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# Diacerein Loaded Novasome for Transdermal Delivery: Preparation, In-Vitro Characterization and Factors Affecting Formulation [#]

**Noor Yousif Fareed [*,1] and Hanan Jalal Kassab[2]**

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[1]Department of Pharmaceutics, College of Pharmacy, University of Basrah, Basrah, Iraq.
[2]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

## Abstract

Diacerein (DCN) is a semi-synthetic anthraquinone derivative of Rhein that is indicated for the management of osteoarthritis. Diacerein exhibits poor dissolution in the GIT fluids and suffers from low bioavailability upon oral administration in addition to the laxative effect of Rhein metabolites. The aim of the present study was to develop novasomal vesicles with optimized entrapment efficiency and size to serve as a carrier for transdermal delivery of diacerein. Novasomal vesicles were prepared by the thin film hydration method thin film hydration. The prepared vesicles were optimized utilizing different surfactant-to-cholesterol molar ratios, sonication types, different sonication times, and varying fatty acid levels. The prepared vesicles were characterized for drug entrapment efficiency, vesicle size, and PDI. The best formula was further investigated for zeta potential ten TEM and compatibility study by FTIR analysis. Results showed that F6 was the best formula regarding it vesicle size 275.2± 2.68nm, entrapment efficiency 69.415± 0.234 %, and PDI 0.309± 0.016. In conclusion, novasomal dispersion could be successfully formulated with the thin film hydration method.

**Keywords: Diacerein, Novasome , Transdermal Delivery, Vesicular System.**

# حويصلات الداياسيرين النوفاسومية كنظام توصيل عبر الجلد: تحضير , تقييم خارج الجسم وتأثير المتغيرات على الصيغ المحضرة#

**نور يوسف فريد*،١ و حنان جلال كساب٢**

#المؤتمر العلمي الثاني لطلبة الدراسات العليا
١ فرع الصيدلانيات ، كلية الصيدلة ، جامعة البصرة ، بصرة ، العراق
٢ فرع الصيدلانيات ، كلية الصيدلة ، جامعة بغداد، بغداد ، العراق

## الخلاصة

يصنف عقار الداياسيرين كمشتق انثراكوينون شبه صناعي لمادة الرين المستخدمة لعلاج حالات السوفان. يعتبر الداياسيرين قليل الذوبانية في سوائل الجهاز الهضمي عندما يؤخذ عن طريق الفم بالاضافة الى التاثير المسهل للرين .الهدف من هذه الدراسة هو تطوير حويصلات النوفاسوم لعقار الداياسيرين بكفائة تحميل عالية و حجم مناسب كنظام توصيل عبر الجلد . تم تحضير النوفاسومات بطريقة الترطيب بالغشاء الرقيق وقد تمت دراسة العديد من المتغيرات مثل كمية معامل التوتر السطحي غير الايوني بالنسبة للكوليسترول , تأثير نوع و وقت الصوتنة بالموجات فوق الصوتية .تم تقييم الحويصلات بالنسبة الى كفائة التحميل ، الحجم و معامل التشتت. كما تم عمل تقييمات اضافية للصيغة المختارة من حيث قياس فرق جهد زيتا ، شكل الحويصلات بواسطة المجهر الالكتروني النافذ وتوافق الدواء مع السواغ بواسطه جهاز مطياف الاشعة تحت الحمراء. اظهرت النتائج النتائج ان التركيبة(٦) أبدت أفضل الخصائص الفيزيائية للحويصلات النوفاسومية من حيث كفائة تحميل بمقدار ٦٩.٤١٥ +٠.٢٣٤ ٪ , حجم الجزيئات ٢٧٥.٢+٢.٨٦ نانوميتر ومعامل التشتت ٠.٣٠٩+٠.٠١٦ . تم الاستنتاج من خلال نتائج الدراسة نجاح طريقة ترطيب الغشاء الرقيق لتحضير نوفاسومات الداياسيرين الحويصلية بكفائة تحميل جيدة و حجم مناسب للتطبيق عبر الجلد.

**الكلمات المفتاحية :الداياسيرين ، النوفاسومية ، نظام توصيل ، النظام الحويصلي**

## Introduction

Diacerein (DCN) is a semi-synthetic anthraquinone derivative (diacetyl derivative of Rhein) inducted for the treatment of osteoarthritis (1). Up on Oral administration, Diacerein is metabolized to rhein (2). The chemical structure of Diacerein and its active metabolite is illustrated in Figure 1. The basic mechanism is related to the ability of Diacerein and its active metabolite rhein to interfere with the main mechanisms involved in cartilage degeneration caused by interleukin-1β (IL-1 β) synthesis and nitrous oxide (NO) production (3). Oral administration of the BCS class II Diacerein is associated with low oral bioavailability (35-50%) due to limited dissolution in the GIT fluid (4).

Furthermore, in the colon, the undissolved rhein is oxidized by bacteria to rhein-9-anthrone inducing a laxative effect, which is associated with diarrhea or soft stools which causes poor compliance and treatment discontinuation(5). Also, in 2015 the European Medicines Agency (EMA) restricted the use of diacerein-containing medicines in osteoarthritis elderly patients, because of major concerns about the frequency and severity of diarrhea (6).

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[1]Corresponding author E-mail: noor.fareed@uobasrah.edu.iq

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Several attempts have been investigated to replace oral administration of Diacerein (7,8). Dermal delivery seems to be an attractive approach. For osteoarthritis management, it's required to design a transdermal delivery system that transposes the stratum corner (9).

Vesicles consist of one or more concentric lipid bilayers formed by amphiphilic molecules surrounding an aqueous phase (10). Colloidal vesicles have the ability to entrap drugs in their bilayer or within the aqueous compartment according to their lipophilic/hydrophilic properties, respectively (11). Vesicular carriers have been investigated in transdermal drug delivery to enhance drug penetration through the stratum corneum (12). Unfortunately, old-generation systems such as liposomes and niosomes showed limited ability to perform this purpose (13).

Novasome is considered a newly developed vesicular structure consisting of non-ionic surfactants and free fatty acids with or without cholesterol (14). It is proposed that free fatty acids will augment transdermal permeation when combined with non-ionic surfactants as they act by fluidizing the lipids of the stratum corneum and increasing the flexibility of the vesicular structure (15,16).

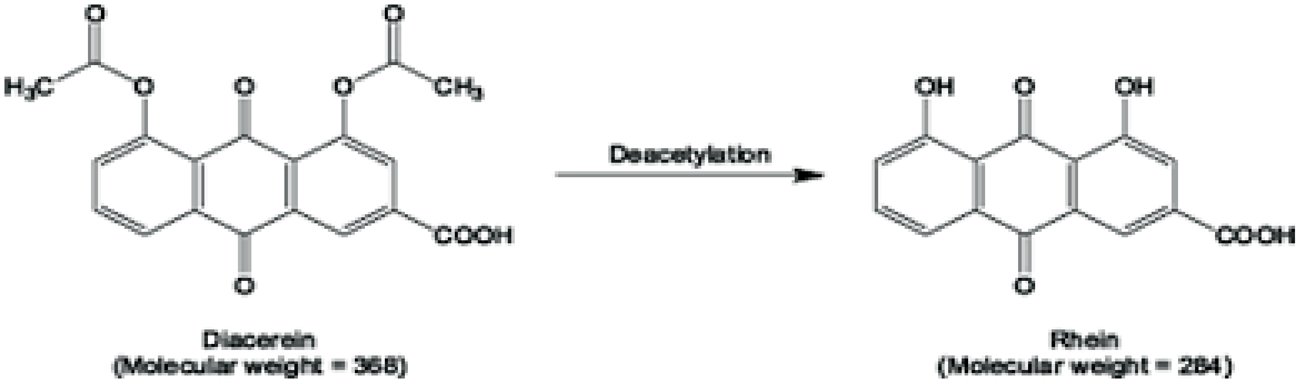

**Figure 1. Chemical structure of diacerein and rhein(2)**

The pharmacokinetic and physicochemical properties of DCN encouraged its transdermal delivery. These include its molecular weight (358.294g/mol), lipophilicity (log *P* = 1.7), and half-life (4.25 hours) (17,18).

The aim of the present study was to develop novasomal carrier for DCN with optimized entrapment efficiency and vesicle size suitable for transdermal delivery, avoiding poor dissolution and diarrhea associated with oral treatment.

## Materials and Methods

### *Materials*

Diacerein (DCN) was purchased from Hangzhou Hyper Chemicals Limited, China. Cholesterol (CH), Span 60(sorbitan monostearate)Xi'an Sonwu biotech Co., Ltd ,China . Stearic acid and Phosphate buffer saline pH 7.4 were purchased from Himedia, India. Methanol and chloroform were obtained from alpha chemicals.

### *Methods*

### *Preparation of DCN Novasomes*

The thin film hydration method was utilized to prepare DCN novasomal formulas. The composition of the prepared formulas is given in Table 1.

The molar ratio was used to design the formulation composition and accordingly, the weight of each component is calculated. The amounts of DCN, span 60, cholesterol, and stearic acid were dissolved in a 15 mL chloroform-methanol mixture (2:1 v/v) and sonicated for 10 min in a bath sonicator (Power sonic 410, Hwashin Technology, Korea) for complete dissolution. The resultant clear solution was added into a 250 mL round bottle flask that was attached to a rotary evaporator (IKA RV8, USA). The solvents were slowly evaporated by rotation at 100 rpm at 60 °C for 30 minutes under reduced pressure till a clear transparent film was produced on the walls of the flask. Then, 20 mL of distilled water was added to the resultant film and allowed to be hydrated by rotation at 150 rpm into water bath previously warmed to 70 °C for 1 hour. The resultant novasomal dispersion was kept in the refrigerator at 4°C overnight for complete annealing of the vesicular wall. Each formula was prepared in triplicate.

**Table 1. The Composition of DCN Novasome Formulas**

| F-code | Drug (mg) | Molar ratio of Cholesterol: Span 60 | Amount of cholesterol (mg) | Amount of Span60 (mg) | Molar ratio of Span 60 : Stearic acid | Amount of stearic acid (mg) | Sonication type | S-time (minute) |
|---|---|---|---|---|---|---|---|---|
| F1 | 10 | 1:2 | 33.8 | 86 | 1:0.25 | 14.123 | None | **0** |
| F2 | 10 | 1:4 | 33.8 | 172 | 1:0.25 | 28.246 | None | **0** |
| F3 | 10 | 1:6 | 33.8 | 258 | 1:0.25 | 42.369 | None | **0** |
| F4 | 10 | 1:4 | 33.8 | 172 | 1:0.25 | 28.247 | Bath sonication | **10** |
| F5 | 10 | 1:4 | 33.8 | 172 | 1:0.25 | 28.247 | Bath sonication | **20** |
| F6 | 10 | 1:4 | 33.8 | 172 | 1:0.25 | 28.247 | Bath sonication | **30** |
| F7 | 10 | 1:4 | 33.8 | 172 | 1:0.25 | 28.247 | Probe sonication | **1** |
| F8 | 10 | 1:4 | 33.8 | 172 | 1:0.25 | 28.247 | Probe sonication | **2** |
| F9 | 10 | 1:4 | 33.8 | 172 | 1:0.25 | 28.247 | Probe sonication | **3** |

***Optimization of formulation variables***

Several independent variables have been tried to optimize the DCN novasome formulations prepared by thin film hydration in terms of vesicle size, entrapment efficiency, and poly-dispersibility index. The variables studied were as follows:

***1-Effect of surfactant molar concentration***

The effect was investigated in F1, F2, and F3 using keeping cholesterol at 100 umoles against 3 levels of surfactant concentration expressed as 200, 400, and 600 umoles. Other variables were kept constant for effective comparison.

***2-Effect of sonication type***

In order to decide the most appropriate size reduction technique, two types of sonication were employed namely, Bath and probe sonication as clarified in Table 1. The parameter set up for the bath sonication process (amplitude 50% and temperature 25 °C), sonication carried out as cycles each one for 10 minutes with 5 minutes off between cycles. On the other hand, probe sonication (Qsonica sonicators, USA) parameters were: amplitude 20%, pulse 1sec. on, 3 sec. off.

***2-Effect of sonication time***

Also, several sonication times were employed for both bath and probe sonication. For bath sonication, 10 minutes (1 cycle), 20 minutes (2 cycles), and 30 minutes (3 cycles) were used for F4, F5, and F6 respectively. In the case of probe sonication, F7, F8, and F9 were subjected to probe sonication for 1 minute, 2 minutes, and 3 minutes, respectively.

***Characterization of DCN novasomes***

Proper characterization of the formulated DCN novasomes is considered essential for quality assurance. All of the prepared DCN novasomal dispersions were evaluated for drug entrapment efficiency, vesicle size, and poly disperseability index. The selected formula was further evaluated for zeta potential, morphology by TEM, and compatibility study by FTIR analysis.

***Determination of vesicle size and PDI***

Dynamic light scattering technology using Zetasizer Nano ZS (Malvern Instruments, UK) was employed in order to determine the mean vesicle size of the prepared DCN Novasomal dispersion. Appropriate dilution with distilled water (1:10) is required for obtaining suitable scattering intensity (19,20). Results were reported as mean ± SD.

***Determination of DCN entrapment efficiency (EE%)***

The EE % of DCN with the prepared novasomal vesicles was estimated by ultrafiltration technique by measuring its free concentration within the supernatants (21). The procedure consists of taking 1mL of the dispersion into the upper chamber of a centrifuge tube matched with an ultrafilter (Millipore Company, USA, MWCO 10 kDa) and centrifugation for 30 minutes at 6000 rpm. Appropriate dilution of the ultrafiltrate with phosphate buffer is required to estimate the concentration of free unentrapped DCN spectrophotometrically at 258.8 nm. The EE% was calculated using equation (1). Results were reported in triplicates as mean ± SD(22).

$$EE\% = \frac{Total\ amount\ of\ drug\ added - Free\ Drug}{Total\ amount\ of\ drug\ added}\ 100\% \qquad \text{equation 1}$$

***Measurement of zeta potential for the selected formulas***

The surface charge of the prepared vesicles was estimated in terms of zeta potential by the estimation of their electrophoretic mobility. This is performed through a Malvern instrument coupled with a laser Doppler anemometer and at a scattering angle of 90° (23).

***Transmission electron microscopy (TEM) studies***

The morphology and topography of the best achieved DCN-loaded novasomes were examined via transmission electron microscopy (Joel JEM 1230; Tokyo, Japan) via the negative staining technique. Briefly, one drop of the diluted novasomal dispersion was loaded onto a carbon-coated copper grid and left for 1 minute to get attached to the carbon substrate. Following this, the sample was allowed to dry at room temperature and examined(24).

***Fourier transform infrared spectroscopy (FTIR)***

FTIR is employed to confirm drug purity and drug excipient compatibility(25). In this study**,** FTIR Spectrum for the pure drug DCN, physical mixture (1:1 molar ratio of drug cholesterol, stearic acid, and Span 60 using the KBr disc method. Also, FTIR spectrum for the dispersion of the selected formula was also tested as a liquid(26).

***Statistical Analysis***

Statistical analysis was performed by Graph Pad Prism 8.0.1 program. The results were expressed as mean ± SD. Analytical statistics in terms of ANOVA test with posthoc Tukey's or Dunnett's multiple comparisons test was used to examine the significance among different formulas. A P-value less than 0.05 was considered to be significant.

## Results and Discussion

A total number of 9 formulas of DCN Novasomes were prepared by the thin film hydration method. The characterization parameters are illustrated in Table 2.

**Table 2.Characterization Parameters for DCN-Loaded Novasomes**

| F-code | Vesicle size (nm) | PDI | EE% |
|---|---|---|---|
| F1 | 433.73± 27.22 | 0.534± 0.049 | 34.255 ± 0.257 |
| F2 | 2416± 162.025 | 1.101± 0.486 | 87.928 ± 0.348 |
| F3 | 6455.33± 737.569 | 0.984± 0.824 | 84.49 ± 0.09 |
| F4 | 727.066± 10.61 | 1.003± 0.182 | 71.155± 0.245 |
| F5 | 532.23± 29.67 | 0.535± 0.0033 | 70.435± 0.205 |
| F6 | 275.2± 2.68 | 0.309± 0.016 | 69.415± 0.234 |
| F7 | 293.9±7.68 | 0.387± 0.023 | 52.75± 0.08 |
| F8 | 150± 2.92 | 0.251± 0.036 | 23.08± 0.1 |
| F9 | 133.16± 2.926 | 0.282± 0.025 | 10.825± 0.275 |

### *A-Effect of formulation variables on vesicle size*

The design of an effect vesicular system for transdermal delivery requires special attention to the required size needed to obtain effect penetration into the skin layers since small particles penetrate more effectively than large ones(27). The mean size of DCN novasomes is illustrated in Table 2.

### *Effect of increasing the amount of surfactant relative to cholesterol*

This effect was investigated in F1, F2, and F3 using keeping cholesterol at 100 umoles against 3 levels of surfactant concentration expressed as 200, 400, and 600 umoles. There was a significant increase in vesicle size (p-value $<0.05$) as the amount of surfactant increased. According to results in **Table 2**, vesicle size raised from 433.73± 27.22 nm in F1 to 2416 ± 162.025 nm and 6455.33± 737.569 nm in F2 and F3, respectively. This could be explained on the basis of the span 60 structure as its long alkyl chain produces large vesicles(28). Furthermore, the amount of stearic acid increases surfactant amount increase leading to more stearic acid deposited on or interpenetrating into the vesicles (29).

### *Effect of sonication type*

Generally, Sonication is required to produce mono-disperse vesicular dispersion after vesicle preparation by thin film hydration method(30). Most frequently, size reduction is by use of probe or bath sonication and results in small unilamellar vesicles (31).

The effect of ultrasound mechanical waves on the lipid membrane is attributed to cavitation or bubble formation. The formed bubbles oscillate nonlinearly and then collapse resulting in violent implosion and local heat production, this process causes a size reduction of the dispersed vesicles (32).

A significant decrease in the mean hydrodynamic diameter (p-value $< 0.05$) is associated with the use of bath sonication in F6 and probe sonication in F9 as compared to the non-sonicated formula F2. Similar findings were also reported in two other researches (33,34).

Despite the efficiency of probe sonication in providing smaller vesicular size as compared to bath sonication, it is important to take into consideration the entrapment efficiency change in each type to decide the most appropriate technique.

### *Effect of sonication time*

In the case of bath sonication, **a** decrease in vesicle size is achieved as the sonication time is increased from 0 minutes in F2 to 10,20, and 30 minutes in F4, F5, and F6, respectively. However, it is statistically significant (p-value $< 0.05$) in F6 compared to F2. Similar findings were also reported in another study (33).

In the case of probe sonication efficiency in reducing vesicle size is significant (p-value $< 0.05$) enhanced by increasing the sonication time from 0 minutes in F2 into 1,2 and 3 minutes in F7, F8, and F9, respectively. These findings are harmonious with the results reported by other researchers (34).

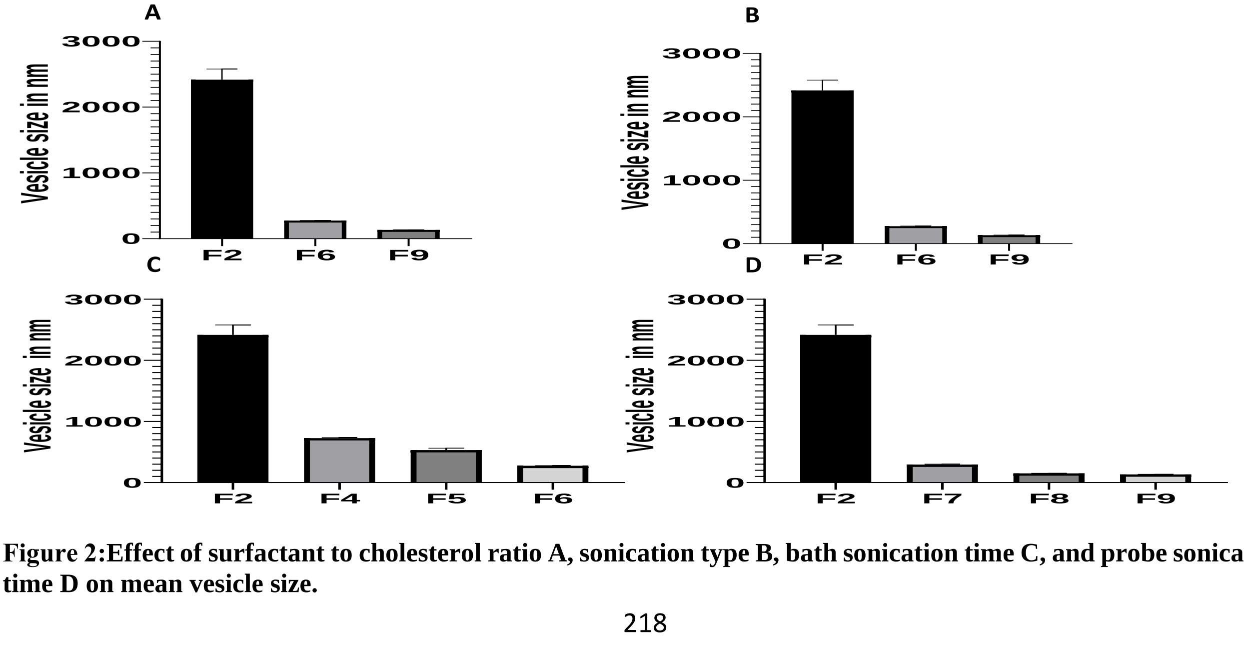

**Figure 2:Effect of surfactant to cholesterol ratio A, sonication type B, bath sonication time C, and probe sonication time D on mean vesicle size.**

### *B-Effect of formulation variables on PDI*

The homogeneity of vesicular size distribution is described by the measurement of PDI value. It is defined as the ratio of standard deviation to mean vesicle size (35).

ANOVA showed a significant decrease in PDI (p-value < 0.05) associated with the use of the size reduction technique represented by bath sonication in F6 and probe sonication in F9 comparison with non-sonicated F2. Similar results were also reported by other researchers (33,34). However, the difference in PDI between F6 and F7 was found to be insignificant by Tukey's multiple comparisons test.

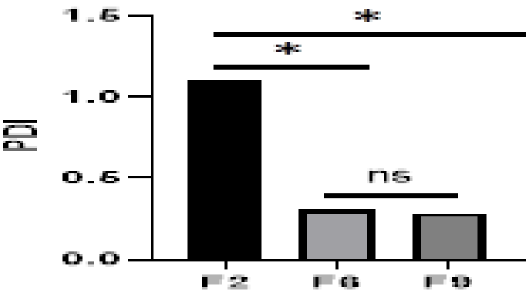

**Figure 3 . Effect of sonication type on the PDI, ns: non-significant, * significant.**

### *C-Effect of formulation variables on EE%*

The ability of novasome to entrap a significant amount of DCN is essential for its prospective use as a transdermal delivery system. Being a lipophilic drug with a log P value of 1.7, DCN is expected to have a preferential partitioning into the lipid phase of the vesicles(36). The percent of DCN entrapped within the novasome is illustrated in **Table 2**.

### *1- Effect of increasing the amount of surfactant relative to cholesterol*

An appropriate balance in the ratio of cholesterol to non-ionic surfactant is required to maximize the entrapment efficiency with vesicles. drug leakage and vesicle fusion are the result of low cholesterol content which consequently causes poor entrapment(37). Statistical analysis using ANOVA followed by Dunnett's multiple comparisons test showed that F1 and F3 EE% are significantly lower than F2. Firstly, the EE% increased from 0.257 ± 34.255 in F1 to 87.928 ± 0.348 in F2 as the amount of surfactant increased from 200 to 400 umoles, respectively. A larger quantity of surfactant results in a lipophilic ambiance for the accommodation higher quantity of lipophilic drugs (38). In contrast, when a smaller amount of surfactant is used, a limited number of vesicles are formed which are unable to entrap sufficient amounts of DCN(39). However, at the molar ratio of cholesterol to surfactant 1:6 in F3, there will be a decrease in the membrane rigidity leading to reduced DCN entrapment inside the prepared novasomes(40).

### *2-Effect of sonication type*

A significant decrease in EE% of DCN within the novasomes (p-value < 0.05) is associated with the use size reduction technique represented by bath sonication in F6 and probe sonication in F9, as compared to the un-sonicated vesicles in F2.

Heat production associated with using probe sonication produces a negative effect on the chemical stability of formula ingredients causing vesicular destruction and melting of stearic acid therefore leakage of the entrapped drug (41). Bath sonication is an effective alternative due to the possibility of controlling sonication parameters while producing a mono-disperse system (42).

### 2-Effect of sonication time

In the case of bath sonication, there is a statistically significant (p-value < 0.05) decrease in the EE% of DCN within the novasome as the sonication time increases from 0 minutes in F2 to 10, 20, and 30 minutes in F4, F5, and F6, respectively. Similar findings were also reported in another study (43).

For probe sonication, the EE% is significantly (p-value < 0.05) decreased by increasing the sonication time from 0 minutes in F2 to 1, 2, and 3 minutes in F7, F8, and F9, respectively. These findings are harmonious with the results reported by other researchers (43). For comparisons above, the ANOVA test was used followed by Dunnett's multiple comparisons test.

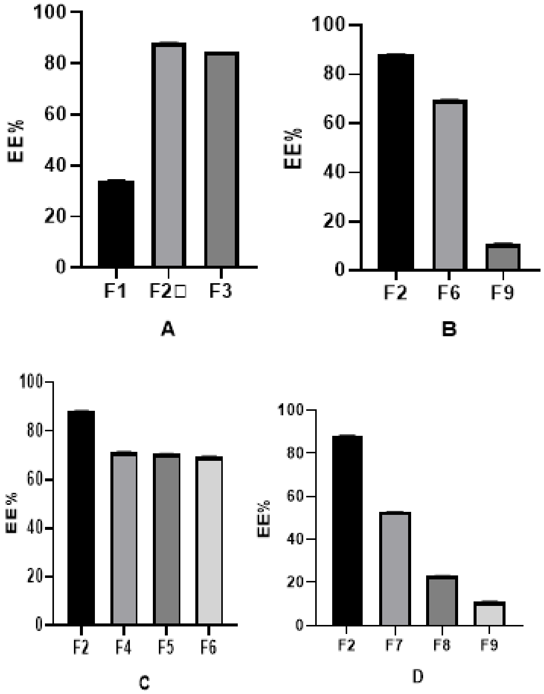

**Figure 4. Effect of surfactant to cholesterol ratio A, sonication type B, bath sonication time C, and probe sonication time D on entrapment efficiency.**

### Zeta Potential of DCN Novasomal Dispersion

Zeta potential can be considered an indirect measurement of the stability of vesicular dispersion. A stable vesicular system is expected when the ZP value is around ±30 mV(44). It can be seen in **Table 3** that the value of zeta potential is high and negative which is related to the intrinsic charge of DCN as it ionizes at the pH of the dispersion medium (the $pK_a$ of DCN is 2.98) (45). Also, The negative charge could be attributed to the ionization of the carboxyl group of the fatty acid as reported by other studies (46). Accordingly, the prepared dispersion is physically stable and associated with low aggregation probability and crystal growth.

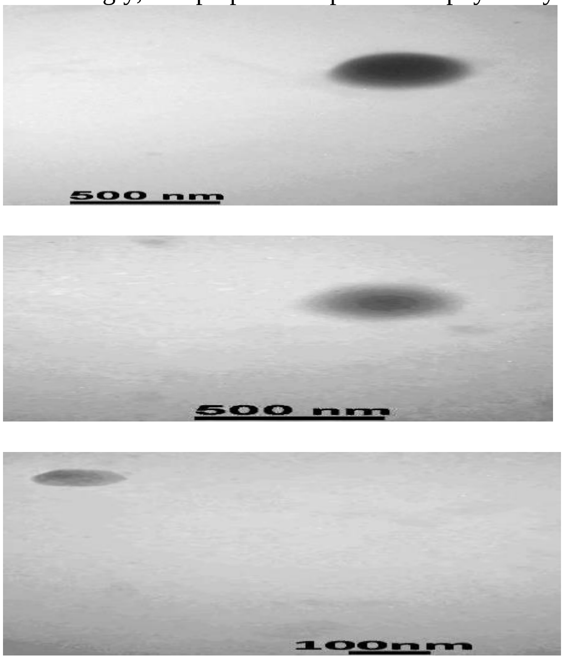

**Figure 5: TEM Images of F6 Selected Novasomal Formula**

**Table 3: Zeta Potential of the Selected DCN Novasomal Formula**

| Formula Code | Zeta potential |
|---|---|
| F6 | -52.763± 4.85 |

### Transmission Electron Microscope Images

The TEM images of the selected novasomal formula are shown in **Figure 5**. The images show spherical vesicles with nano size range which represent novasome vesicular structure.

### Fourier Transform Infrared Spectroscopic (FTIR) Analysis.

The FTIR spectra of the pure DCN, physical mixture (DCN, span 60, cholesterol, stearic acid), and selected formula F6 are shown in **Figure 6.** The FTIR spectrum of DCN (A) displayed characteristic peaks represented by ester group C=O stretching at 1770.65 $cm^{-1}$. Ketone group C=O stretching at 1678 $cm^{-1}$, 759.95 $cm^{-1}$ (m- substituted benzene) and 702.09 $cm^{-1}$ (benzene) (47). These peaks are also reported in other literature which indicates the purity of DCN and the absence of any kind of impurity (48). The characteristic peaks of DCN were also present in the physical mixture with the excipients used in the formula indicating the absence of interaction(49). However, the characteristic peaks were not found in the spectra of the selected formula (C) indicating drug entrapment within the vesicles (50).

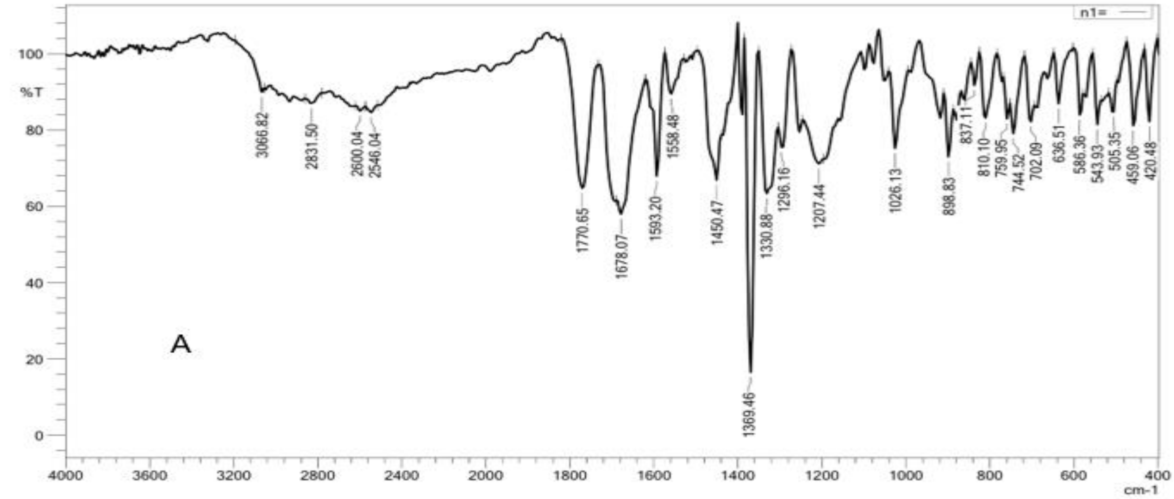

**Figure 6. FTIR Spectra of A-Diacerein , B- Physical mixture , C- Selected Formula**

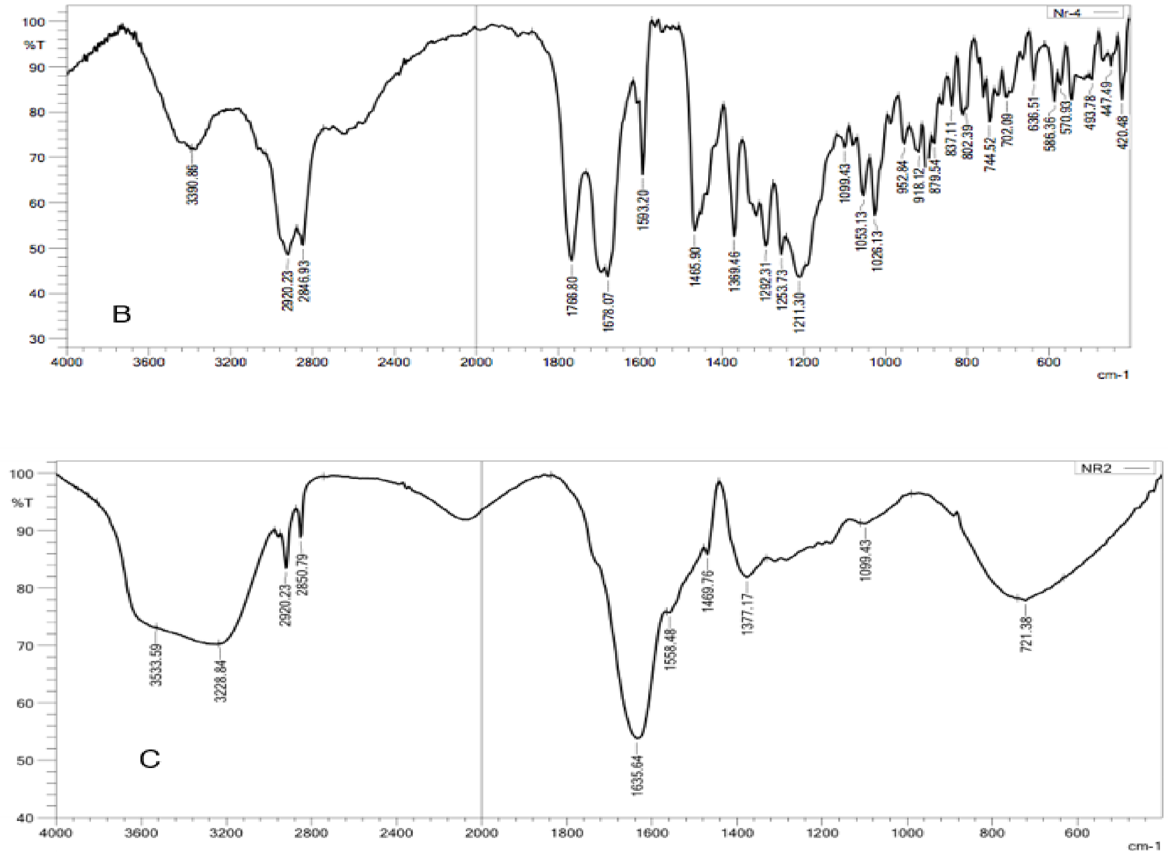

**Figure 6. FTIR Spectra of A-Diacerein, B- Physical mixture, C- Selected Formula**

## Conclusions

In this study, the vesicular carriers were suggested to enhance DCN delivery through the skin to avoid the side effects associated with its oral administration. Novasomal vesicle was successfully prepared by thin film hydration method using span 60 as a surfactant and stearic acid as fatty acid with cholesterol as a membrane stabilizer. The properties of the vesicle in terms of size entrapment and PDI were optimized through a combination of formulation and process variables. the optimized formula F6 showed vesicle size suitable for transdermal delivery with low PDI and acceptable EE%.TEM study confirmed spherical vesicle formation. FTIR study confirmed compatibility while the high ZP indicates a low probability of aggregation and enhanced physical stability. Accordingly, DCN-loaded novasomal dispersion could be developed as a platform for transdermal drug delivery to avoid oral side effects.

## Recommendations

The selected formula (F6) needs to be further investigated for incorporation into a suitable vehicle and subjected to an ex-vivo permeation study and in-vivo anti-inflammatory study.

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## Ethics Statements

The authors declare that all procedures followed in the current study were performed *in vitro* and no need for ethical approval from an ethics committee.

## Conflict of Interest

The authors declare that there were no conflicts of interest regarding the publication of this paper.

## Author Contributions

The authors confirm their contribution to the paper as follows: study conception and design: Hanan Jalal Kassab. Experimental work, results interpretation and statistical analysis, draft manuscript preparation: Noor Yousif Fareed. Both authors reviewed the results and approved the final version of the manuscript.

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