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Design, Development and Characterization of Vinorelbine as a Nano-Lipid Carrier (NLC)

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Abstract

A full factorial 2^3 design was used to study the effect of independent variables, in which eight formulas were prepared by emulsification-ultrasonication method using different concentrations of poloxamer 188, the concentration of soya lecithin, time of sonication, the mean particle size and entrapment efficiency were measured as responses. A drug release study was performed using a dialysis membrane diffusion process for the prepared formulas (Vn-NLC1 to 8), in which formulas Vn-NLC 3, Vn-NLC 4, Vn-NLC 7 and Vn-NLC 8 gave the slowest burst effect and continued for 48 hours; however, Vn-NLC 7 had smallest mean particle size, polydispersity index and high entrapment efficiency and zeta potential were 88 ±3 nm, 0.018 ±0.006, -32.2 ±1, and 92.54% respectively, so it was chosen as optimum formula. The small size and sustained release character of the optimum Vn-NLC 7 formula may give a better accumulation in lung tissue for a longer time**,** so greatly enhance vinorelbine activity and reduce toxicity.

Keywords: Vinorelbine, nano-lipid carrier (NLC), lung cancer, Minitab, Plackett-Burman design, factorial design

Introduction

Lung cancer creates a significant health problem worldwide, considered the second most frequent cancer in women and men and the most common cause of cancer-related deaths, probably related to the lack of earlier diagnosis. Having an 18.6% five-year survival rate, half of patients die within the first year due to a lack of specific symptoms at an early stage and are diagnosed at an advanced stage. Broadly, lung cancer is classified into non-small lung cancer (most common, accounting for 85% of cases) and small lung carcinoma. Chemotherapy is preferred at all stages of the disease and even after surgery to reduce relapse and metastasis. However, they are inefficient because only a small fraction of the administered dose reaches affected tissues and does not stay long enough to produce sufficient tumor eradication [1, 2]. Lipid vesicles were used as carriers to enhance drug penetration by mimicking the properties of cell membranes that facilitate drug penetration across the biological membrane and ensure that the encapsulated drug reaches the target site in an efficient and controlled manner. In addition, the lipid vesicles improve drug retention at the target site, in which the lipophilic nature of these carriers enables them to interact with cellular membranes, resulting in prolonged residence time and so reducing systemic side effects and improving therapeutic outcomes [3]. Among these is a nano-lipid carrier (NLC), in which a liquid (oil) and solid lipid are used instead of just one lipid. It has various advantages, including controlled release, biodegradability, improved targeting, high stability in different conditions with the capability to form a concentrated dispersion, and high entrapment efficiency of both lipophilic and hydrophilic compounds [4]. NLC is a new generation of formulation that offers flexibility in loading efficiency, drug release, and improved performance of the final dosage form [5].

Vinorelbine is a semisynthetic vinca alkaloid (BCS class II) with a partition coefficient (LogP 4), dissociation constant (pka 5.4) and broad antitumor activity as well as 40% oral bioavailability [6]. Its structure is shown in Figure 1. It has a higher efficacy than other vinca alkaloids against non-small lung cancer, exerting an antitumor effect through inhibition of microtubule assembly by binding tubulin and preventing cell division. It has extensive extravascular distribution with a large volume of distribution from 25-40 L/kg; the highest amount occurs in excretory organs like the liver and kidney. Successful cancer killing by vinorelbine usually requires long-term exposure and retention in these cells [7].

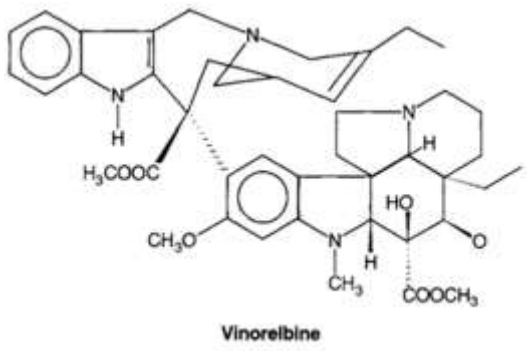

Figure 1. Vinorelbine chemical structure [8].

This work aims to apply a full factorial to design and suggest an optimized formula to be prepared and evaluated to have a nano-lipid carrier (NLC) for vinorelbine to increase drug penetration and retention in tumor tissue so as to achieve better antitumor activity that may lower dose-related side effects.

Materials and methods

Materials

Vinorelbine (Vn) was purchased from Hefei TNJ Chemical Industry Co., Ltd (Anhui, P.R.China), Glyceryl monostearate (GMS), soybean lecithin (using parenterally), octanoic acid oil (O.A.) were purchased from Hangzhou Hyper Chemicals Limited (Hangzhou, China), Pluronic F68 (poloxamer 188) was purchased from BASF (Ludwigshafen, Germany), Amicon 15 centrifugal filters and Millex syringe filter (0,45µm and 0,22 µm) were purchased from Sigma-Aldrich International GmbH (Taufkirchen, Germany). All other reagents and chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Method

Determination of drug melting point

Vinorelbine melting point was determined using the capillary tube as stated by the USP by melting point instrument (Stuart.SMP3. UK.) [9]. In addition, differential scanning calorimetry (DSC) was used to verify the pure drug's thermal behavior using DSC-60 (DSC-60 Shimadzu.Japan).

Calibration curve construction

The vinorelbine calibration curve was prepared in pH 7.4 of phosphate buffer saline (PBS) as well as in ethanol. A series of the drug concentrations (4, 8, 12, 16, 20, 24, 28, 32, 36, 40 µg/mL) were prepared from its stock solution (1 mg/ml). The samples were analyzed spectrophotometrically (Shimadzu 1800, Japan) at the determined λ max [10].

Selection of the best solid lipid and liquid lipid /oil

For the nanocarrier preparation, selecting the most appropriate lipid depends on the best vinorelbine solubility in different lipids (solid and liquid).

Solubility study for vinorelbine in different liquid lipids/oils (liquid phase)

The solubility of vinorelbine in various liquid lipids/oils (oleic acid, soybean oil, medium chain triglyceride, octanoic acid, olive oil, and triacetin) was determined by adding an excess amount of vinorelbine (20mg/mL) and shaking reciprocally at 100 rpm, 25±0.5° C for 48 hrs. in a shaking water bath after vortex mixing for 10 minutes. Then, the supernatant was filtered by a 0.45 µm filter syringe [11], diluted with ethanol, centrifuged at 4000 rpm for 20 minutes, and measured by UV-visible spectroscopy at 268 nm to determine the concentration of vinorelbine in each oil [12].

Solubility study for vinorelbine in different solid lipid

The solubility of vinorelbine in different solid lipids (stearic acid, palmitic acid, and GMS) was determined under normal light using the naked eye to visualize the clear solubilized vinorelbine in melted lipid, in which the vinorelbine was added in specific amounts to the lipid, which is heated at 3°C above its melting point in a controlled temperature water bath [13].

Screening for the best Surfactants and Co–surfactant

Different surfactants and co-surfactants (Tween ®20 and Tween ®80, poloxamer 188, cremophore E.L., transcutol, soya lecithin, propylene glycol, and PEG 400 were) screened for emulsification ability. Briefly, 150 mg of oily phase was added to 150 mg of each surfactant and co-surfactant with gradual heating at about 50 ºC to assist in mixture homogenization. From each mixture, 100 mg was diluted with 100 mL of deionized water at the same temperature in a conical flask, and the emulsification ability was determined by the number of flask inversions required to yield a homogenous emulsion. Then, after two hours of standing, the transmittance was determined at 650 nm using a visible spectrometer against deionized distilled water as blank [14].

Preliminary experimental parameters screening using a Plackett-Burman design

To start with a preliminary experimental parameters screening, which was done by the Plackett-Burman design (part of Minitab software version 21), where seven variables (sonication power, sonication time, homogenization rate, homogenization time, ratio of solid lipid to liquid lipid phase, lecithin and poloxamer 188 concentrations) were selected to study their effects on the response (droplet size) and accordingly twelve formulations were suggested (as shown in Table 1) [15].

Table 1 Experimental parameter variables designed by Plackett-Burman design

Run order (formulation no.)	sonication power (W)	sonication time (min)	Homogeniz-ation rate (rpm)	Homogeniz-ation time (min)	Solid: liquid lipid ratio (%w/w)	Lecithin (%w/v)	Poloxamer 188 (%w/v)
P1	0.3	15	32000	5	25	0.5	0.5
P2	0.3	5	8000	25	10	0.5	0.5
P3	0.7	15	8000	5	10	0.5	0.1
P4	0.7	5	8000	5	25	0.1	0.5
P5	0.5	10	20000	15	17.5	0.3	0.3
P6	0.7	5	32000	25	25	0.1	0.1
P7	0.3	5	32000	5	25	0.5	0.1
P8	0.3	15	8000	25	25	0.1	0.5
P9	0.7	5	32000	25	10	0.5	0.5
P10	0.7	15	32000	5	10	0.1	0.5
P11	0.3	5	8000	5	10	0.1	0.1
P12	0.7	15	8000	25	25	0.5	0.1

Note: all formulas containing 10mg of vinorelbine as a drug

Method of preparation of nano-lipid carrier loaded with vinorelbine

The suggested twelve preliminary NLC formulas by Plackett-Burman were prepared by the emulsification-ultrasonication method [16], where the solid lipid (GMS) and liquid lipid (octanoic acid) mixed in different ratios in a glass beaker containing chloroform as an organic solvent, then vinorelbine (10mg) was added to a 50 mg lipid mixture. After evaporating the organic solvent using a hot plate magnetic stirrer at 75±1 ℃ (above GMS melting point) and 1000rpm, 50mL of the aqueous phase (with the same temperature) containing different ratios of poloxamer 188 (as surfactant) and soya lecithin (as co-surfactant) was added drop by drop with continuous stirring. The coarse emulsion was formed, which was then homogenized using a high-speed homogenizer with different homogenization speeds and times, followed by sonication using a high-energy ultrasonication at a different sonication power and time (on-off 4-2 sec) using a cold bath to control the temperature [17]. The effect of the seven variables (in the twelve formulas) was evaluated through their effect on droplet size (as a response).

Determination of average particle size of the prepared nano-lipid carrier loaded with vinorelbine

The average particle size of the twelve prepared nano-lipid carrier formulas (P1-P12) was analyzed at room temperature (25 ℃) using a laser particle size analyzer ABT-9000; the samples were diluted with deionized distilled water. All the results were obtained in triplicate [18].

Further ingredients optimization using a full-factorial design

Out of the seven variables suggested by the Plackett-Burman design and their corresponding effects on droplet size, the most important variables were four variables, including the concentration of poloxamer 188, concentration of soya lecithin, time of sonication and power of sonication. As the sonication power above 300 Watt may destroy the lipid system [19], so it was kept constant at 300 Watt, in addition to solid: liquid lipid ratio (at 75:25), homogenization rate and time (at 15000 rpm for 5min) were also kept constant as the chosen values. The effect of the three remaining variables on the mean particle size and entrapment efficiency (as responses) was evaluated using a simple factorial (2^3) design for further formulation optimization (as shown in Table 2). Accordingly, eight formulations (Vn-NLC1 to 8) were prepared (using a similar method as described earlier) and evaluated for their particle size and entrapment efficiency (as major responses). Then, ANOVA (one-way and two-way) was applied to analyze the effect of independent variables on the responses, and each response coefficient was studied at a 95% confidence level. The values with non-significant P values (greater than 0.05) were removed from the analysis. In addition to statistical analysis, graph plotting analysis was done, including normal plot and interaction plot [20].

Table 2. Suggested optimized formulation variables by full factorial (2^3) design for the formulation of Vn-NLCs

Run Order	Formula code	poloxamer 188 (%w/v)	soya lecithin (%w/v)	Time of ultra-sonication (min)
1	Vn-NLC1	0.1	0.1	5
2	Vn-NLC2	0.5	0.1	5
3	Vn-NLC3	0.1	0.5	5
4	Vn-NLC4	0.5	0.5	5
5	Vn-NLC5	0.1	0.1	15
6	Vn-NLC6	0.5	0.1	15
7	Vn-NLC7	0.1	0.5	15
8	Vn-NLC8	0.5	0.5	15

Characterization of the prepared nano-lipid carrier for vinorelbine (Vn-NLC1 to Vn-NLC8)

Determination of particle size, polydispersity index (PDI), and zeta potential

The average diameter and PDI of NLC were analyzed at room temperature (25 ℃) using a laser particle size analyzer ABT-9000, USA. The zeta potential was measured using Malvern zeta sizer, U.K. Before the measurement, the samples were diluted ten times with deionized distilled water. All the results were obtained in triplicate. [21].

Entrapment Efficiency Determination

A sample of 10 mL was placed in the upper chamber of a 15 mL Amicon® centrifugal tube with a 10 KDa filter from Sigma-Aldrich, Germany. The mixture was centrifuged at 3500 rpm for 15 min at 4°C. Then, 0.5 mL was taken from the lower chamber and diluted to 5 mL with ethanol. The samples were then analyzed spectrophotometrically at a wavelength of 268 nm using a Shimadzu spectrophotometer 1800 [22, 23].

Effect of the selected variables on in-vitro release study

In vitro drug release for vinorelbine from the prepared Vn-loaded NLC formulas (Vn-NLC1 to 8) was studied using a dialysis membrane diffusion process, in which 10 mL of each dispersion formula was transferred into a 12-14 KDa dialysis bag (previously soaked for 12 hr in the dissolution medium). After that, the release study was performed in 400 mL of phosphate buffer saline PBS (pH 7.4 at 37ºC) in dissolution apparatus type II stirred at 100 rpm. A 3 mL sample was withdrawn using a 0.22 µm filter syringe and replaced with fresh medium at a predetermined time interval, which continued up to 48 hours. The samples were suitably diluted and analyzed spectrophotometrically at 268 using a Shimadzu UV-spectrophotometer [24].

Selection of optimum formula

The best formula was selected on the basis of the smallest particle size, highest entrapment efficiency (%EE) and suitable drug release

Results and Discussion

Determination of the melting point of vinorelbine

The melting point of vinorelbine powder was found to be (182 °C), which agreed with that reported [25]. This indicated the purity of the drug powder. The DSC analysis also showed a single sharp peak at the drug melting point, indicating crystallinity and there is no sign of polymorphism (as shown in Figure 2).

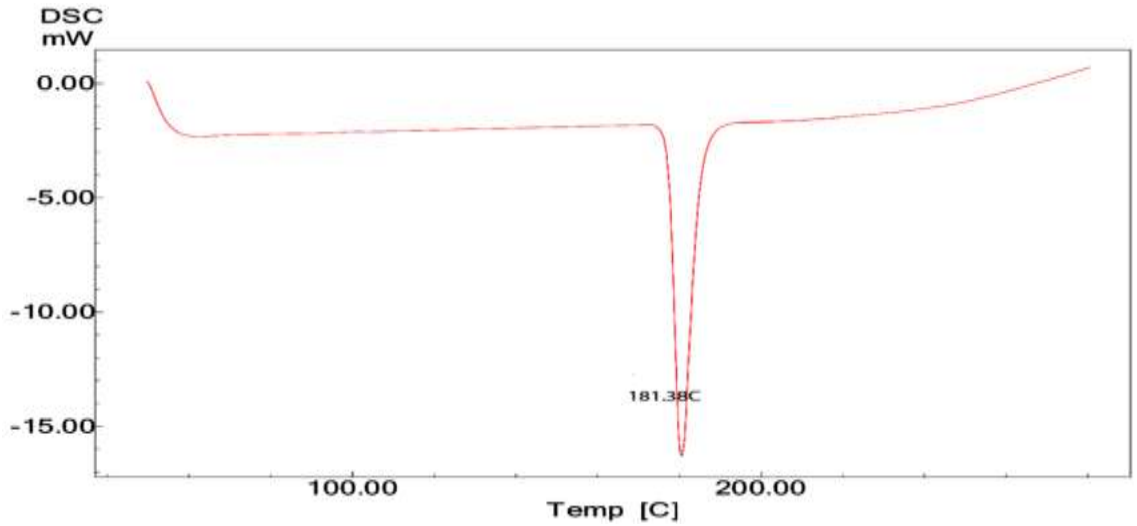

Figure 2. The DSC thermogram of the pure drug

Calibration curve construction

The calibration curves of vinorelbine in PBS at pH 7.4 and in ethanol were demonstrated in Figure 3. Straight lines were obtained by plotting absorbance versus the concentration of the drug (µg/ml). A high determination coefficient (R^2) indicated that the calibration curves obey (Beer's law) within the concentration range [26].

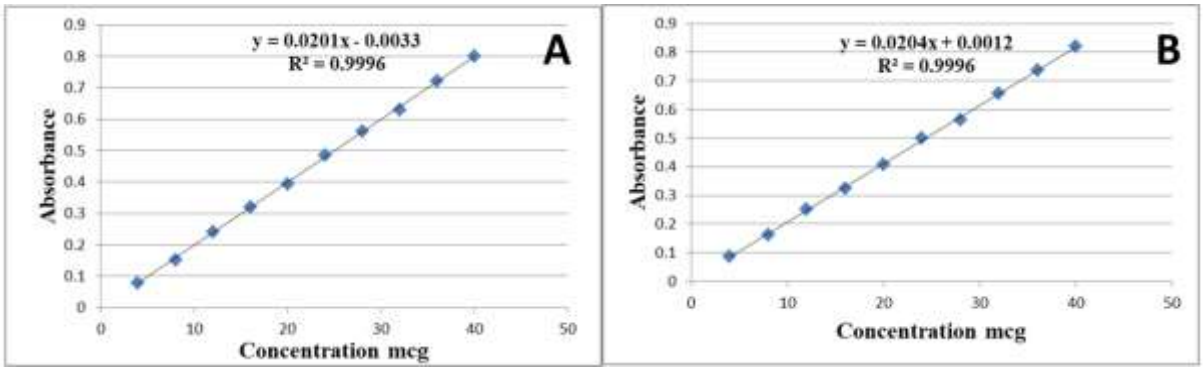

Figure 3. Calibration curves of vinorelbine in (A) ethanol and (B) PBS at pH 7.4

Vinorelbine solubility in liquid and solid lipids

GMS showed the highest solubility of vinorelbine, while octanoic acid (caprylic acid) showed the highest solubility for vinorelbine (as shown in Figure 4); GMS showed the highest drug solubility due to the long unsaturated hydrocarbon chain (C21), which is capable of physically bonding with the hydrocarbon portion of vinorelbine (lipophilic drug belonging to class II category) while the two oxygen and hydroxyl groups of the glycerol portion of GMS that capable of hydrogen bonding with many hydrogen bond donor and acceptors groups present in vinorelbine. In addition, the high solubility of vinorelbine in octanoic acid (liquid lipid) was probably due to its nature as a medium-chain fatty acid that usually shows high solubility for lipophilic compounds [27-29]. Both GMS and octanoic acid were generally regarded as safe (GRAS), non-toxic, inexpensive, in addition to the suitable melting point of GMS; therefore, both were selected in this work.

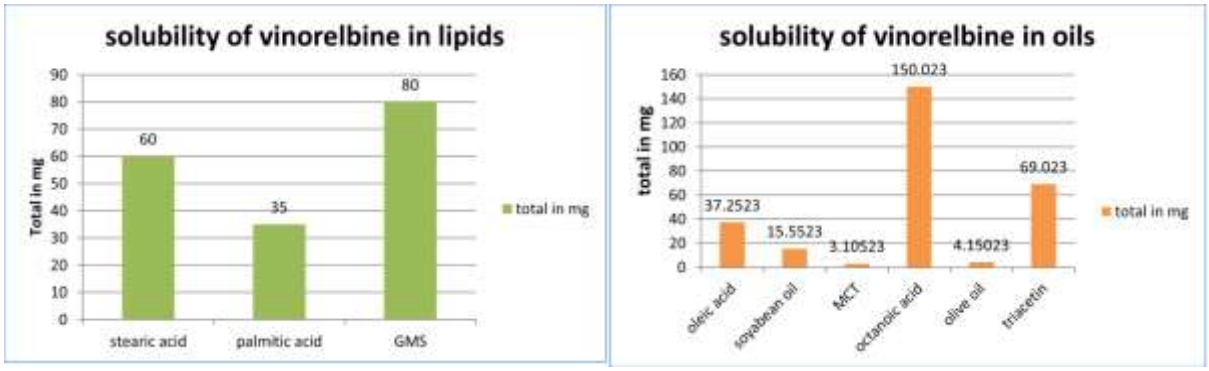

Figure 4. Vinorelbine solubility in different liquid and solid lipids

Screening of Surfactants and Co–surfactant

The results of emulsification ability for different surfactants and co-surfactants (Table 3), in which poloxamer 188 and soya lecithin were chosen as surfactants and co-surfactants, respectively since they showed fewer inversions during the experiment than others, where five flask inversions for poloxamer 188 and seven for soya lecithin were required to achieve homogenous emulsion with high transmittance (99.1% for poloxamer 188 and 98.1% for soya lecithin). The smaller number of inversions needed to yield homogenous mixtures indicated ease of emulsification property and better thermodynamic stability [30]. While other surfactants and co-surfactants showed poor emulsification properties despite the higher transmittance values as they required a higher number of flask inversions [31].

Table 3 Results of screening surfactants and co–Surfactants emulsification ability.

Type of Surfactant/Co–surfactant	No. of Inversions	%Transparency
Tween ®20	34	93.4
Tween®80	13	96.5
Poloxamer 188	5	99.1
cremophore®EL	23	97.5
Transcutol®	49	96.7
Soy Lecithin	7	98.1
propylene glycol	19	97.5
PEG 400	27	98.7

Preliminary experimental parameters optimization using a Plackett-Burman design.

The particle size (as a response) for the twelve formulas (P1–P12) suggested by the Plackett-Burman design is presented in Table 4.

Table 4. Results of determination of particle size for the prepared formulation (P1 – P12), which were suggested by Plackett-Burman design

Formula no.	Droplet size nm	Formula no.	Droplet size nm	Formula no.	Droplet size nm
P1	158±2	P5	177±7	P9	250±6
P2	199±3	P6	199±10	P10	223±3
P3	158±8	P7	158±5	P11	199±1
P4	281±9	P8	177±12	P12	140±4

The order pattern for the efficacy of the seven variables on particle size is presented in the Pareto chart of Plackett-Burman (Figure 5), where the effect of the variables is arranged in the following order: poloxamer 188 > sonication time > lecithin concentration > sonication power > solid: liquid lipid ratio ≈ homogenization rate ≈ homogenization time. The effect of homogenization rate and homogenization time as well as solid: liquid lipid ratio was significantly ($P<0.05$) lower than the other four variables (with no significant difference between them). Therefore, they kept constant at their reported optimum level [32-34], which were homogenization rate and time (15000 rpm for 5min) and solid: liquid lipid ratio (75:25). While the other four variables poloxamer 188 concentration (most predominant effect), sonication time, soya lecithin concentration and sonication power showed most predominant effect on particle size. Concerning the effect of sonication power, it was also kept constant at the reported level (300 Watt) to avoid the destruction of the lipid system [35].

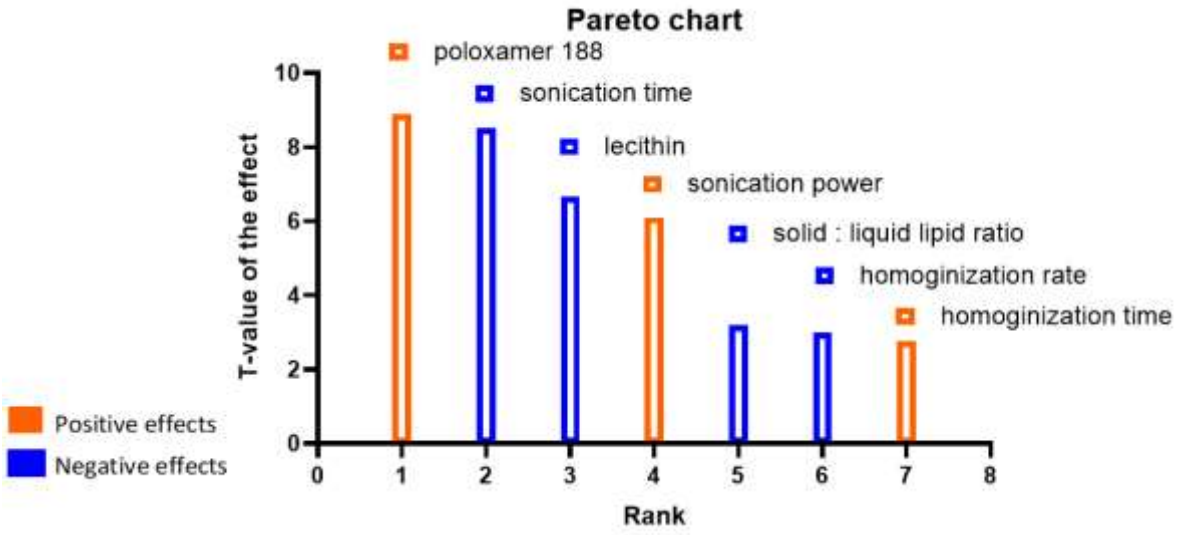

Figure 5. Pareto chart of Plackett-Burman design presenting the order of efficacy of the variables on particle size.

Further ingredients optimization using a full-factorial design

The eight formulas (Vn-NLC1 to 8) suggested by factorial design were prepared and evaluated as in Table 5.

Table 5. Characterization of the prepared NLC formulas (Vn-NLC1 to 8), which were suggested by 2^3 full-factorial design.

RunOrder	Formula code	MPS nm	% E.E	Zeta potential mV	PDI
1	Vn-NLC1	183±5	90.87%±2.0	-20.09±1.0	0.167±0.020
2	Vn-NLC2	213±9	89.54%±1.6	-22.7±0.6	0.013±0.050
3	Vn-NLC3	140±12	98.87%±4.0	-24.36±0.5	0.136±0.020
4	Vn-NLC4	180±10	90.87%±0.7	-22.17±0.3	0.188±0.010
5	Vn-NLC5	157±7	85.77%±3.0	-31.04±0.5	0.211±0.040
6	Vn-NLC6	182±11	79.54%±2.0	-25.19±1.2	0.158±0.020
7	Vn-NLC7	88±3	92.54%±0.5	-32.2±1.0	0.018±0.006
8	Vn-NLC8	110±3	82.44%±3.0	-29.93±1.4	0.15±0.015

MPS: mean particle size, % E.E.: entrapment efficiency and PDI: polydispersity index.

The analysis of the experiment design was applied in accordance with the measured two major responses (particle size and %EE) while zeta potential and PDI were in the acceptable range for all the eight formulas, referring to their good stability [36,37]. The analysis was done as follows:

Model validation and data optimization analysis

The response range of all eight NLC formulas (Vn-NLC1 to 8) for mean particle size and entrapment efficiency were 88 - 213 nm and 98.87% to 79.54 %, respectively. Minitab software was used to analyze the efficacy of the variables on responses, depending on the values of R^2, adjusted R^2 and predicted R^2 for each response. The results in Table 6 show that the regression coefficient values, including R^2, adjusted R^2, and predicted R^2 were very close to one (with an acceptable standard deviation SD ±), indicating linearity, which means a direct linear effect of variables on responses and reflecting the successful design of the formulations [38].

Table 6. Regression coefficient values for each response as predicted by Minitab software

For mean particle size		For entrapment efficiency%
R^2 value	0.9924	0.9962
R^2(adj) value	0.9822	0.9388
R^2(pred) value	0.9457	0.9388
SD (±)	0.057989	0.000070

To assess the effect of variables on both responses in the formulation process, normal plots were applied (by Minitab software), in which a variable with significant effect appears as a point that deviates far from the straight line (either positively or negatively), while variable with non-significant effect appears as a point that falls close to the straight line indicating non-significant random variation. The straight line represents the theoretical distribution of effects under the assumption that all effects are due to random variation [39]. The results (presented in Figures 6 and 7) revealed that all the variables have a significant effect on both responses through their distance away from the plot straight line.

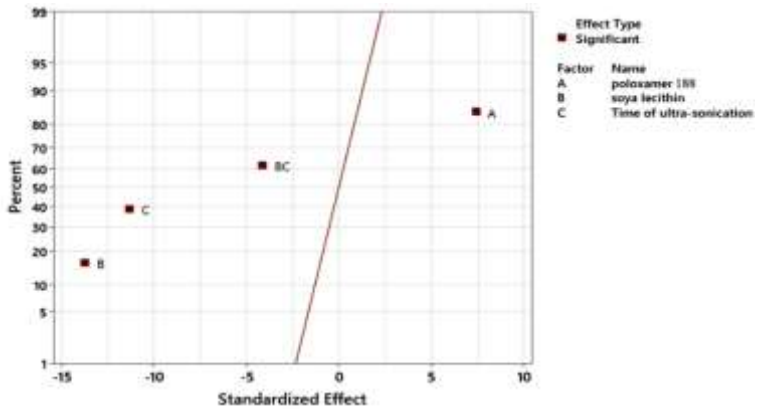

Figure 6. Normal plots for the standardized effects of the three variables on mean particle size (MPS)

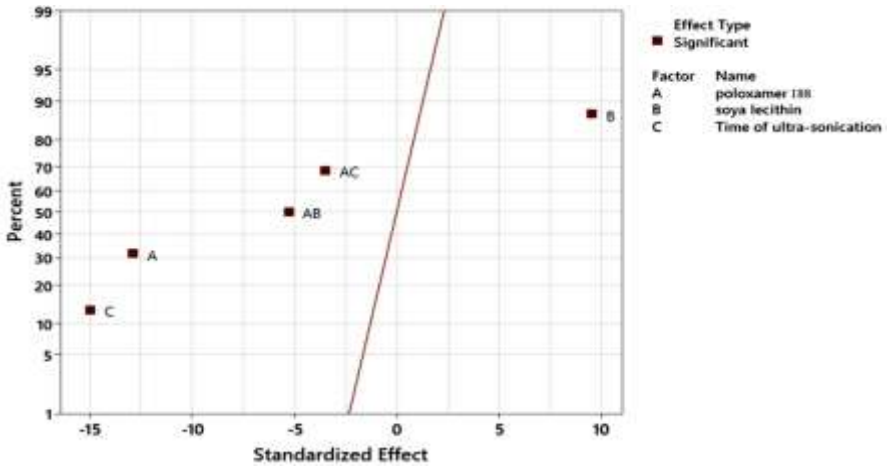

Figure 7. Normal plots for the standardized effects of the three variables on %EE.

In order to analyze the significance of each variable on the responses, analysis of variance (ANOVA) was applied. The results presented in Tables 7 and 8 show that all selected variables had a significant effect (P<0.05) on both particle size and %EE (as responses).

Table 7. Analysis of variance (ANOVA) results for particle size

Analysis of Variance	DF	Adj S.S.	Adj MS	F-Value	P-Value	Significance
Model	4	12130.5	3032.63	97.43	0.002	Significant P<0.05
Linear	3	11602.4	3867.46	124.26	0.001	
poloxamer 188	1	1711.1	1711.12	54.98	0.005	
soya lecithin	1	5886.1	5886.13	189.11	0.001	
Time of ultra-sonication	1	4005.1	4005.13	128.68	0.001	
Two Way Interactions	1	528.1	528.12	16.97	0.026	
soya lecithin and Time of ultra-sonication	1	528.1	528.12	16.97	0.026	
Error	3	93.4	31.13			
Total	7	12223.9				
poloxamer 188 and soya lecithin	1	0.001389	0.001389	27.93	0.034	
poloxamer 188 and Time of ultra-sonication	1	0.000612	0.000612	12.32	0.072	
Error	2	0.000099	0.000050			
Total	7	0.025989				

Table 8. Analysis of variance (ANOVA) results for Entrapment efficiency %

Analysis of Variance	DF	Adj S.S.	Adj MS	F-Value	P-Value	Significance
Model	5	0.025889	0.005178	104.14	0.010	Significant P<0.05
Linear	3	0.023888	0.007963	160.14	0.006	
poloxamer 188	1	0.008230	0.008230	165.53	0.006	
soya lecithin	1	0.004512	0.004512	90.75	0.011	
Time of ultra-sonication	1	0.011145	0.011145	224.15	0.004	
Two Way Interactions	2	0.002001	0.001001	20.12	0.047	
poloxamer 188 and soya lecithin	1	0.001389	0.001389	27.93	0.034	
poloxamer 188 and Time of ultra-sonication	1	0.000612	0.000612	12.32	0.072	
Error	2	0.000099	0.000050			
Total	7	0.025989				

Abbreviations: D.F.: degree of freedom, adj. S.S.: adjusted sum of R^2, adj. M.S.: adjusted mean sum of R^2, F: Fisher's ratio, P-value: significance value.

Further analysis of the effect of the independent variables on mean particle size

Upon further analysis by Minitab software, which suggested the polynomial equation for the effect of three variables on mean particle size as follows:

MPS = 195.75 + 73.13 A - 54.4 B - 2.037 C - 8.13 B*C ……..eq. (1)

Where MPS: mean particle size, A: concentration of poloxamer 188, B: concentration of soya lecithin, C: sonication time

Consequently, the plot of the pattern of the effect of each variable on MPS appears in Figure 8, where increasing the concentration of poloxamer 188 caused a significant (P<0.05) increase in the mean particle size (MPS) while increasing soya lecithin concentration or sonication time favors a decrease in MPS. The results can be explained as follows: increasing poloxamer 188 concentration may enhance more polymer chain linking and accelerate aggregation of NLC by raising the viscosity of the aqueous phase and, therefore, increasing the mean particle size of the prepared NLC [40]. In addition, increasing soya lecithin concentration and sonication time may lead to the formation of smaller micro-emulsions at high levels and yield smaller sizes [41].

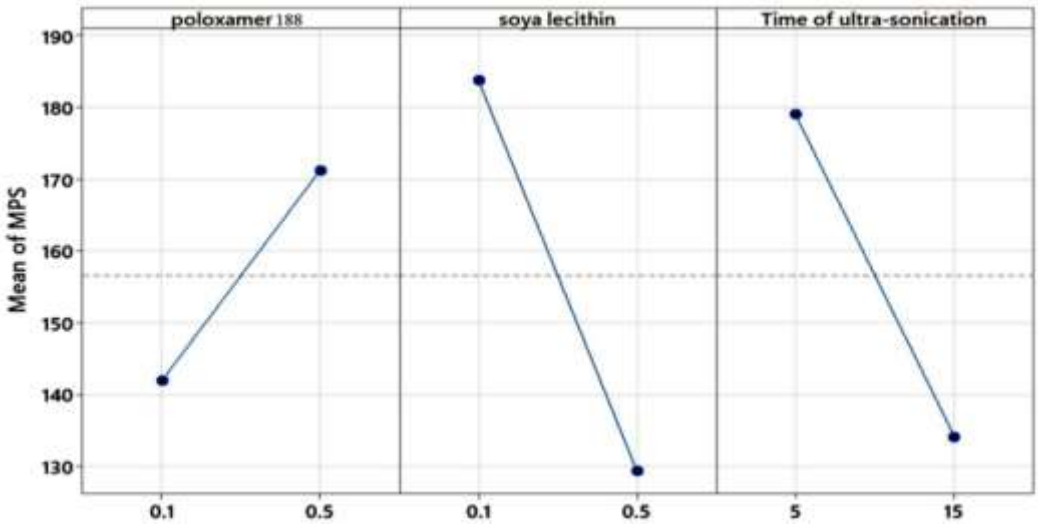

Figure 8. Plot the pattern of the effect of the variables on MPS.

A further plot was produced by Minitab, which is the interaction plot (combination of two variables) between two variables on MPS (Figure 9.). The results showed a significant (P<0.05) effect for the interaction of soya lecithin concentration and sonication time on MPS, which appeared as two non-parallel lines. While the interaction of poloxamer 188 concentration and soya lecithin concentration or the interaction of poloxamer 188 concentration and sonication time had a non-significant (P>0.05) effect on MPS since it appeared as parallel lines (the grey side) [42].

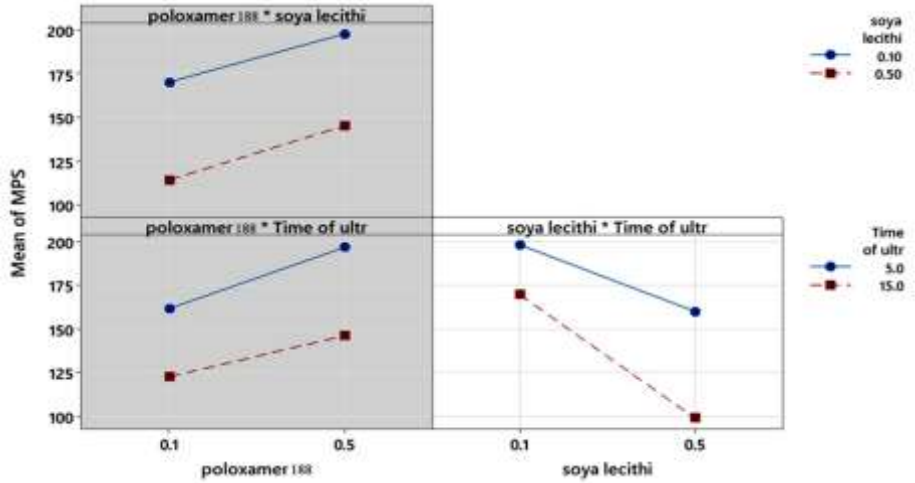

Figure 9. Interaction plot between two variables on MPS.

3D response surface plots as well as 2D contour plots for the effect of the interaction of two variables on MPS (Figure 10), wherein (A) the smallest particle size (<100 nm, the very faint green color) observed when the concentration of soya lecithin was 0.5% and poloxamer 188 concentration was 0.1%. While in (B), the smallest particle size was 100-120 nm when poloxamer 188 concentration was 0.1% and the time of sonication was 15 min. In (C), the smallest particle size (<100 nm) was observed when the concentration of soya lecithin was 0.5% and the sonication time was 15 min. These results complied with that obtained with formula Vn-NLC 7.

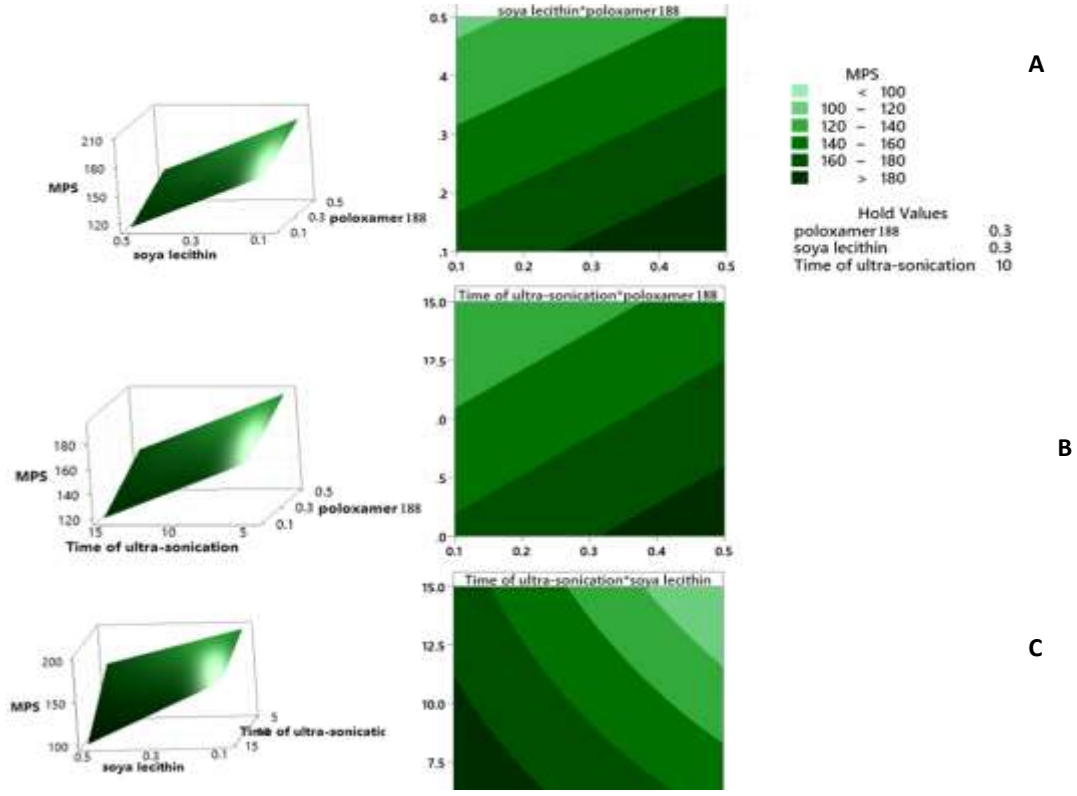

Figure 10. 3D response surface plots and 2D contour plots for the interaction effect of two variables: (A) soya lecithin and poloxamer 188 concentration, (B) ultrasonication time and poloxamer 188 and (C) ultrasonication time and soya lecithin concentration on MPS

Further analysis of the effects of the independent variable on entrapment efficiency

Further analysis by Minitab software, which suggested the polynomial equation for the effect of three variables on entrapment efficiency, was as follows:

$$\%EE = 0.9193 + 0.0259\,A + 0.2176\,B - 0.004840\,C - 0.3294\,A \times B - 0.00875\,A \times C \quad \text{eq. (2)}$$

Where % E.E.: entrapment efficiency, A: poloxamer 188, B: soya lecithin, C: sonication time.

Consequently, the plot of the pattern of the effect of each variable on %EE appeared in Figure 11**,** which shows that the increase in soya lecithin concentration caused a significant ($P<0.05$) increase in %EE. While increasing poloxamer 188 concentration or sonication time decreased entrapment efficiency. This can be attributed to the fact of the high lipophilicity of soya lecithin (as co-surfactant) that may enhance the solubility of the lipophilic drug (vinorelbine), leading to an increase in %EE [43]. However, poloxamer 188 had a negative effect on %EE because of its high water solubility, which led to more drugs being portioned in the aqueous phase during the emulsification process, thereby lowering the entrapment efficiency [44]. The longer sonication may cause expulsion of the organic phase leakage of the encapsulated drug, leading to a lower %EE [45].

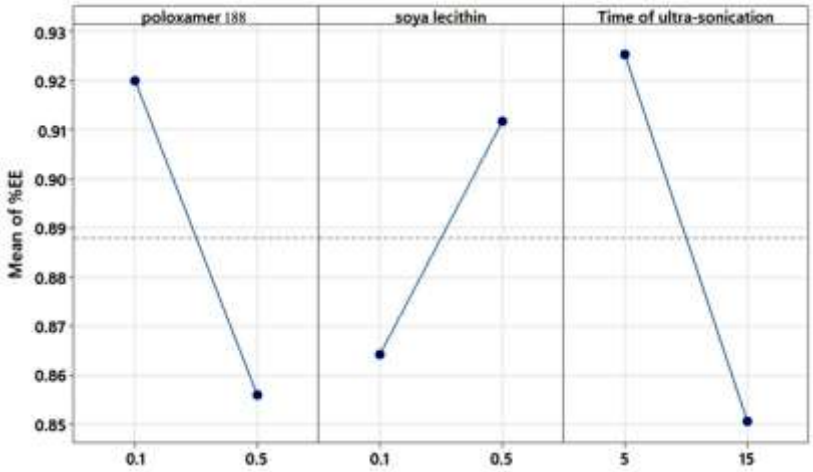

Figure 11. Plot of the pattern of variables effect on EE%.

The interaction between two variables and their effect on %EE is presented in Figure 12, where parallel lines were obtained for the interaction between soya lecithin concentration and sonication time, indicating a non-significant effect for the combination of these two variables on %EE [46]. While the interaction between poloxamer 188 and soya lecithin concentration, as well as between poloxamer 188 and sonication time, showed non-parallel lines indicating their significant effect on %EE.

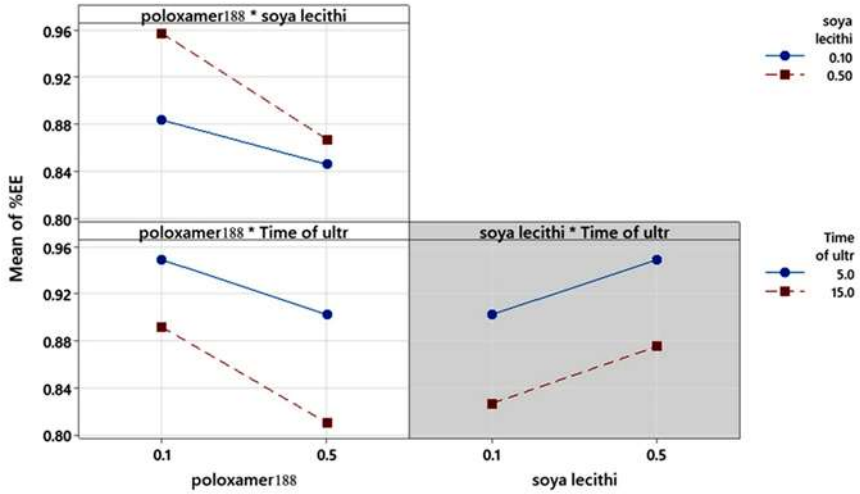

Figure 12. Interaction plot between two variables on EE%.

The 3D response surface and 2D contour plots for the interaction of two variables on %EE (Figure 13) showed that in (A) the highest %EE (>94%, dark green color) observed when the concentration of soya lecithin was 0.5% and poloxamer 188 concentration was 0.1%. While in (B) the highest %EE (>94%) was observed when sonication time was 5 min and the concentration of poloxamer 188 was 0.1%. In (C) the highest %EE (>94%) was observed when soya lecithin concentration was 0.5% and sonication time was 5 min. These results complied with that obtained with the formula Vn-NLC3.

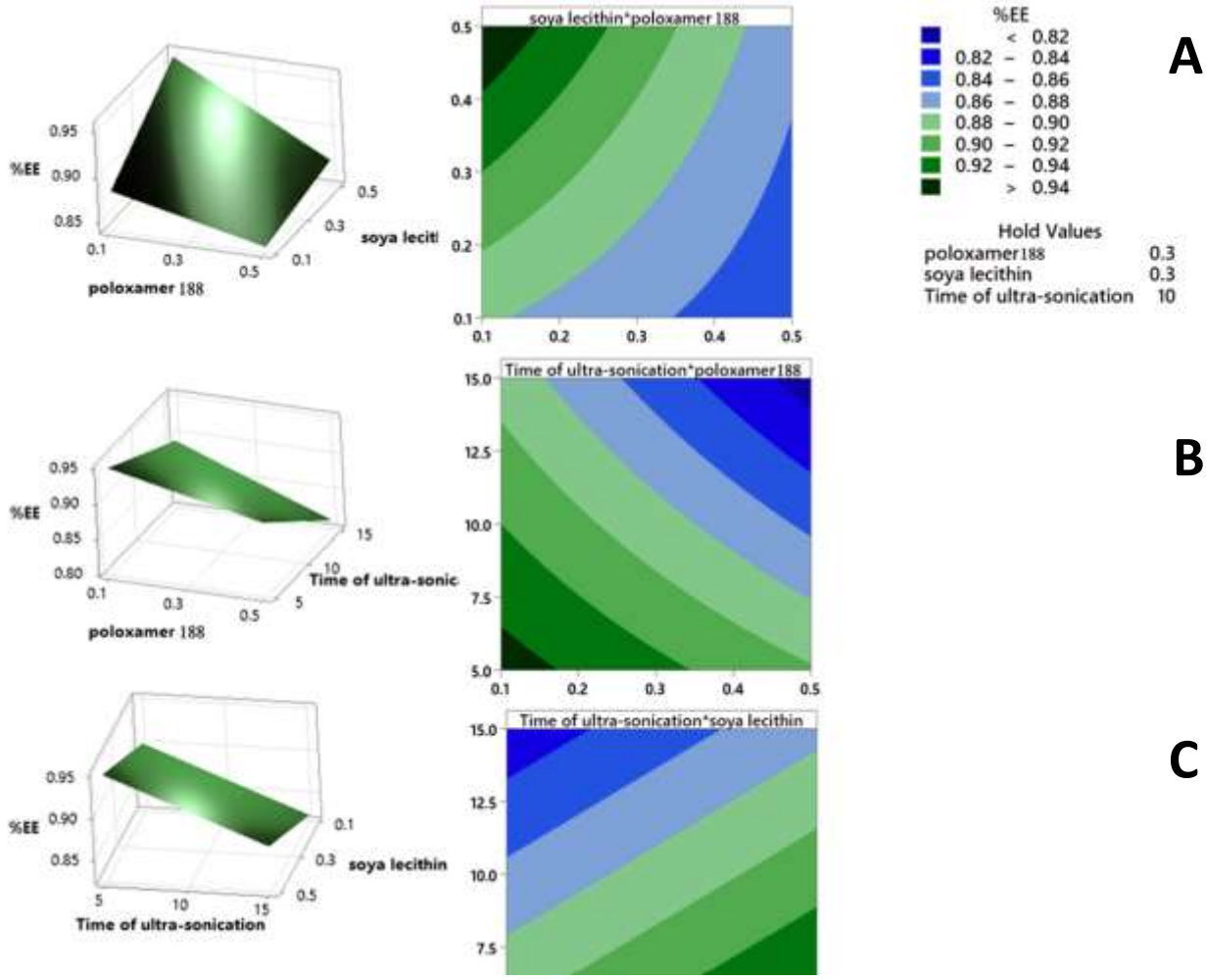

Figure 13. 3D response surface plots and 2D contour plots for the interaction effect of two variables: (A) soya lecithin and poloxamer 188 concentration, (B) ultrasonication time and poloxamer 188 and (C) ultrasonication time and soya lecithin concentration on EE%.

The effect of experimental variables on in-vitro drug release

The effect of the three variables (soya lecithin concentration, poloxamer 188 concentration and sonication time) on the drug release from the prepared Vn-NLC1 to Vn-NLC8 formulas is presented in Figure 14. In general, the liberation of vinorelbine from NLC occurred in two-phase patterns, in which there was an initial burst release pattern within the first four hours due to drug diffusion from the outer layer of NLC followed by sustained release character for up to 48 hours, which could be related to slow penetration of the aqueous medium into the hydrophobic lipid accompanied by slow dissolution and diffusion of drug from lipid core. Concerning the effect of sonication time on drug release, it was observed that Vn-NLC1 and Vn-NLC5 (same content but different sonication time 5 and 15 min respectively) showed similar release pattern [47] (similarity factor f_2 70.83) with no significant difference (P>0.05) after 4 h and 48 h, although increasing sonication time led to significant (P<0.05) decrease in particle size and %EE, due to the stable lipid structure of the prepared NLC [48-50] . The same was observed with Vn-NLC2 and Vn-NLC6 (f_2 65.50), Vn-NLC3 and Vn-NLC7 (f_2 82.67) as well as Vn-NLC4 and Vn-NLC8 (f_2 77.84).

Concerning the effect of poloxamer 188 concentration, it was observed that as the concentration of poloxamer 188 increased (keeping other variables constant) the drug release was decreased significantly (P<0.05) as in Vn-NLC1 that contain 0.1%w/v in comparison to Vn-NLC2 containing 0.5%w/v, where Vn-NLC1 showed 67.75% initial release after 4 h and 96.18% drug release after 48 h in comparison to Vn-NLC2 that showed 51.90 % initial release after 4 h and 85.08 % drug release after 48 h, knowing that both had the same soya lecithin concentration (0.1% w/v) and subjected to the same sonication time (5 min). The decrease in drug release could be related to the effect of poloxamer 188 concentration on particle size which mentioned earlier, where increasing concentration of poloxamer 188 caused significant (P<0.05) increase in the particle size leading to

reducing drug dissolution [51]. The same result was observed for Vn-NLC3 and 4, Vn-NLC5 and 6 as well as Vn-NLC7 and 8.

Concerning the effect of soya lecithin concentration, it was observed that as the concentration of soya lecithin increased (keeping other variables constant) the drug release was decreased significantly ($P<0.05$) as in Vn-NLC1 that contain 0.1%w/v soya lecithin in comparison to Vn-NLC3 containing 0.5%w/v, where Vn-NLC1 showed 67.75% initial release after 4 h and 96.18% drug release after 48 h in comparison with Vn-NLC3 that showed 44.38% and 80.49% drug release within 4 and 48 h respectively, knowing that both had the same poloxamer 188 concentration (0.1% w/v) and subjected to the same sonication time (5 min). The decrease in drug release could be related to the hydrophobicity of soya lecithin that held tightly the lipophic drug in the lipid vesicle leading to slower drug release rate [52,53]. The same result was observed for Vn-NLC2 and 4, Vn-NLC5 and 7 as well as Vn-NLC6 and 8.

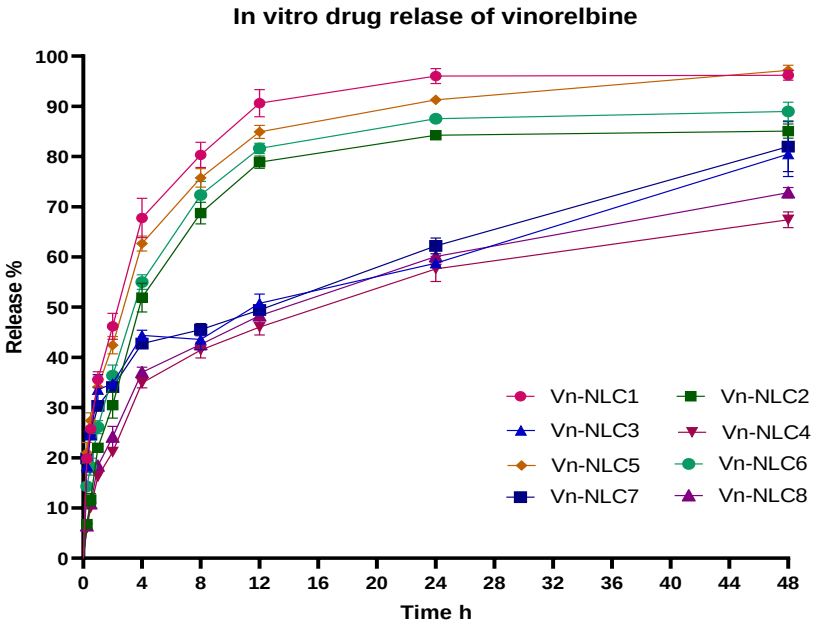

Figure 14. Drug release profile from the prepared formulas Vn-NLC 1 to 8 formulations in phosphate buffer saline (pH7.4)

Selection of optimum Vn-NLC formula

The best formula was Vn-NLC7 selected on the basis of the smallest particle size 88±3, PDI 0.018 ±0.006 (Figure 15), high entrapment efficiency 92.54% ±0.5, zeta potential -32.2±1and suitable drug release, where the smallest particle size may give faster penetration through lung tissue, that may accumulate for a longer time due to the slow burst release that continued up to 48 hours leading to a better anticancer activity [54].

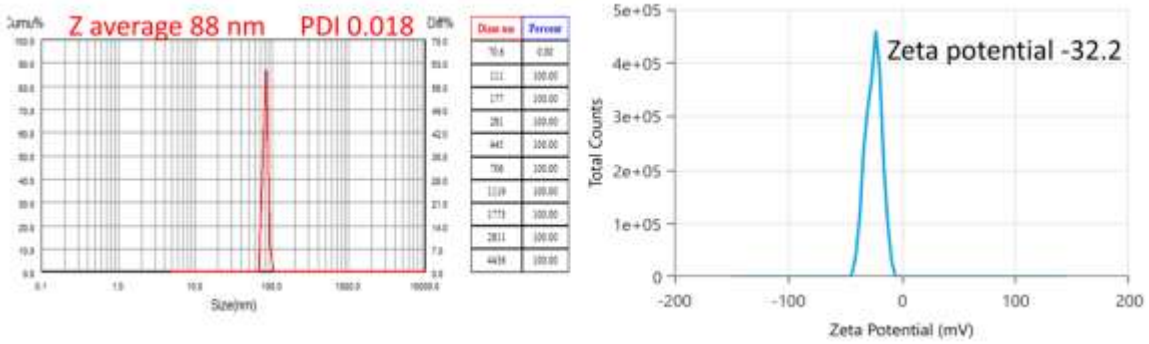

Figure 15. Average particle size, PDI, and zeta potential for the selected formula.

The mathematical modeling for the results of drug release from Vn-NLC7 is presented in Table 9, in which, according to the result, the drug release follows the Higuchi model, which is common for NLC to follow such model [55].

Table 9. The Release Kinetic Data of the Selected Formulations

Formulation	ZERO R^2	FIRST R^2	HIGUCHI R^2	KORSMEYER R^2
Vn-NLC7	0.767	0.01	0.912	0.303

Conclusions

This work succeeded in preparing a nano-lipid carrier for vinorelbine and complied with the suggested design and analysis of Minitab software. The optimized formula had the smallest particle size and high entrapment efficiency that may enhance drug penetration and retention of the drug inside the tissue as it gave slow initial release that continued up to 48 hrs. therefore subjecting tumor cells to long-term exposure to vinorelbine, leading to successful cancer eradication.

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Conflicts of Interest

There were no conflicts of interest related to this research.

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Author Contribution

All authors contributed equally in all steps of the research and in reviewing the results. Also, they have approved the final version of the manuscript before submission

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تصميم وتطوير وتقييم دواء الفينورلبين باستخدام ناقل نانوي دهني (NLC)

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الخلاصة

تم استخدام التصميم العاملي الكامل (تصميم ٢^٣) لدراسة تأثير المتغيرات المستقلة، حيث تم تحضير ثماني صيغ بطريقة الاستحلاب بالموجات فوق الصوتية باستخدام (عوامل مختلفة) تراكيز مختلفة من البولوكسامر ١٨٨، صويا الليسيثين ، ووقت الموجات فوق الصوتية، وتم قياس متوسط حجم الجسيمات وكفاءة احتباس الدواء كاستجابات التصميم. تم إجراء دراسة تحرير الدواء للصيغ التي تم تحضيرها (Vn-NLC1 الى ٨) ، حيث أعطت الصيغ Vn-NLC 3 وVn-NLC٤ وVn-NLC7 وVn-NLC٨ تحرر دوائي بطيء واستمر لمدة ٤٨ ساعة؛ ومع ذلك، كان لـ Vn-NLC 7 أصغر متوسط حجم (MPS) وأقل تشتت حجمي (PDI) وكفاءة احتباس دوائي عالية(E.E. %) ، وكانت السعة التحميلية وجهد الزيتا ٨٨ ±٣ نانومتر، ٠,٠١٨ ±0.006، -٣٢,٢ ±١، و٩٢,٥٤٪ على التوالي، لذا تم اختيارها كصيغة مثالية. قد يمنح الحجم الصغير

التحرر الدوائي البطيء لصيغة Vn-NLC 7 المثلى تراكمًا أفضل في أنسجة الرئة لفترة أطول، وبالتالي يعزز نشاط الفينورلبين بشكل كبير ويقلل من السمية.

الكلمات المفتاحية: الفينورلبين، الناقل النانوي الدهني(NLC) ، سرطان الرئة، ميني تاب، تصميم بلاكيت-بورمان، التصميم العاملي.