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Original Article

# DIACEREIN-LOADED NIOSOMES (DC-NS): A NEW TECHNIQUE TO SUSTAIN THE RELEASE OF DRUG ACTION

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**ABSTRACT**

**Objective:** The study's main goal is to develop a suitable niosomes (NS) encapsulated drug for anti-inflammatory effects such as diacerein (DC) and to evaluate the system's vesicle size (VS), entrapment efficiency (EE %), physical stability and *in vitro* release.

**Methods:** Tween (40 and 60), cholesterol, and stearylamine were used in a 1:1:0.1 molar ratios as non-ionic surfactants. Thin film hydration was used to create the NS.

**Results:** The higher EE% was observed with NS (F11) prepared from tween 60, cholesterol and 2.5 min sonication. These formulations' release patterns were Higuchi diffusion and first order. For the stability study, NS formulations were stored at temperature between 2-8 °C for 60 d retains the most drugs when compared to room and high temperature conditions.

**Conclusion:** The findings of this study have conclusively shown that after NS encapsulation of DC, drug release is prolonged at a constant and controlled rate.

**Keywords**: Diacerein, Niosome, Cholesterol, Tween, Stearylamine and Stability


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## INTRODUCTION

To obtain a stable blood or tissue level that is therapeutically both effective and nontoxic for extended periods of time, the drug therapy's foremost goal is to reach a certain blood or tissue level. The development of an appropriate dosage regimen is a critical component in achieving this goal [1]. As delivering a medication at the correct dosage rate for the duration of the treatment is the goal of novel drug delivery systems [2], the targeted drug delivery is administering drugs locally in the correct concentration by blocking other routes of entry to reduce toxicity while maximizing the effectiveness of the treatment. It is the process of selectively delivering a drug or drugs to target cells according to their identity [3]. The molecules' function could be implemented by an active carrier that could transport the molecules to the location of action and then release them to perform their function [4].

Modifying the parent compound chemically so that the derivative is only activated at the target site is called a chemical method. There are three types of targeting methods: chemical, covalent bonding, and physical. However, liposomes, nano-particles, resealed erythrocytes, magnetic microspheres, platelets and monoclonal antibodies are all different physical carriers. It is because of the NS drug delivery system's significant advantages over conventional drug delivery systems that it has recently drawn a lot of attention. Hence, it is said that NS are non-ionic surfactant vesicles that have a water-based component and a lipid membrane. This vesicle complex reportedly encapsulates a wide range of molecules within the liquid membrane. Lamellar structures formed by a non-ionic surfactant, cholesterol and stearylamine mixed together and then hydrated in water are known as vesicles and called NS or non-ionic surfactants [5]. DC blocks IL-1 synthesis and release directly, limits IL-1-induced activities, and is shown in animal models of osteoarthritis as well as in human subjects with osteoarthritis of finger joints and knees to have a disease-modifying effect [6].

The study's main goal was to formulate niosomes of diacerein (DC-NS) (IL-1 inhibitor), optimize the formulation, characterize them, and evaluate the system's *in vitro* performance.

## MATERIALS AND METHODS

### Materials

Diacerein (DC) (NUTRA Specialities Private Limited) was obtained as a gift sample from ADCO (Cairo, Egypt). Tweens (40 and 60) were purchased from El-Nasr Pharmaceutical Chemicals Co (ADWIC, Cairo, Egypt). Cholesterol was purchased from MERCK (Kenilworth NJ, USA). Stearylamine was purchased from Fluka, Sigma-Aldrich (chemie Riedstr.2, Germany). The solvents and other reagents used in this study were of analytical grade. All the ingredients were used without further purification. Phosphate buffered saline (PBS) with a pH of 7.4 was prepared according to the instructions in the Indian Pharmacopia 2020 [7].

### Methods

### Preparation of DC-NS

DC-NS was synthesized via the thin film hydration method. The drug was taken in a 1:1 molar ratio with nonionic surfactants and cholesterol. Different NS formulations were formulated by thin film hydration technique reported by Azmin *et al.,* [8]. Quantities of surfactant, either tween 40 or 60, and cholesterol were added to 10 ml of chloroform in a round bottom flask; the resulting solution was accurately weighed [9]. Under reduced pressure and at a temperature of 60±5 °C, the solvent mixture was evaporated in a rotary evaporator (Bibby Sterilin LTD, Stone Staffordshire, England). Then the flask rotated until a smooth, dry film was obtained. At 60 °C, with gentle shaking on a water bath, the film was hydrated with 25 ml of PBS 7.4 containing DC (0.5 %). After sonicating the NS suspension, it was transferred to a suitable glass container and placed in an ice bath for heat dissipation. The sonicated dispersion was then allowed to stand at room temperature for approximately 2 h to form NS. The formulation was refrigerated [10].

A technique of Box-Behnken design [11] taking three prime selected formulation variables (factors) at three different levels was employed to devise and carry out the experimental work for the formulation of DC-NS. These major factors include the percent of charge inducer ($X_1$), HLB values ($X_2$) and sonication time ($X_3$). So, fifteen formulae of different combinations were prepared, by taking values of the variables $X_1$, $X_2$ and $X_3$ at different levels, as shown in table 1.

**Table 1: DC-NS formulation according box-behnken design**

| Formula No. | Variable level in coded form | | | | | |
|---|---|---|---|---|---|---|
| | X1 (charge inducer) | | X2 (HLB value) | | X3 (Sonication time) | |
| | Actual (%) | Coded | Actual | Coded | Actual (min) | Coded |
| F1 | 5 | 0 | 14.9 | -1 | 5 | 1 |
| F2 | 0 | -1 | 15.25 | 0 | 5 | 1 |
| F3 | 5 | 0 | 15.6 | 1 | 5 | 1 |
| F4 | 0 | -1 | 15.25 | 0 | 0 | -1 |
| F5 | 0 | -1 | 15.6 | 1 | 2.5 | 0 |
| F6 | 5 | 0 | 14.9 | -1 | 0 | -1 |
| F7 | 10 | 1 | 15.25 | 0 | 5 | 1 |
| F8 | 10 | 1 | 15.25 | 0 | 0 | -1 |
| F9 | 10 | 1 | 15.6 | 1 | 2.5 | 0 |
| F10 | 5 | 0 | 15.6 | 1 | 0 | -1 |
| F11 | 0 | -1 | 14.9 | -1 | 2.5 | 0 |
| F12 | 10 | 1 | 14.9 | -1 | 2.5 | 0 |
| F13 | 5 | 0 | 15.25 | 0 | 2.5 | 0 |
| F14 | 5 | 0 | 15.25 | 0 | 2.5 | 0 |
| F15 | 5 | 0 | 15.25 | 0 | 2.5 | 0 |

**Characterization of DC-NS**

**Photo microscopy**

A photo microscopy (Leica, Dreieich, Germany) was used to characterize vesicle dispersions for vesicle formation and morphology. In order to examine and photograph samples under an optical microscope equipped with a camera, NS formulation samples were examined under an optical microscope with a camera set to magnifications ranging from 40 to 100 times [12].

**Determination of vesicle size**

The size of NS vesicles was determined by using the Malvern Mastersizer (X ver.2.15, Malvern instruments Ltd. Malvern, UK) [12].

**Determination of DC entrapment efficiency**

Separating the formulated DC-NS from un-entrapped drug was accomplished by centrifugation at 7000 rpm for 30 min by using a centrifuge (BiofugePrimo, Heraeus). The isolated layers were washed twice with PBS 7.4 and centrifuged again [13]. The amount of entrapped DC was estimated indirectly by measuring the un-entrapped drug in the washing by using UV spectrophotometer (Shimadzu UV-1650 P. C, Japan) according to the following equation (Eq. 1):

$$EE\ (\%) = (C_{total} - C_{free\ drug}/C_{total}) \times 100 \quad \text{..... (Eq. 1)}$$

Where $C_{total}$ is the total amount of the drug loaded and $C_{free\ drug}$ is the quantity of the free drug in the washing.

***In vitro* release of DC-NS**

Every single NS formula was used in this test. The treatments were carried out separately, then they were all washed thoroughly, and the amount of DC trapped was determined (as mentioned above). When the entire drug had been metabolized, the remaining amount was considered to be the total amount of drug. Each preparation's pellet was then suspended in 500 ml of phosphate buffer solution (PBS) 7.4. Dissolution test system (Hanson research–Hanson virtual instruments, SR8 plus, USA) was used to carry out the experiment. The device was adjusted to a rate of 80 rpm and the temperature was adjusted to 37 °C. At different time intervals, a 5 ml sample of each NS suspension was taken. The samples were separated and filtered through a 0.45 µm filter; the amount of DC released at each time interval was determined, and the amount of DC retained was calculated for each formula per time interval.

**Optimization of DC-NS**

The Statistical Correlation between Independent Variables (Charge inducer percent X1, HLB value X2 and Sonication time X3) and dependent response of DC-NS (Particle size Y1, EE% Y2 and *in vitro* release after 8 h Y3) using Statistical package STATGRAPHICS plus STATGRAPHICS plus (version 4, Manugistics Inc., Rockville, MD, USA) were all made.

**Physical stability of DC-NS**

The physical stability of the formulated DC-NS was investigated to determine whether it leached down from NS, in liquid form, during storage. NS formulation samples were sealed in glass vials and stored for two months at three different temperatures: refrigeration (4 °C), room temperature, and elevated temperature (40 °C). After removing samples from each vial at predetermined intervals of 15, 22, 30, 45, and 60 d, the residual quantity of DC in the vesicles was determined as previously described after separation from un-entrapped drug [14].

**RESULTS AND DISCUSSION**

**Characterization of DC-NS**

**Photo microscopy**

Fig. 1 shows a photomicrograph of DC-NS prepared by the thin film hydration method. They are show that the NS were spherical in shape and existed in both dispersed and aggregate collections.

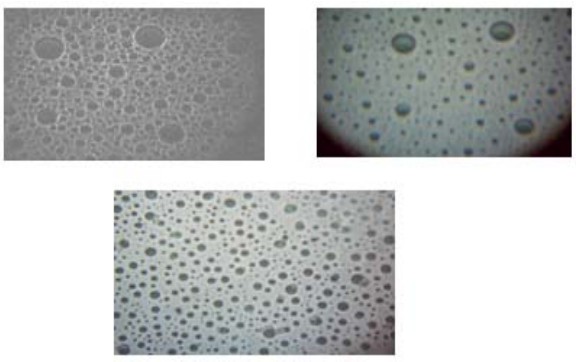

**Fig. 1: Photomicrograph of DC-NS**

**Determination of vesicle size**

The means particle diameters of NS, composed of tween 40 and 60 with cholesterol are shown in table 2. The results reveal that formula 9 (tween 40, HLB= 15.6) has the smallest particle diameter (7.33 um) while Formula 11(tween 60, HLB= 14.9) has the largest particle diameter (23.66 um). These findings could be attributed to the hydrophilic surfactant, which solubilized cholesterol and reduced particle size [15].

**Determination of EE%**

The EE% of all NS formulations formed of tween 40 and 60 with cholesterol are reported in table 3. The results reveal that formula 11 (tween 60, HLB= 14.9) has the highest EE% (58.43%) while Formula 15 (tween 60and40, HLB= 15.25) has the smallest EE% (9.52 %). These findings could be attributed to the affinity of cholesterol to lipophilic surfactant [16].

**Table 2: Particle diameter of DC-NS***

| Formula | Particle size (um) |
|---|---|
| F1 | 18.63±0.23 |
| F2 | 18.99±0.45 |
| F3 | 14.21±0.54 |
| F4 | 16.98±0.63 |
| F5 | 16.7±0.43 |
| F6 | 15.24±0.53 |
| F7 | 16.25±0.66 |
| F8 | 19.54±0.75 |
| F9 | **7.33**±062 |
| F10 | 12.72±1.76 |
| F11 | **23.66**±2.42 |
| F12 | 21.07±2.43 |
| F13 | 21.84±2.31 |
| F14 | 23.16±1.58 |
| F15 | 20.38±1.21 |

*Results are represented as mean±SD, n = 3

**Table 3: EE% of DC-NS***

| Formula | EE% |
|---|---|
| F1 | 55.42±3.42 |
| F2 | 46.99±4.23 |
| F3 | 29.52±1.89 |
| F4 | 52.05±3,98 |
| F5 | 20.96±1.87 |
| F6 | 39.76±2.54 |
| F7 | 24.94±1.43 |
| F8 | 17.23±1.64 |
| F9 | 10.24±0.53 |
| F10 | 49.64±3.32 |
| F11 | **58.43**±2.43 |
| F12 | 22.29±1.56 |
| F13 | 12.65±0.65 |
| F14 | 9.76±0.74 |
| F15 | **9.52**±0.76 |

*Results are represented as mean±SD, n = 3

***In vitro* release of DC-NS**

Fig. 2, 3, and 4 show the results of an *in vitro* study on the release of DC-NS vesicles formulated with Tween 40, Tween 60, and a combination of them. The percentages of the drug released after 8 h (Q8h) from the formulated NS vesicles are shown in table 4.

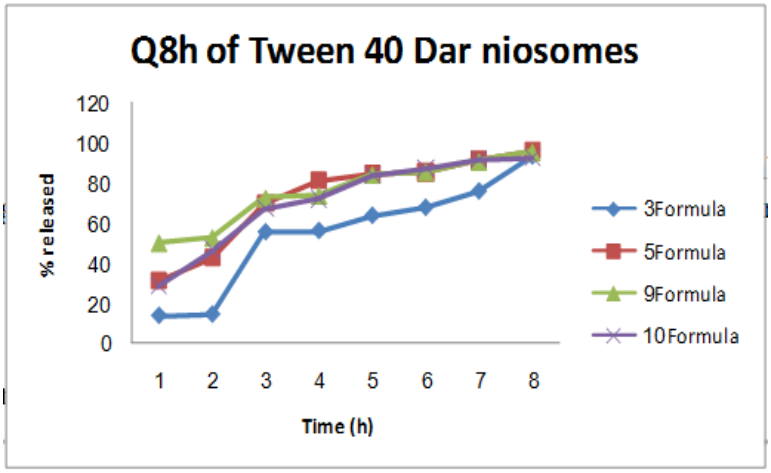


**Fig. 2: *In vitro* release of DC from tween 40 NS after 8 h**

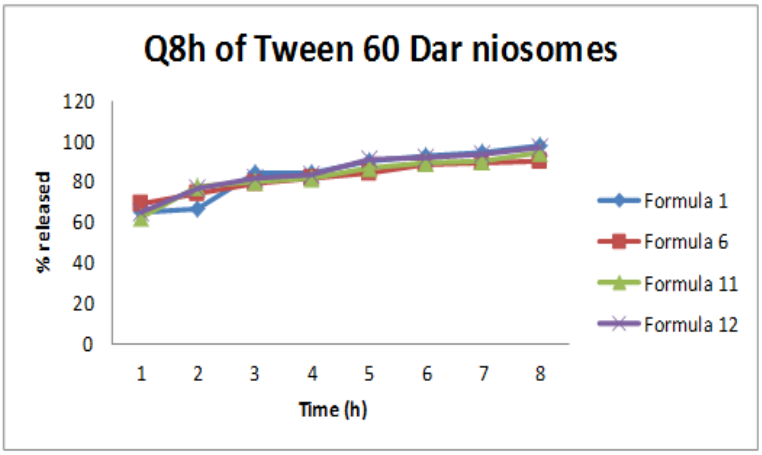


**Fig. 3: *In vitro* release of DC from tween 60 NS after 8 h**

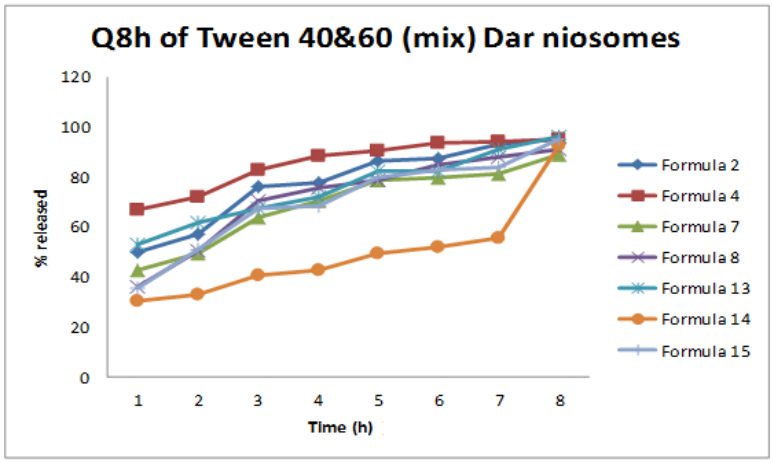


**Fig. 4: *In vitro* release of DC from tween 40 and60 (mix) NS after 8 h**

**Table 4: *In vitro* release of DC-NS after 8 h***

| Formula | Q8 h (%) |
|---|---|
| F1 | 97.5±5.42 |
| F2 | 95.2±6.32 |
| F3 | 93.1±5.43 |
| F4 | 95.4±4.75 |
| F5 | 96.2±7.32 |
| F6 | 90.1±5.43 |
| F7 | 89.1±4.62 |
| F8 | 91.3±3.72 |
| F9 | 95.6±4.67 |
| F10 | 92.3±3.54 |
| F11 | 94.2±3.65 |
| F12 | 96.8±5.87 |
| F13 | 96.1±4.88 |
| F14 | 92.7±5.76 |
| F15 | 95.2±4.96 |

*Results are represented as mean±SD, n = 3

The results of *in vitro* release have shown that formula F1 (HLB= 14.9) has the highest release 97.5% compared to formula F7 (HLB= 15.25) which has the lowest *in vitro* release 89.1%. This result may be due to the increasing lipophilicity of surfactant mixture in formula F1 which was accompanied by an increase in the solubility of DC and therefore an increase in its release from formula F1 [17].

**Optimization**

**Factorial characterization of DC-NS**

The observed results and experimental trials for the DC formulations are shown in table 5. The dependent studied variables were Y1 (particle size), Y2 (EE%) and Y3 (release after 8 h) which are based on the experimental design. The range of the responses for Y1 was 23.66 um in F11 (maximum) and 7.33 um in F9 (minimum) while in Y2, the range of the responses was 58.43 % in F11 (maximum) and 9.52 % in F15 (minimum). The range of the responses for Y3 was 97.5 % in F1 (maximum) and 89.1 % in F7 (minimum).

The relationship between the dependent and independent variables was further elucidated by using the main effect plot. Fig. 5-13 showed the effects of factors X1, X2 and X3 on the response Y1, Y2 and Y3.

**Table 5: Full factorial design layout, effect of X1, X2 and X3 on Y1***

| Formula no. | Variable level in coded form | | | Particle size (um) Y1 | E. E. (%) Y2 | Release (%) Y3 |
|---|---|---|---|---|---|---|
| | X1 | X2 | X3 | | | |
| F1 | 0 | -1 | 1 | 18.63±0.22 | 55.42±2.47 | **97.5±7.23** |
| F2 | -1 | 0 | 1 | 18.99±0.32 | 46.99±2.57 | 95.2±6.62 |
| F3 | 0 | 1 | 1 | 14.21±0.52 | 29.52±1.54 | 93.1±5.64 |
| F4 | -1 | 0 | -1 | 16.98±0.86 | 52.05±3.54 | 95.4±4.88 |
| F5 | -1 | 1 | 0 | 16.7±0.41 | 20.96±1.78 | 96.2±5.74 |
| F6 | 0 | -1 | -1 | 15.24±0.64 | 39.76±1.87 | 90.1±2.74 |
| F7 | 1 | 0 | 1 | 16.25±0.74 | 24.94±1.77 | **89.1±5.66** |
| F8 | 1 | 0 | -1 | 19.54±0.44 | 17.23±0.89 | 91.3±4.78 |
| F9 | 1 | 1 | 0 | 7.33±0.54 | 10.24±0.54 | 95.6±7.87 |
| F10 | 0 | 1 | -1 | 12.72±0.85 | 49.64±2.66 | 92.3±6.78 |
| F11 | -1 | -1 | 0 | 23.66±0.97 | 58.43±3.87 | 94.2±7.76 |
| F12 | 1 | -1 | 0 | 21.07±1.43 | 22.29±1.65 | 96.8±6.48 |
| F13 | 0 | 0 | 0 | 21.84±0.52 | 12.65±0.46 | 96.1±5.54 |
| F14 | 0 | 0 | 0 | 23.16±1.43 | 9.76±0.87 | 92.7±7.99 |
| F15 | 0 | 0 | 0 | 20.38±1.46 | 9.52±0.46 | 95.2±7.88 |

*Results are represented as mean±SD, n = 3

Fig. 5, 6 and 7 showed the main effects, interaction effects and quadratic effects of charge inducer (X1), HLB values (X2) and sonication time (X3) on the particle size. According to these figures, it was obvious that (X2) had the main effects on the particle size; it was also noted that increasing X1 from 0% to 10% resulting in decreasing particle size from 22.5 um to 19.5 um (negative effect) as a result of the increasing repulsion between particles [18]; increasing X2 from 14.9 to 15.6 resulted in increasing particle size

from 21.6 um to 22.78 um then decreasing to 14.7 um (negative effect); and increasing X3 from 0 min to 10 min resulted in increasing particle size from 18.4 um to 21.8 um then decreasing to 19.3 um (positive effect).

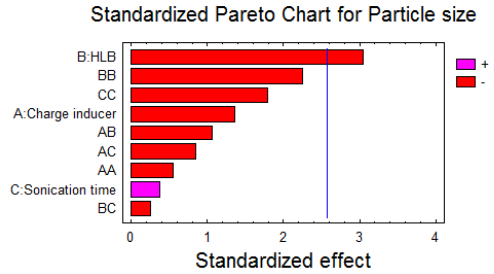


**Fig. 5: Standardized pareto chart showing the quadratic effect and interaction effect of X1, X2 and X3 on the particle size**

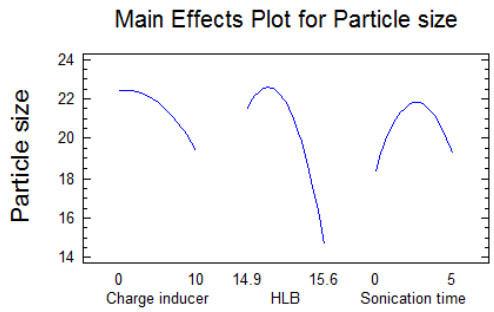


**Fig. 6: Main effect plot showing the effect of X1, X2 and X3 on the particle size**

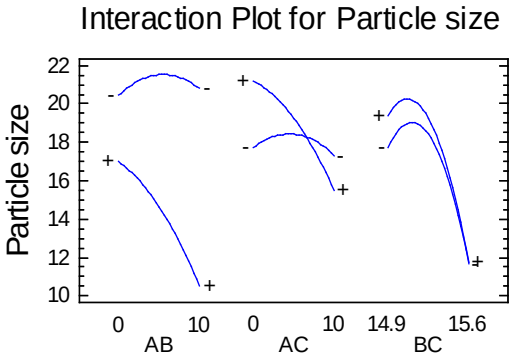


**Fig. 7: Main effect plot showing the interaction effect of X1, X2 and X3 on the particle size**

The ANOVA for particle size was shown in table 6. The statistical significance of each effect was determined by comparing the mean square to an estimate of the experimental error. In this case, the HLB value (X2) had a p-value less than 0.05, indicating that it was significantly different from zero at the 95% confidence level. According to the R-squared statistic, the fitted model explains 80.75% of the variability in particle size. The adjusted R-squared statistic is 46.12 %, which better suited comparing the models with different numbers of independent variables. The standard error of the estimate is 3.167, and the standard deviation of the residuals is 3.167. The mean absolute error (MAE) of 1.516 is the average value of the residuals. The Durbin-Watson (DW) statistic examines the residuals to see if there is any significant correlation based on their order in your data file. Because the DW value is greater than 1.4 (2.436), the residuals are unlikely to have a significant autocorrelation.

**Table 6: Analysis of variance for particle size**

| Source | Sum of square | DF | Mean square | F-ratio | p-value |
|---|---|---|---|---|---|
| A: Charge inducer | 18.4225 | 1 | 18.4225 | 1.84 | 0.2334 |
| B: HLB value | 93.6396 | 1 | 93.6396 | 9.33 | 0.0283 |
| C: Sonication time | 1.38611 | 1 | 1.3861 | 0.14 | 0.7253 |
| AA | 2.97694 | 1 | 2.9769 | 0.30 | 0.6093 |
| AB | 11.4921 | 1 | 11.4921 | 1.15 | 0.3334 |
| AC | 7.0225 | 1 | 7.0225 | 0.70 | 0.4409 |
| BB | 50.6958 | 1 | 50.6958 | 5.05 | 0.0745 |
| BC | 0.664225 | 1 | 0.6642 | 0.07 | 0.8072 |
| CC | 32.2504 | 1 | 32.2504 | 3.22 | 0.1329 |
| Total error | 50.1551 | 5 | 10.0310 | | |
| Total (corr.) | 260.63 | 14 | | | |

R-squared = 80.7562 %, R-squared (adjusted for d. f.) = 46.1173 %, Standard Error of Est. = 3.16718, Mean absolute error = 1.51644, Durbin-Watson statistic = 2.43633 (P=0.1147)

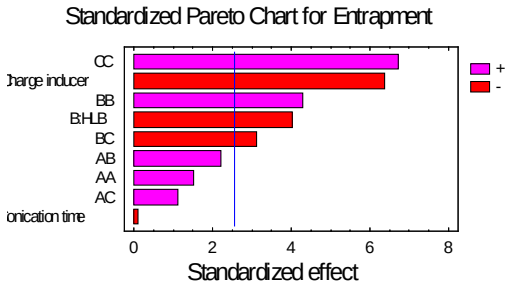


**Fig. 8: Standardized pareto chart showing the quadratic effect and interaction effect of particle size X1, X2 and X3 on the EE%**

**Effect of X1, X2 and X3 on Y2 (EE%)**

Fig. 8, 9 and 10 showed the main effects, interaction effects and quadratic effects of charge inducer (X1), HLB values (X2) and sonication time (X3) on the EE%. According to these figures, it was obvious that $(X3)^2$, X1, $(X2)^2$, X2 and X2X3 respectively had the main effects on the EE%. It was also noted that increasing X1 from 0% to 10% has resulted in decreasing EE% from 28.2 to 2.2 % (negative effect); increasing X2 from 14.9 to 15.6 has decreased EE% from 31.8 to 9.2 then increase to 15.8 (negative effect) while increasing X3 from 0 to 5 min has resulted in decreasing EE% from31.1 to 10.9% then increasing to 31% (no effect).

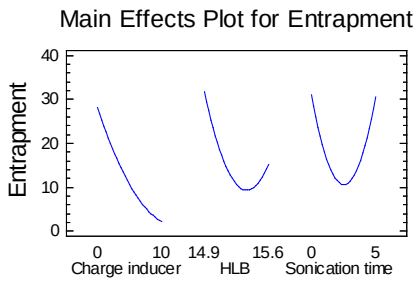


**Fig. 9: Main effect plot showing the effect of X1, X2 and X3 on the EE%**

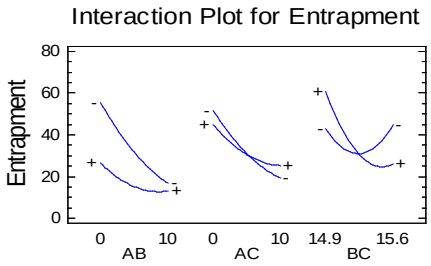


**Fig. 10: Main effect plot showing the interaction effect of X1, X2 and X3 on the EE%**

Table 7 showed the ANOVA for the EE%. Comparing the mean square of each effect to an estimate of experimental error allowed determining whether or not it was statistically significant. In this case, it was noted that five effects (the charge inducer X1, HLB value (X2), $(X2)^2$, sonication time (X2 X3) and $(X3)^2$) had p-value less than 0.05 indicating that it is significantly different from zero at 95% confidence level.

The fitted model accounts for 96.37 % of the variability in the EE %, according to the R-squared statistic. The adjusted R-squared statistic is 89.85 %, making it more suitable for the comparison of the models with different numbers of independent variables. The estimation error shows a residual standard deviation of 5.75. As a result, the residuals have a mean absolute error of 2.83. According to their order in the data file, the Durbin-Watson (DW) statistic examines the residuals in order to determine if there is any significant correlation. Because the DW value is less than 1.4 (1.2586), the residuals are likely to have significant autocorrelation.

**Table 7: Analysis of variance for EE%**

| Source | Sum of square | DF | Mean square | F-ratio | p-value |
|---|---|---|---|---|---|
| A: charge inducer | 1344.9900 | 1 | 1344.9900 | 40.71 | 0.0014 |
| B: HLB value | 536.9360 | 1 | 536.9360 | 16.25 | 0.0100 |
| C: sonication time | 0.4095 | 1 | 0.4095 | 0.01 | 0.9157 |
| AA | 75.6719 | 1 | 75.6719 | 2.29 | 0.1906 |
| AB | 161.5440 | 1 | 161.5440 | 4.89 | 0.0780 |
| AC | 40.7682 | 1 | 40.7682 | 1.23 | 0.3172 |
| BB | 605.8540 | 1 | 605.8540 | 18.34 | 0.0078 |
| BC | 320.0520 | 1 | 320.0520 | 9.69 | 0.0265 |
| CC | 1496.5000 | 1 | 1496.5000 | 45.30 | 0.0011 |
| Total error | 165.1940 | 5 | 33.0388 | | |
| Total (corr.) | 4555.0400 | 14 | | | |

R-squared = 96.3734 percent, R-squared (adjusted for d. f.) = 89.8455 percent, Standard Error of Est. = 5.74794, Mean absolute error = 2.83456, Durbin-Watson statistic = 1.25863 (P=0.0300)

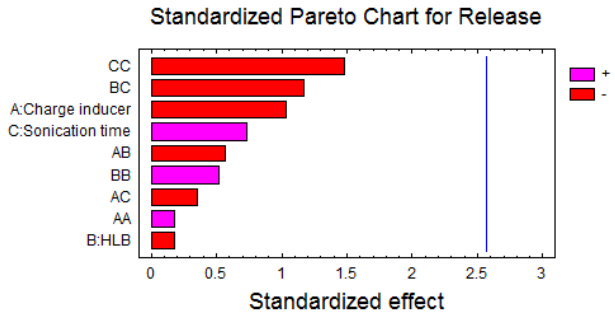


**Fig. 11: Standardized pareto chart showing the quadratic effect and interaction effect of X1, X2 and X3 on the release after 8 h**

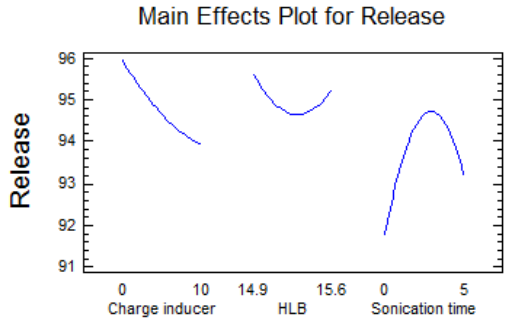


**Fig. 12: Main effect plot showing the effect of X1, X2 and X3 on the release after 8 h**

**Effect of X1, X2 and X3 on Y3 (release after 8 h)**

Fig. 11, 12 and 13 showed the main effects, interaction and quadratic effects of charge inducer (X1), HLB value (X2) and sonication time (X3) on the release after 8 h. According to these figures, it was obvious that no factor had an effect on the release after 8 h. It was also noted that increasing X1 from 0% to 10% has resulted in decreasing release after 8 h from 96% to 93.9 (negative effect); increasing X2 from 14.9 to 15.6 has decreased the release after 8 h from 95.6% to 94.6% then increasing to 95.4% (negative effect); and increasing X3 from 0 to 5 min has resulted in increasing release after 8 h from 91.8 % to 94.8% then decreasing to 93.2% (positive effect).

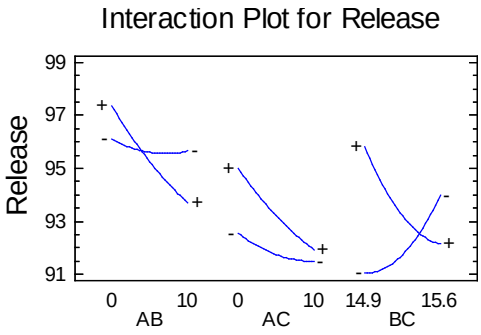


**Fig. 13: Main effect plot showing the interaction effect of X1, X2 and X3 on the release after 8 h**

There was a statistically significant ANOVA for the release after 8 h in table 8. In order to determine the statistical significance of each effect, the mean square was compared to an estimate of the experimental error. None of the factors in this case had a p-value below 0.05, which means they were not significantly different from zero at the 95 % confidence level. In terms of R-squared, the model as fitted accounts for 54.89% of variability in the release after 8 h. For the comparisons of models with different numbers of independent variables, adjusted R-squared statistics are 0%. The

estimated standard deviation of the residuals is 2.827 while the estimated standard error of the estimate is 2.827. The residual mean absolute error (MAE) was 1.428. The DW statistic examines the residuals to see if there is any significant correlation in the data, which are listed in the order they appear in the data file. As the DW value is above 1.4 (1.943), residual autocorrelation is unlikely.

**Table 8: Analysis of variance for release after 8 h**

| Source | Sum of square | DF | Mean square | F-ratio | p-value |
|---|---|---|---|---|---|
| A: Charbe inducer | 8.405 | 1 | 8.4050 | 1.05 | 0.3523 |
| B: HLB values | 0.245 | 1 | 0.2450 | 0.03 | 0.8679 |
| C: Sonication time | 4.205 | 1 | 4.2050 | 0.53 | 0.5009 |
| AA | 0.262564 | 1 | 0.2626 | 0.03 | 0.8633 |
| AB | 2.56 | 1 | 2.5600 | 0.32 | 0.5960 |
| AC | 1 | 1 | 1.0000 | 0.13 | 0.7380 |
| BB | 2.17026 | 1 | 2.1703 | 0.27 | 0.6246 |
| BC | 10.89 | 1 | 10.8900 | 1.36 | 0.2958 |
| CC | 17.601 | 1 | 17.6010 | 2.20 | 0.1980 |
| Total error | 39.9817 | 5 | 7.9963 | | |
| Total (corr.) | 88.6373 | 14 | | | |

R-squared = 54.893 %, R-squared (adjusted for d. f.) = 0.0 %, Standard Error of Est. = 2.82778, Mean absolute error = 1.42889, Durbin-Watson statistic = 1.94334 (P=0.3431)

By applying the optimized response, the optimized formula containing DC-NS is obtained by using the independent variables as follow: Charge inducer (0 %), HLB (15.6) and sonication time (0 min). Table 9 showed the observed and the predicted values of the responses for the optimized formula of DC-NS that suggested by factorial design.

**Table 9: Observed and predicted values of the responses for the optimized DC-NS**

| Response | Observed | Predicted | Residual |
|---|---|---|---|
| Particle size (Y1) | 14.8 | 12.8 | 2 |
| Entrapment(Y2) | 60.5 | 58.43 | 2.07 |
| Percent release after 8 h (Y3) | 97.8 | 95.58 | 2.22 |

**Release kinetics**

By analyzing a linear regression study, it has been possible to determine the proper order of drug release from various formulations. All *in vitro* release results were analyzed by using the first, zero and Higuchi diffusion model equations. According to the results, the drug was released from the niosome via a zero, first order, and Higuchi diffusion model, as shown in table 10.

**Table 10: The calculated correlation coefficients for the *in vitro* release of DC-NS prepared by Box-Behnken design employing different kinetic orders or systems**

| Formula | Zero | First | Higuchi's |
|---|---|---|---|
| F1 | 0.9413 | -0.9807 | 0.96491 |
| F2 | 0.9523 | -0.9893 | 0.97799 |
| F3 | 0.9456 | -0.9138 | 0.95636 |
| F4 | 0.9357 | -0.9889 | 0.97021 |
| F5 | 0.9240 | -0.9783 | 0.96068 |
| F6 | 0.9752 | -0.9914 | 0.99345 |
| F7 | 0.9635 | -0.9821 | 0.98422 |
| F8 | 0.9363 | -0.9921 | 0.97255 |
| F9 | 0.9660 | -0.9749 | 0.97888 |
| F10 | 0.9411 | -0.9933 | 0.97676 |
| F11 | 0.9339 | -0.9797 | 0.96564 |
| F12 | 0.9556 | -0.9816 | 0.98298 |
| F13 | 0.9919 | -0.9474 | 0.99044 |
| F14 | 0.8810 | -0.7369 | 0.83798 |
| F15 | 0.9581 | -0.9460 | 0.98205 |

**Table 11: Physical stability study of DC-NS***

| Time | Drug retained (%) | | |
|---|---|---|---|
| | 4 °C | 25 °C | 40 °C |
| 7 d | 60.5±3.43 | 60.7±3.66 | 60.5±3.77 |
| 15 d | 60.2±2.44 | 60±2.76 | 59.5±2.69 |
| 21 d | 60.2±3.54 | 59.8±1.52 | 58±3.21 |
| 30 d | 60±3.28 | 59.7±2.87 | 55±1.29 |
| 45 d | 59.8±2.54 | 59.2±3.56 | 48±0.54 |
| 60 d | 59.7±2.83 | 59±2.75 | 45±2.48 |

*Results are represented as mean±SD, n = 3.

**Physical stability study of DC-NS**

A physical stability study of the formulated NS was performed to investigate the drug leaching from NS during storage at refrigerator temperature, room temperature, and elevated temperature. The percentage of DC retained after a period of 7, 15, 22, 30, 45 and 60 d in MLVs NS composed of tween 40 with cholesterol in molar ratio 1:1 are shown in table 11. Further, the results of a two-month study show that the maximum percentage of the retained drug was observed at refrigerated conditions rather than room or elevated temperature. This could be due to the increased fluidity of lipid bilayers at higher temperatures, which leads to increased drug leakage [19].

## CONCLUSION

All of this research has conclusively demonstrated that after DC encapsulation, drug release is prolonged at a constant and controlled rate. The study has suggested that different NS formulations can provide consistent and prolonged release of DC. In this way, the entrapped drug will stay in the body for a longer period of time, which lessens any potential side effects and magnifies the medication's positive effects. This finding suggests that the NS drug delivery system could potentially be a successful vehicle for the novel drug delivery system.

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## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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