ONH particles. This difference may be due to some physicochemical properties like the molecular weight since stabilizers with a high molecular weight like Soluplus® (180,000 g/mol) may cause NCs bridging because of their long molecular chain while stabilizers with a low molecular weight like Brij® 35 (1199 g/mol) difficult to create a physical barrier that prevents particles from being mutually attracted to each other (Choi JY *et al.,* 2008). Koli 64, with a molecular weight of (45,000-70,000 g/mol), was the most suitable one.

Characterization of the optimized formulation further supported these observations. The low zeta potential value (of -8.45±1.4m). The stability of NCs contributes to the steric hindrance of the Koli 64 tails, which may cause repulsion of the particle. (Gol D *et al*., 2018)

Dissolution studies confirmed a markedly improved release profile of ONH-NCs compared to pure drug and the PM, which can be explained by the increased surface area-to-volume ratio and enhanced wettability of NCs. This enhancement in dissolution rate agreed with that obtained from cilnidipine NCs. (Al Hazzaa *et al.,* 2023). On the other hand, a similar dissolution profile was obtained by pure ONH and the PM, indicating that the presence of a stabilizer in its dry form does not affect the release of the drug.

Spectroscopic and thermal analyses also provided evidence of molecular interactions and reduced crystallinity. FTIR spectra suggested hydrogen bonding between ONH and Koli 64, supporting the stabilizing role of the polymer. DSC and XRD analyses demonstrated reduced crystallinity in ONH-NCs compared to pure ONH, confirming successful nanosizing and stabilization. (Khan *et al*.) (Najm *et al*., 2015). Finally, FESEM images revealed spherical and homogeneously distributed nanoparticles, contrasting with the irregular and larger crystalline particles of the pure drug.

CONCLUSION

The study results indicate the possibility of applying NCs technology to enhance the solubility and dissolution rate of ONH, which was successfully optimized by using the previously described experimental design. The optimized formula was prepared using Koli 64 as a stabilizer in a ratio of 0.75:1 stabilizer: drug, 1:7.5 solvent- anti-solvent and 700 rpm. The crystallinity of the final product was confirmed by DSC and XRD.

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Authors' contributions

Zainab A. Almusawi contributed to the D-optimal design expert®, data collection and statistical analysis. Eman B.H. Alkhedairy contributed to the study concept, supervised the project, interpreted the data and critically revised the manuscript for important intellectual content. Both authors contributed to drafting the manuscript, reviewed the final version and approved it for submission.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval

According to the regulations of the Institutional Research Committee at College of Pharmacy / University of Baghdad ethical approval was not required for this in-vitro study involving no human or animal subjects; hence, no ethical approval number is applicable.

Conflict of interest

The authors declare that they have no conflicts of interest.

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