FORMULATION, CHARACTERIZATION, STABILITY, INVITRO EVALUATION AND OPTIMIZATION OF DIACEREIN NIOSOMES

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ABSTRACT

Niosomes or non-ionic surfactants vesicles are microscopic lamellar structures formed on the admixture of a non-ionic surfactant, cholesterol and stearylamine with subsequent hydration in aqueous media. The delivery of drugs by "vesicular drug delivery system" such as niosomes provides several important advantages over conventional drug therapy. **Diacerein** is an Interleukin-1 inhibitor and it is highly effective in relieving the symptoms of osteoarthritis. Diacerein, in contrast to NSAIDs, are potent inhibitor of IL-1 beta induced nitric oxide production by chondrocytes and cartilage, without reducing prostaglandin E2 production.

The main objective of this study was to design suitable niosome-encapsulated drug delivery for anti-inflammatory drugs like Diacerein and evaluate the vesicle size, entrapment efficiency, in vitro release and physical stability of the system. Non-ionic surfactants used were Tween (40&60), cholesterol and stearylamine in molar ratio 1:1:0.1. The niosomes were prepared by thin film hydration method. The higher entrapment efficiency was observed with niosome (F11) prepared from tween 60, cholesterol and 2.5 min sonication. The release pattern shown by these formulations were first order & Higuchi diffusion controlled mechanism. The physical stability study show that niosomal preparation stored at refrigerated temperature for 60 days show maximum drug retained compare to room temperature and elevated temperature conditions. Finding of all this investigation conclusively demonstrate prolongation of drug release at a constant and controlled rate after niosomal encapsulation of Diacerein.

Keywords: Formulation, Stability, Niosome, Cholesterol, Diacerein, Tween, Stearylamine

INTRODUCTION

The basic goal of drug therapy is to achieve a steady state blood or tissue level that is therapeutically effective and nontoxic for an extended period. The design of proper dosage regimen is an important element in accomplishing this goal **(Satturwar *et al.*, 2002)**. Novel drug delivery systems aim to deliver the drug at a rate directed by the needs of the body during the period of treatment and channel the active entity to the site of action **(Biju *et al.*, 2006)**. Targeted drug delivery implies for selective and effective localization of pharmacologically active moiety at preidentified (preselected) targeted (s) in therapeutic concentration while restricting its access to nontarget normal cellular linings thus minimizing toxic effects and maximizing therapeutic index. Targeted drug delivery is an event where a drug carrier complex/conjugate delivers drug (s) exclusively to the preselected targeted cells in a specific manner **(Vyas and Khar, 2004)**. To pursue optical drug action, functional molecules could be transported by a carrier to the site of action and released to perform their task **(Shahiwala and Misra, 2002).**

The targeting methods may be classified as chemical methods, co-valent bonding and physical methods. Chemical methods involve chemical modification of the parent compound to a derivative, which is activated only at the target site. Various physical methods make use

of the carriers such as liposomes, niosomes, resealed erythrocytes, nano-particles, platelets, magnetic microspheres, and monoclonal antibodies. Recently niosomal drug delivery system (A particulate colloidal carrier system) is drawing attention due to its significant advantages over conventional drug delivery system. It is reported that niosomes are non-ionic surfactant vesicles inclosing an aqueous phase and a wide range of molecules could be encapsulated within aqueous spaces of lipid membrane vesicles. Niosomes or non-ionic surfactants vesicles are microscopic lamellar structures formed on the admixture of a non-ionic surfactant, cholesterol and stearylamine with subsequent hydration in aqueous media **(Sheena *et al.*, 1998)**. Diacerein directly inhibits IL-1 synthesis, release, down modulate IL-1 induced activities and have been shown to posses disease modifying effect in experimental models of osteoarthritis and in human subjects with finger joint and knee osteoarthritis, **(Fidelix *et al.*, 2006)**

The present study was aimed for formulating niosomes of diacerein (IL-1 inhibitor), optimizing the formulation, characterizing them and assessing in vitro performance of the system.

MATERIALS AND METHODS

Materials and Equibement

Diacerein (NUTRA Specialities Private Limited) was obtained as a gift sample from ADCO, Egypt. Tweens (40 and 60) were procured from El-Nasr Pharmaceutical Chemicals Co (ADWIC), Cairo, Egypt. Cholesterol was procured MERCK, E.Merck, and Darmstadt. Stearylamine: purchased from Fluka, Sigma-Aldrich chemie Riedstr.2, Germany. Solvents and other reagents were of analytical grade. All the Ingredients were used without further purification. Phosphate Buffer saline (PBS) pH 7.4 was prepared as described in the Indian Pharmacopia1996. Statistical package STATGRAPHICS plus (version 4, Manugistics Inc., Rockville, MD, USA). Rotary evaporator:(Bibby Sterilin LTD, Stone Staffordshire-England).UV spectrophotometer(Shimadzu UV-1650 P.C.Japan). Bio centrifuge (BiofugePrimo, Heraeus). Mastersizer(X ver.2.15, Malvern instruments Ltd.Malvern, UK). Rotary shaker Bath(Stuart SBS30, Bibby Sterilin LTD, Stone Staffordshire- England). Dissolution test system (Hanson research – Hanson virtual instruments, SR8 plus USA). Probe Sonicator(Cole-Parmer Instrument Co., Chicago-Illinois).

Methods

Preparation of Diacerein Niosomes

Niosomes were prepared by using thin film hydration method. Drug, nonionic surfactants and cholesterol were taken in molar ratio 1:1. Different niosomal formulations were prepared by thin film hydration technique reported by **Azmin *et al,* (1985)**. Accurately weighed quantities of surfactant (either tween 40 or 60) and cholesterol, were dissolved in 10ml of chloroform in a round bottom flask **(Abdulhasan *et al.*, 2010)**. The solvent mixture was evaporated in a rotary evaporator under reduced pressure at a temperature of 60 ± 5 ^{0}C and the flask rotated until a smooth, dry film was obtained. The film was hydrated with 25 ml of PBS 7.4 containing Diacerein (0.5%) at 60 ^{o}C with gentle shaking on a water bath**.** The niosomal suspension was then transferred to a suitable glass container and sonicated using probe sonicator in an ice bath for heat dissipation. The sonicated dispersion was then allowed to stand for about 2 hours at room temperature to form niosomes. The formulation was stored in refrigerator **(Sakthivel *et al.*, 2012)**.

A technique of Box-Behnken design **(Box and Behnken, 1960)** taking three prime selected formulation variables (factors) at three different levels was used to design the experimental work for the preparation of Diacerein entrapped niosomes. 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): Diacerein entrapped niosomes 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**	**Coded**
Formula 1	5%	0	14.9	-1	5 min	1
Formula 2	0%	-1	15.25	0	5 min	1
Formula 3	5%	0	15.6	1	5 min	1
Formula 4	0%	-1	15.25	0	0 min	-1
Formula 5	0%	-1	15.6	1	2.5 min	0
Formula 6	5%	0	14.9	-1	0 min	-1
Formula 7	10%	1	15.25	0	5 min	1
Formula 8	10%	1	15.25	0	0 min	-1
Formula 9	10%	1	15.6	1	2.5 min	0
Formula 10	5%	0	15.6	1	0 min	-1
Formula 11	0%	-1	14.9	-1	2.5 min	0
Formula 12	10%	1	14.9	-1	2.5 min	0
Formula 13	5%	0	15.25	0	2.5 min	0
Formula 14	5%	0	15.25	0	2.5 min	0
Formula 15	5%	0	15.25	0	2.5 min	0

Characterization of Diacerein Niosomes

Photo microscopy

Vesicle dispersions were characterized by photo microscopy for vesicle formation and morphology. Samples of Niosomal formulations were examined under optical microscope by means of fitted camera and photographed at magnification of 40 to 100 X **(Abdelbary and Elgendy, 2008).**

Determination of vesicle size

This is performed for characterization of vesicle's size. Vesicle size of niosomes were determined by using Malvern Mastersizer **(Abdelbary and Elgendy, 2008).**

Determination of Diacerein entrapment efficiency

The prepared Diacerein niosomes were separated from unentrapped drug by centrifugation at 7000 rpm for 30 minutes. The isolated layers were washed twice with PBS 7.4 and recentrifuged again **(El-Ridy *et al.*, 2008; Hu *et al.*, 1999 and Silver *et al.*, 1985).** The amount of Diacerein entrapped was estimated indirectly by measuring the unentrapped

drug in the washing and subtracting it from the total initial amount of Diacerein used at the start of the niosomes preparation.

In vitro release of Diacerein from niosomes

All niosomal formulae were employed in this examination. Each preparation was separated, washed and the amount of Diacerein entrapped was determined (as mentioned above).

The amount of drug retained at zero time was considered as the total amount of drug. The pellet of each preparation was then suspended using phosphate buffer solution (PBS) 7.4 to exactly 500 ml. The Rotary shaker was used to carry out the experiment. The device was adjusted to a rate of 80 stroke/min and the temperature was adjusted to 37-40^{o}C. A 5 ml sample from each of the niosomal suspension was taken at different time interval. The samples were separated and filtered through 0.45 µm filter, the amount of Diacerein released was determined at each time interval and the amount of Diacerein retained was then calculated at each time interval for each formula.

Optimization of Diacerein niosomes:

Statistical Correlation between Independent Variables (Charge inducer percent X1, HLB value X2 and Sonication time X3) and dependent response of Diacerein niosomes (Particle size Y1, Entrapment efficiency Y2 and In vitro release after 8 hrs Y3) using Statistical package STATGRAPHICS plus.

Physical Stability of Diacerein Niosomes

Physical stability of the prepared diacerein niosomes were carried out to investigate the leaching of down from niosomes (in a liquid form) during storage. The samples of niosomal formulations were sealed in a glass vial and stored at refrigeration temperature (4o C), room temperature and elevated temperature (40oC) for a period of 2 months. Samples from each vial were withdrawn at definite time intervals, 15, 22, 30, 45 & 60 days, the residual amount of the drug in the vesicles was determined as described previously after separation from unentrapped drug **(Singh *et al., 2011).***

RESULTS AND DISCUSSION

Characterization of Diacerein Niosomes

Photo microscopy

The photomicrograph of Diacerein niosomes prepared by thin film hydration method is shown in figure (1). They reveal that the niosomes were spherical in shape and exist in disperse and aggregate collections.

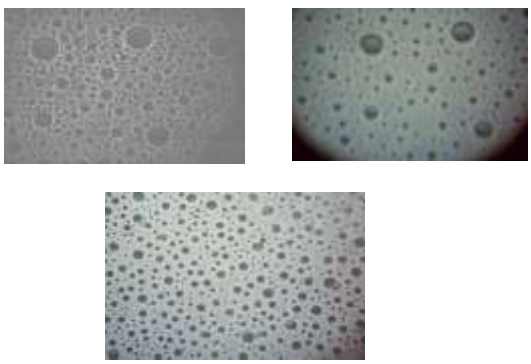

Figure (1): Photomicrograph of diacerein niosomes

Determination of vesicle size

The means particle diameters of niosomes, composed of tween 40 and 60 with cholesterol are shown in table (2). The results reveal that formula 9(tween 40) has the smallest particle diameter (7.33 um) while Formula 11(tween 60) has the largest particle diameter (23.66 um).

Determination of Entrapment efficiency:

The entrapment efficiencies of all niosomal formulations composed of tween 40 and 60 with cholesterol are reported in Table (3). The results reveal that formula 11(tween 60) has the highest entrapment efficiency (58.43 %) while Formula 15(tween 60&40) has the smallest entrapment efficiency (9.52 %).

Table (2): Particle diameter of Diacerein niosomes

Formula	**Particle size**
Formula 1	18.63 um
Formula 2	18.99 um
Formula 3	14.21 um
Formula 4	16.98 um
Formula 5	16.7 um
Formula 6	15.24 um
Formula 7	16.25 um
Formula 8	19.54 um
Formula 9	**7.33 um**
Formula 10	12.72 um
Formula 11	**23.66 um**
Formula 12	21.07 um
Formula 13	21.84 um
Formula 14	23.16 um
Formula 15	20.38 um

Table (3): Entrapment efficiency of diacerein niosomes

Formula	**% entrapped**
Formula 1	55.42
Formula 2	46.99
Formula 3	29.52
Formula 4	52.05
Formula 5	20.96
Formula 6	39.76
Formula 7	24.94
Formula 8	17.23
Formula 9	10.24
Formula 10	49.64
Formula 11	**58.43**
Formula 12	22.29
Formula 13	12.65
Formula 14	9.76
Formula 15	**9.52**

In vitro release of Diacerein from niosomes

Results of an in vitro study on the release of diacerein niosomal vesicles prepared using Tween 40 and Tween 60 and mix of them are shown in Figs. 2, 3 and 4, respectively. The percentage of drug released after 8 h (Q8h) from the prepared niosomal vesicles are shown in Table (4).

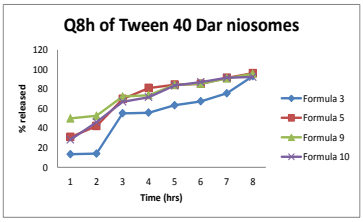

Figure (2): In vitro release of Diacerein from tween 40 niosomes after 8 hrs

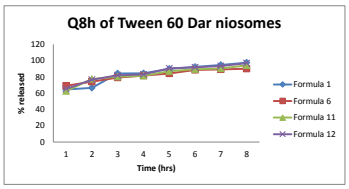

Figure (3): In vitro release of Diacerein from tween 60 niosomes after 8 hrs

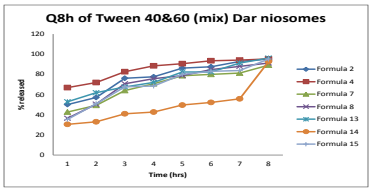

Figure (4): In vitro release of Diacerein from tween 40 &60 (mix) niosomes after 8 hrs.

Table (4) : In vitro release of Diacerein from niosomes after 8 hrs

Formula	Q8 hr(%)
Formula 1	**97.5**
Formula 2	95.2
Formula 3	93.1
Formula 4	95.4
Formula 5	96.2
Formula 6	90.1
Formula 7	**89.1**
Formula 8	91.3
Formula 9	95.6
Formula 10	92.3
Formula 11	94.2
Formula 12	96.8
Formula 13	96.1
Formula 14	92.7
Formula 15	95.2

Optimization:

Factorial Characterization of Diacerein niosomes

The experimental runs and the observed responses for the Diacerein formulations are shown in table (5). The dependent variables studied were Y1 (particle size), Y2 (Entrapment efficiency) and Y3 (Release after 8 hrs) 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 using main effect plot. Figures (5-13) showed the effects of factors X1, X2 and X3

on the response Y1, Y2 and Y3. The results given in these figures were manipulated in details as following:

Table (5): Full factorial design layout

Formula No.	**Variable level in coded form**					
	X1	**X2**	**X3**	**Particle size (Y1)**	**E.E.(Y2)**	**Release (Y3)**
Formula 1	0	-1	1	18.63 um	55.42	**97.5**
Formula 2	-1	0	1	18.99 um	46.99	95.2
Formula 3	0	1	1	14.21 um	29.52	93.1
Formula 4	-1	0	-1	16.98 um	52.05	95.4
Formula 5	-1	1	0	16.7 um	20.96	96.2
Formula 6	0	-1	-1	15.24 um	39.76	90.1
Formula 7	1	0	1	16.25 um	24.94	**89.1**
Formula 8	1	0	-1	19.54 um	17.23	91.3
Formula 9	1	1	0	**7.33 um**	10.24	95.6
Formula 10	0	1	-1	12.72 um	49.64	92.3
Formula 11	-1	-1	0	**23.66 um**	**58.43**	94.2
Formula 12	1	-1	0	21.07 um	22.29	96.8
Formula 13	0	0	0	21.84 um	12.65	96.1
Formula 14	0	0	0	23.16 um	9.76	92.7
Formula 15	0	0	0	20.38 um	**9.52**	95.2

Effect of X1, X2 and X3 on Y1 (particle size)

Figure (5) standardized Pareto chart and figures (6-7) showed the main effects, interaction effects and quadratic effects of charge inducer (X1), HLB values (X2) and sonication time (X3) on the particle size. From the figures it was obvious that (X2) had the main effects on the particle size. Also it was noted that increasing X1 from 0% to 10% resulted in decreasing particle size from 22.5 um to 19.5 um (negative effect); 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).

Table (6) showed the ANOVA for the particle size. The statistical significance of each effect was tested by comparing the mean square against an estimate of the experimental error. In this case it was noted that the HLB value (X2) had p-value less than 0.05 indicating that it significantly different from zero at 95% confidence level. The R-squared statistic indicates that the model as fitted explains 80.75 % of the variability in the particle size. The adjusted R-squared statistic, which is more suitable for comparing models with different number of independent variables, is 46.12 %. The standard error of the estimate shows standard deviation of the residuals to be 3.167. The mean absolute error (MAE) of 1.516 is the average

value of the residuals. The Durbin-Watson (DW) statistic tests the residuals to determine if there any significant correlation based on the order in which they occur in your data file. Since the DW value is greater than 1.4 (2.436), there is probably not any serious autocorrelation in the residuals.

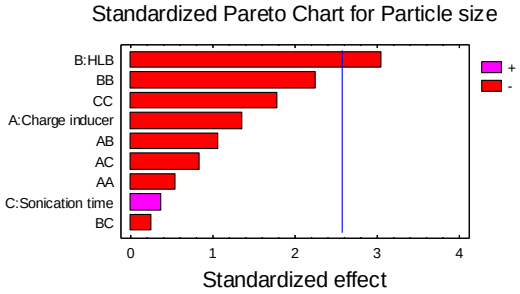

Figure (5): Standardized pareto chart showing the quadratic effect and interaction effect of X1, X2 and X3 on the particle size

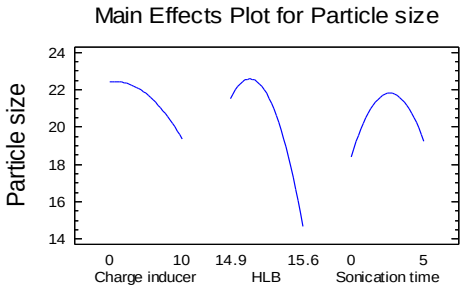

Figure (6): Main effect plot showing the effect of X1, X2 and X3 on the particle size

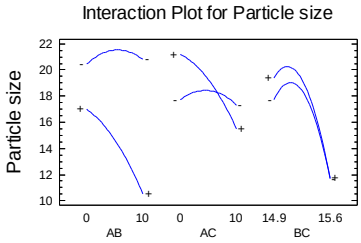

Figure (7): Main effect plot showing the interaction effect of X1, X2 and X3 on the particle size

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

Effect of X1, X2 and X3 on Y2 (entrapment efficiency)

Figure (8) standardized Pareto chart and figures (9-10) showed the main effects, interaction effects and quadratic effects of charge inducer (X1), HLB values (X2) and sonication time (X3) on the entrapment efficiency. From the figures it was obvious that $(X3)^2$, X1, $(X2)^2$, X2 and X2X3 respectively had the main effects on the entrapment efficiency. Also it was noted that increasing X1 from 0% to 10% resulted in decreasing entrapment efficiency from 28.2 to 2.2 (negative effect); increasing X2 from 14.9 to 15.6 decrease entrapment efficiency from 31.8 to 9.2 then increase to 15.8 (negative effect) and increasing X3 from 0 to 5 min resulted in decreasing entrapment efficiency from31.1 to 10.9 then increasing to 31 (no effect).

Table (7) showed the ANOVA for the entrapment efficiency. The statistical significance of each effect was tested by comparing the mean square against an estimate of the experimental error. In this case it was noted that 5 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 significantly different from zero at 95% confidence level.

The R-squared statistic indicates that the model as fitted explains 96.37 % of the variability in the entrapment efficiency. The adjusted R-squared statistic, which is more suitable for comparing models with different number of independent variables, is 89.85 %. The standard error of the estimate shows standard deviation of the residuals to be 5.75. The mean absolute error (MAE) of 2.83 is the average value of the residuals. The Durbin-Watson (DW) statistic tests the residuals to determine if there any significant correlation based on the order in which they occur in your data file. Since the DW value is less than 1.4 (1.2586), there is probably serious autocorrelation in the residuals.

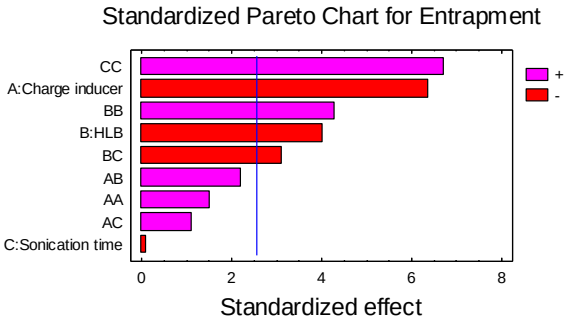

Figure (8): Standardized pareto chart showing the quadratic effect and interaction effect of particle size X1, X2 and X3 on the entrapment

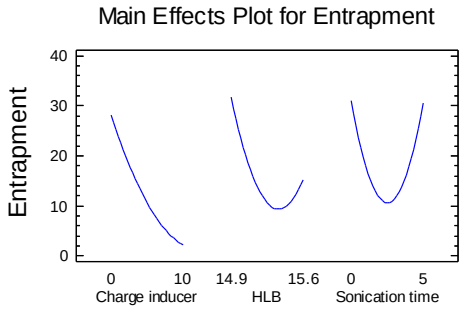

Figure (9): Main effect plot showing the effect of X1, X2 and X3 on the entrapment

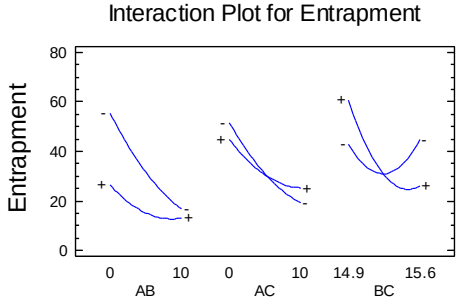

Figure (10): Main effect plot showing the interaction effect of X1, X2 and X3 on the entrapment

Table (7): Analysis of variance for Entrapement efficiency

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)

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

Figure (11) the standardized pareto chart and figures (12-13) showed the main effects, interaction effects and quadratic effects of charge inducer (X1), HLB value (X2) and sonication time (X3) on the release after eight hours. From the figures it was obvious that no factor had effects on release after eight hours. Also it was noted that increasing X1 from 0% to 10% resulted in decreasing release after eight hours from 96% to 93.9 (negative effect); increasing X2 from 14.9 to 15.6 decrease release after eight hours from 95.6% to 94.6% then increasing to 95.4%(negative effect); and increasing X3 from 0 to 5 min resulted in increasing release after eight hours from 91.8 % to 94.8% then decreasing to 93.2% (positive effect).

Table (8) showed the ANOVA for the release after eight hours. The statistical significance of each effect was tested by comparing the mean square against an estimate of the experimental error. In this case it was noted that none of the factors had p-value less than 0.05 indicating that it not significantly different from zero at 95% confidence level.The R-squared statistic indicates that the model as fitted explains 54.89 % of the variability in the release after 8 hours. The adjusted R-squared statistics, which are more suitable for comparing models with different number of independent variables, is 0 %. The standard error of the estimate shows standard deviation of the residuals to be 2.827. The mean absolute error (MAE) of 1.428 is the average value of the residuals. The Durbin-Watson (DW) statistic tests the residuals to determine if there any significant correlation based on the order in which they occur in your data file. Since the DW value is greater than 1.4 (1.943), there is probably not any serious autocorrelation in the residuals.

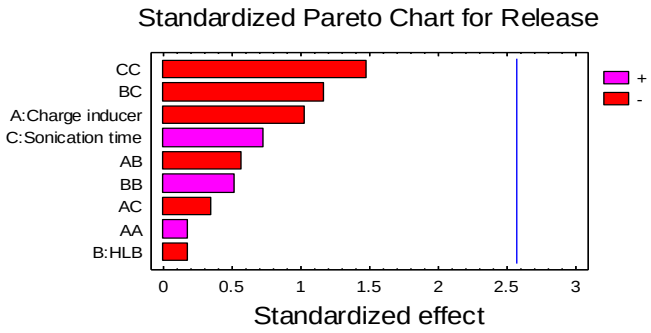

Figure (11): Standardized pareto chart showing the quadratic effect and interaction effect of X1, X2 and X3 on the release after 8 hours.

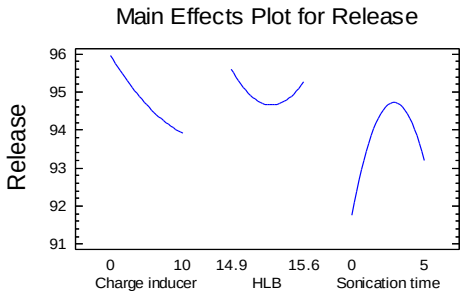

Figure (12): Main effect plot showing the effect of X1, X2 and X3 on the release after 8 hours.

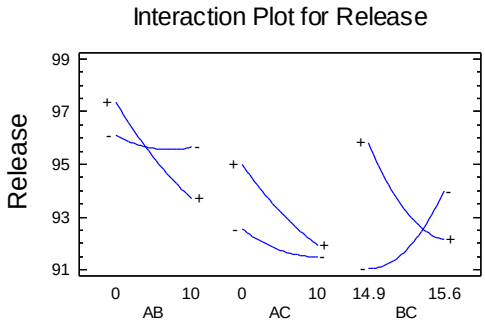

Figure (13): Main effect plot showing the interaction effect of X1, X2 and X3 on the release after 8 hours

Table (8): Analysis of variance for Release after 8 hours

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

By applying the optimize response, the optimized formula containing Diacerein-entrapped niosomes 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 Diacerein niosome that suggested by Factorial design.

Table (9): Observed and predicted values of the responses for the optimized Diacerein niosomes

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

Release Kinetics

We determined the proper order of release of drug from different formulations by analyzing linear regression study. Zero, first and Higuchi diffusion controlled model equations were applied to all in vitro release results. From the results we can conclude that the drug was released from niosome by a zero, a first order and *Higuchi diffusion* controlled mechanism Table (10).

Table (10): The Calculated Correlation Coefficients for The In-Vitro Release of Diacerein Niosomes 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**

Physical Stability Study of Diacerein Niosomes

Physical stability study of the prepared niosomes was carried out to investigate the leaching of drug from niosomes during storage at refrigerator condition, room temperature and elevated temperature. The percentage of Diacerein retained after a period of 7, 15, 22, 30, 45 & 60 days in MLVs niosomes composed of tween 40 with cholesterol in molar ratio 1:1 are shown in table (11). Also the results indicate that maximum percentage drug retained was observed at refrigerated conditions than room temperature and elevated temperature, after 2 months study. This may be due to the higher fluidity of lipid bilayers at higher temperature resulting into higher drug leakage.

Table (11): Physical stability study of Diacerein noisome

Time	Drug Retained		
	4 °C	**25 °C**	**40 °C**
7 days	60.5%	60.7%	60.5%
15 days	60.2%	60%	59.5%
21 days	60.2%	59.8%	58%
30 days	60%	59.7%	55%
45 days	59.8%	59.2%	48%
60 days	59.7%	59%	45%

CONCLUSION

All this investigation conclusively demonstrate prolongation of drug release at a constant and controlled rate, after encapsulation of Diacerein. The study suggests that niosomal formulation can provide consistent and prolonged release of Diacerein from different niosomal formulations. It will lead to sustained action of the entrapped drug that reduce the side effects associated with frequent administration of the drug and potentiate the therapeutic effects of the drug.

It shows that niosomal drug delivery system may be a promising carrier for the novel drug delivery system.

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صياغة وتوصيف ودراسة الثبات والاتاحة المعملية للنيوزومات المحتوية علي الدياسيرين

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النيوزومات هي حويصلات تتكون من منشطات سطحية غير أيونية ويمكن الحصول عليها من هدرجة المنشطات السطحية مع أو بدون ادراج الكوليسترول أو الدهون الأخرى.كما أنها مماثلة لليبوزومات التي يمكن استخدامها كناقلة للأدوية المحايدة والمحبة للدهون وتعد النيوزومات وسيلة واعدة لتوصيل الدواء ومن مميزاتها أنها غير سامة ومتوافقة ومتحللة حيويا. الدياسيرين هومانع افرازإينترليوكين-١ وهو فعال للغاية في تخفيف أعراض التهاب المفاصل. وعلى النقيض من الأدوية المضادة للالتهاب،علي الرغم من أن الدياسيرين والرايين، الناتج النشط للدياسيرين، مثبطات قوية لإفراز أكسيد النيتريك الناتج من التحفيز الخاص بالانترلوكين-١بيتا علي الغضروف لكنه لا يؤثر علي إنتاج البروستاغلاندين-إي ٢.

لذلك كان الهدف من هذا العمل هو تحضير النيوزومات كحاملات لعقارالدياسيرين وتقييم حجم الجزيئات وفاعلية الاحتواء والإتاحة المعملية لانطلاق الدياسيرين من النيوزومات بعد ٨ ساعات بهدف تحسين تأثيره العلاجى كمضاد لالتهابات المفاصل.

وقد تم تحديد أقصى طول موجي لامتصاص وتشييد المنحنيات المعيارية للدياسيرين فى محلول منظم ذو أس هيدروجيني ٧.٤ وهو ٢٥٨ نانوميتر وتم التحضيرالنيوزومات باستخدام طريقة هيدرة الفيلم المتكون .وأظهرت نتائج نظام الاستجابة السطحية لنظام بوكس بنكن ان اعلي فاعلية احتواء للدياسيرين ظهرت في تجربة ١١ المحضرة باستخدام الكوليسترول والتوين ٦٠ والذبذبات لمدة٢.٥ دقيقة كما أظهرت النتائج أن النيوزومات المحتوية علي الدياسيرين أكثر ثباتا في درجات الحرارة الباردة عن درجات الحرارة العالية او درجة حرارة الغرفة .