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RESEARCH PAPER

Formulation and Characterization Nano Niosomes Loaded with Diclofenac Sodium

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ABSTRACT

Diclofenac sodium is a member of a non-steroidal anti-inflammatory drug (NSAID). It has been widely prescribed NSAID worldwide. It has analgesic, anti-inflammatory and antipyretic effects. Topical application has a relatively short duration of action needed for multiple uses. This study aimed to produce Nano-vesicles loading with Diclofenac sodium to achieve a control release behaviour. The coacervation phase separation method was used to prepare proniosomes vesicles from different surfactants, lecithin, cholesterol, Ethanol and buffer phosphate (pH 7.4) with 1 % glycerine where characterized surface by using TEM and DSL. The surfactant type, surfactant amount, and lecithin amount were adjusted to produce different preparations having various properties. Response surface methodology (RSM) by Design-Expert® software was employed for the design of the experiment (DOE). A transmission electron microscope was employed to characterize the morphology and surface characteristics of the Niosomes. Entrapment efficiency and in vitro release were, also, analysed. Results revealed Niosomes with a particle size of about 96.8 nm and an entrapment efficiency of 80.53 %. The in vitro release study revealed a control-released behaviour for up to 32 h, as compared to plain hydrogel (diclofenac powder in hydrogel base) that was completely released in 4 h. In a conclusion, the preparation of Niosomes vesicles loaded with diclofenac sodium was successfully prepared in the nanoscale range and control release was successfully achieved.

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INTRODUCTION

Proniosomes are the dehydrated form of Niosome. Proniosomes are composed of non-ionic surfactants that can be hydrated to produce a dispersion of the aqueous Niosome [1]. Proniosomal gels have resistance to stress produced by skin flexion, and mucociliary movement and enhance percutaneous absorption because they are composed of non-ionic surfactants. Proniosomes have high stability, better percutaneous absorption and ease of application [2,3]. Proniosomes were considered a smart delivery system that can improve the administration of drug particles through the skin layer. The surfactant plays a fundamental role in penetrating through the stratum corneum and

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can control the percutaneous drug release, while the lipids component can act as depots, providing a release effect at the site of action [4]. When proniosomes gel was applied to the skin, they were changed to Niosomes due to the effect of the sufficiently hydrated environment of the skin, thus delaying the release of their payload [5].

Diclofenac sodium, non-selectively, inhibits both forms of COX (COX-1 and COX-2) enzymes, preventing the conversion of arachidonic acid into prostaglandins [6]. It is sparingly soluble in water, soluble in ethanol, freely soluble in methanol and slightly soluble in acetone. The melting point is about 283 °C [7]. The bioavailability ranged from 50 to 60 % of oral administration. Diclofenac sodium is rapidly excreted after dosing. The plasma half-life is approximately 1 to 2 h. The plasma compartment is primarily restricted to Diclofenac sodium, where the plasma protein binding is more than 99 % [8].

MATERIALS AND METHODS

Materials

Absolute Ethanol was purchased from Hayman Ltd. UK, and Cholesterol and Glycerine were purchased from HiMedia Laboratories, India. Diclofenac sodium was purchased from Sama Al-Fayhaa, Iraq. Lecithin was purchased from M/S

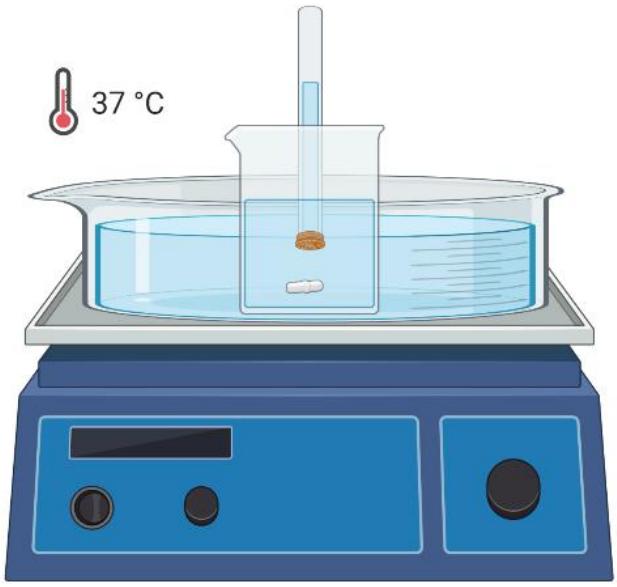


Fig. 1. *In vitro* drug release study by using Modified Franz diffusion cell.

Table 1. Variable selected for the formulation of proniosomes (independent and dependent factors).

Factors			
Independent variables (mg)		Level used	
		Low (-1)	High (+1)
A =	Span 60	0	1000
B =	Tween 80	0	1000
C =	lecithin	100	600
	Dependent variables	Goal (Desirability)	
Y1 =	Vesicles size (nm)	Minimize	
Y2 =	Entrapment efficiency (EE %)	Maximize	

Provisor pharma, India. Span 60 and Tween 80 were purchased from Thomas Baker (Chemicals) Pvt. Ltd, India.

Methods

Preparation of Proniosomes

The Design-Expert® software (Stat-Ease, Inc., version 6.0.10) was used to design the experiments. Factors with two levels were selected and fed to the software. These factors were surfactant type, surfactant amount and lecithin amount and were considered independent variables. The outcomes of these factors were the yield value. The yield value was entrapment efficiency and particle size and considered as the dependent variable. The target or desirability of the optimization was entrapment efficiency (maximized) and particle size (minimised) as illustrated in Table 1 [9].

High and low HLB surfactants such as Tween 80 (HLB 15) and Span 60 (HLB 4.7) were chosen based on hydrophilic-lipophilic balance (HLB) value [5]. The D-optimal RSM by Design-Expert® software was selected to statistically analyse the effect of independent variables on dependent variables (properties) of proniosomes. D-optimal has estimated a lack of fit and replicated some formulas.

Method of preparation of proniosomes

Proniosomes were prepared by weighing different amounts of the surfactant, lecithin, and cholesterol with a fixed amount of diclofenac sodium and mixing them in 1 ml of absolute ethanol in a wide-mouthed cup. The amount of each component was suggested by the software. The mixture was, then, sonicated in the water bath sonicator (Shanghai Kudos LTD, Chin) for approximately 5 min to dissolve them. Then, the cup was tightly closed and put in a shaker water bath (GLF, Germany) at 60 – 70 °C for 5 – 10 min with frequent shaking until all the ingredients were completely dissolved and a clear dispersion was formed. Next, the aqueous phase (PBS pH 7.4 with 0.1 % glycerol) was gradually added and left in the water bath until a clear dispersion was formed. Ultimately, the dispersion was left to maturation at a temperature of 25 ± 2 °C for 24 h and the proniosomes gel was formed [10]. This method was repeated for all formulas.

Particles size and size distribution analysis

Proniosomes preparations were hydrated in PBS (pH 7.4) and dispersed in a water bath sonicator (Shanghai Kudos LTD, Chin) for 5 min. Particle sizes and distribution were analysed using the particle size analyser apparatus (Angstrom Advance Inc. USA). Where the dynamic light scattering was measured at a constant temperature of 25 °C and the angle of scattering was 90°. All measurements were measured in triplicate and the mean ± standard deviation of the size and polydispersity index (PDI) were recorded [11,12].

Entrapment efficiency

Proniosomes preparations were hydrated in 10 ml of PBS (pH 7.4) and warmed at 40 °C for about 5 min to form niosomes. The encapsulation efficiency of niosomes was achieved by the centrifugation method [13]. The niosomal dispersion was centrifuged (Hittech, Germany) at 15,000 rpm a

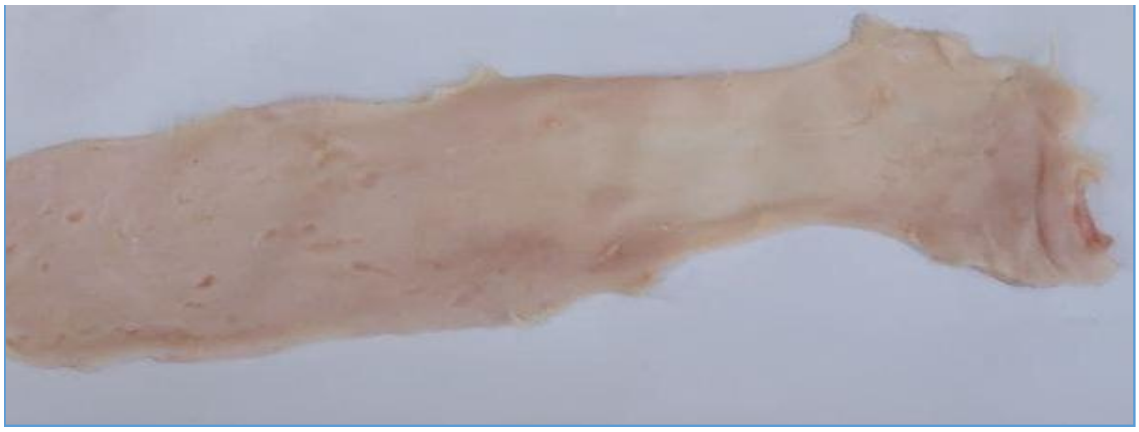


Fig. 2. The skin of Albino rat, which used for *ex vivo* permeation study.

4 °C for 45 min to separate unentrapped drugs as supernatant. The supernatant was separated, filtered (0.22 µg filter paper) and sufficiently diluted with PBS (pH 7.4) to spectrophotometrically read absorption (Shimadzu, Japan) at 275 nm, to determine the concentration of the unentrapped drug.

Preparation of the optimal formula

The D-optimal RSM by Design-Expert® software, statistically, analysed the data of entrapment efficiency and particle size of all preparations. The optimum desirability value of the independent variable for the preparation of proniosomes gel was, then, determined. This software allowed the analysis of all variables at the same time. The desirability for optimal formula was maximizing entrapment as well as minimizing particle size of hydrated niosomal vesicle. The optimal formula was regenerated by the coacervation phase separation method and used for further investigations.

Evaluation of optimal Proniosome formula

Fourier transforms infrared spectroscopy study

Diclofenac sodium powder was mixed with potassium bromide powder, milled and compressed into a 13 mm diameter disc. Then, the disc was mounted into an FTIR instrument (Shimadzu, Japan) and analysed at 4000 - 400 cm^{-1} to investigate drug purity. The compatibility test was also investigated by analysing Span 60, Tween 80, Lecithin, cholesterol, physical mixture and optimal proniosomes preparation [14,15].

The morphology and surface characteristics (Transition electron microscopy)

The morphology and surface characterisation of the optimal proniosomes preparation was observed by using TEM (JEOL Model - JEM 2100–200KV, Japan). The TEM sample was composed of a drop of optimal proniosomes on a carbon-coated copper grid, left to settle and dry for 3-5 min in an ambient atmosphere. Then, viewed and photographed [16,17].

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
span 60	is in range	0	1000	1	1	3
tween 80	is in range	0	1000	1	1	3
lecithin	is in range	100	600	1	1	3
EE%	maximize	48.3136	89.1186	1	1	3
Size	minimize	54.6	4149.67	1	1	3

Solutions

Number	span 60	tween 80	lecithin	EE%	Size	Desirability	
1	673.60	376.17	414.69	79.1831	106.92	0.864	Selected

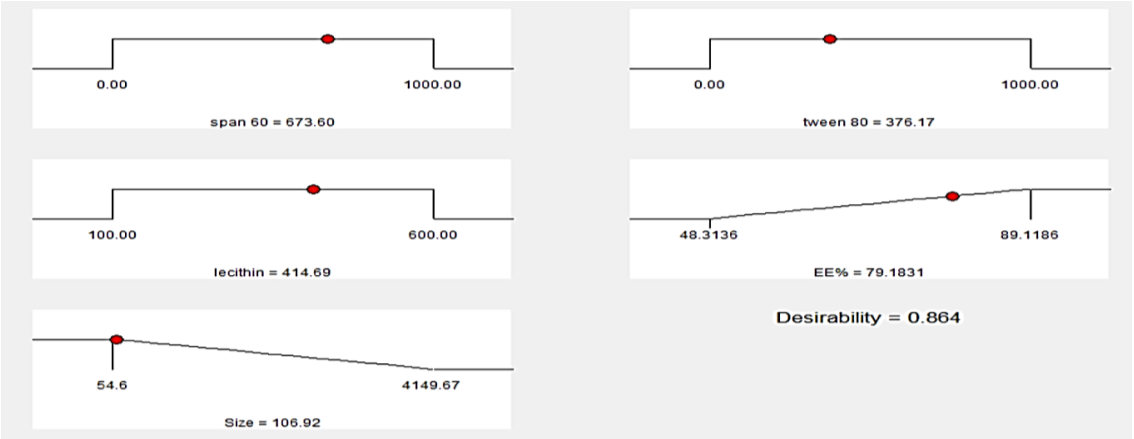


Fig. 3. Predicted values (dependent and independent variable) for optimal preparation.

In vitro release study

In vitro, drug release studies were carried out by employing a modified Franz diffusion cell with minor modification. The Franz diffusion cell consisted of a two-compartment receptor and donor that were separated by a membrane. The area of diffusion was 1.4 cm^2 and the receptor volume was 15 ml. The dialysis membrane cut-off 8000-14000 Dalton (Sigma- Aldrich Corp. St. Louis, MO, USA) was used as shown in Fig. 1.

The dialysis membrane was washed and soaked for 24 h in PBS (pH 7.4). On one side of the dialysis membrane, a specific weight amount of optimal proniosomes formula was placed. The receptor medium was composed of PBS (pH 7.4). The temperature of the compartment was maintained at 37 ± 2 °C by submerging it in the water bath. Heating and shaking were provided by using a hot plate with a magnetic stirrer and thermostatic. The receptor fluid was stirred by a magnetic bead fitted to a magnetic stirrer (aLFA, China). At each sampling time interval, 0.5 ml of samples were withdrawn and immediately replaced by equal volumes of fresh PBS (pH 7.4). Finally, the samples withdrawn were analysed spectrophotometrically (Shimadzu, Japan) at 275 nm [18].

Spreadability test

The spreadability of the optimal proniosomes formula was determined by measuring the diameter of the gel circle between two glass plates have size (10 cm^2). The sample of gel (500 mg) was located on a glass plate (in a 1 cm diameter circle) and another glass plate was covered. Then, 500 g of weight was applied to the upper glass plate until there was no further spreading. The new diameter of the circle in (cm) was recorded [19-21]. The spreadability test was measured for optimal proniosomes formula and voltaren® gel. The triplicated measurement of spreadability was taken and the mean ± SD was measured.

Viscosity study

The viscosity study was performed by using rotational viscometer apparatus (Fungilab, Spain) with spindle R6 [22]. The rotation speed of the spindle was increased from 3 to 100 rpm and decreased from 100 to 3 pm, the time interval of each measurement was 10 seconds, and the temperature was 25 °C. The triplicated measurement of viscosity was done and the mean ± SD was recorded [19].

Ex vivo permeation study

The modified Franz diffusion cell was employed to study the *ex vivo* permeation (as described in the section In vitro release study) with minor modification, thus, a rat's skin was used instead of the synthetic membrane. Specific amounts (equivalent to 1 mg of diclofenac sodium) of optimal proniosomes formula and diclofenac sodium hydrogel formula (containing only pure

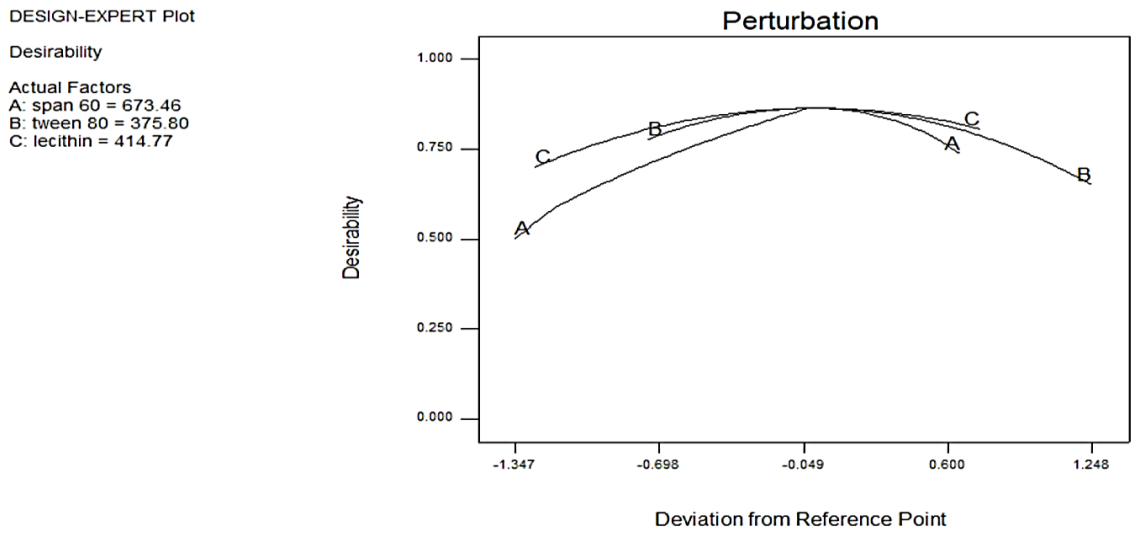


Fig. 4. The perturbation plot showed the effect of the independent variable on the desirable.

diclofenac powder as plain or control gel) were studied. The surgical blades dissected the rat's skin, and the subcutaneous tissues and fat were removed by a scalpel (Fig. 2).

Stability study

The stability study was carried out according to ICH guidelines. Optimal proniosomes formula was stored under different temperatures (4 ± 2 °C, 25 ± 2 °C, 32 ± 2 °C) for up to three months. Samples were analysed for different time intervals for physical appearance, pH value, vesicle size and entrapment efficiency [23,24].

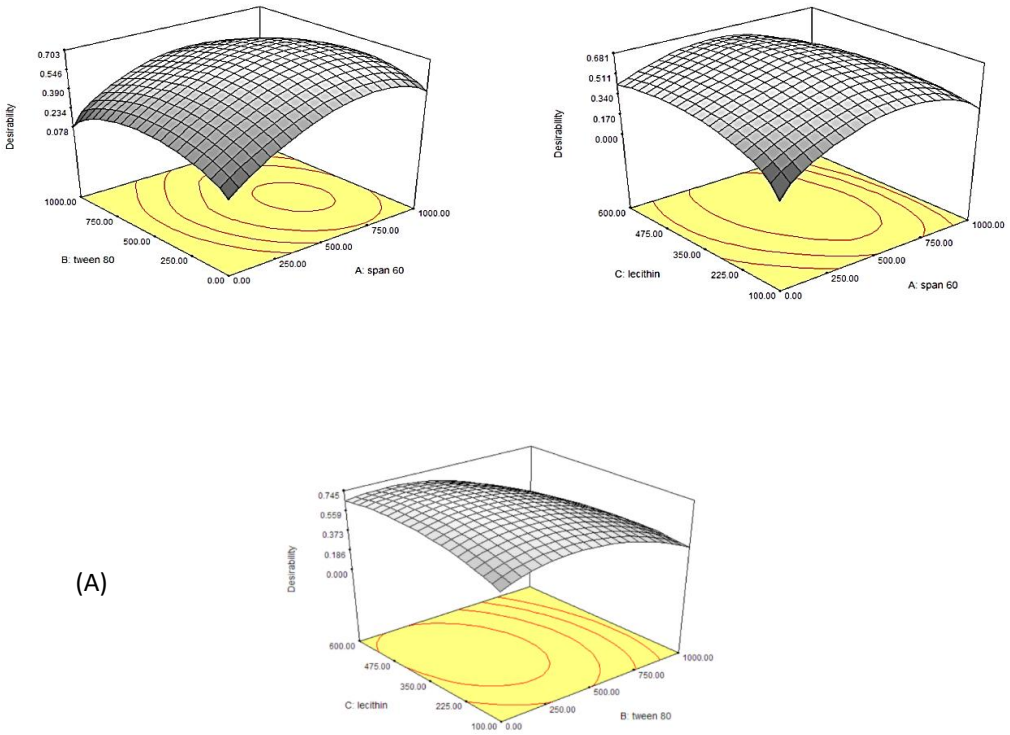
Statistical Analysis

All preparations and formulas were chosen according to the recommendation of the Design Expert® software. One-way ANOVA (at $p \leq 0.05$) was carried out to determine the effect of the independent variables on the outcome (dependent variables). Microsoft Excel software (version 2016) was used to analyse the data and produce the required mean, standard deviation, t-test and similarity ƒ2 tests.

RESULT AND DISCUSSION

Preparation of the optimal preparation

The numerical optimization technique that uses the desirability function was employed to prepare the optimal diclofenac proniosomes with the desired responses. The target of the desirability of optimization is to get the proniosome preparation



(A)

(C)

Fig. 5. 3D response surface plots illustrated the effect amount of (A) Span 60 and Tween 80, (B) Span 60 and lecithin, (C) Tween 80 and lecithin on desirability.

with minim particle size and maxim the entrapment efficiency.

The predicted levels of the independent variable (surfactant type, surfactant amount and lecithin amount) and predicted value dependent variable for optimal formulations that are predicted from D-optimal RSM were predicted by Design-Expert® software and shown in Table 2 and Fig. 3.

Table 2. Predicted values (dependent and independent variable) for optimal preparation.

The main effect of the independent variable on desirability was found that the Span 60 had the major effect on the desirability, thus, a positive relationship was yielded. On the other hand, Tween 80 and lecithin have a relatively moderate negative relationship on Proniosome. They have played an important role with cholesterol in the stability of the bilayer membrane [3]. The lecithin has a high phase transition temperature making the bilayer membrane less leakage and is employed as a permeation enhancer [1,2]. The amount of lecithin had moderated effect (parabolic effect) on the desirability as illustrated in Figs. 4 and 5.

To check the validity (Robustness) of predicted levels (independent and dependent variables) of optimal preparation, three batches of proniosomes were prepared according to of proposed levels of the independent variable and the observed dependent variable was analysed (entrapment efficiency and particles size) are shown in Table 2.

Evaluation of optimal proniosomes preparation

Particles size and entrapment efficiency of the optimal Proniosomes preparation

The particle size and entrapment efficiency EE% of the optimal proniosomes preparation were analysed to compare the observed and the predicted data generated by Design-Expert®

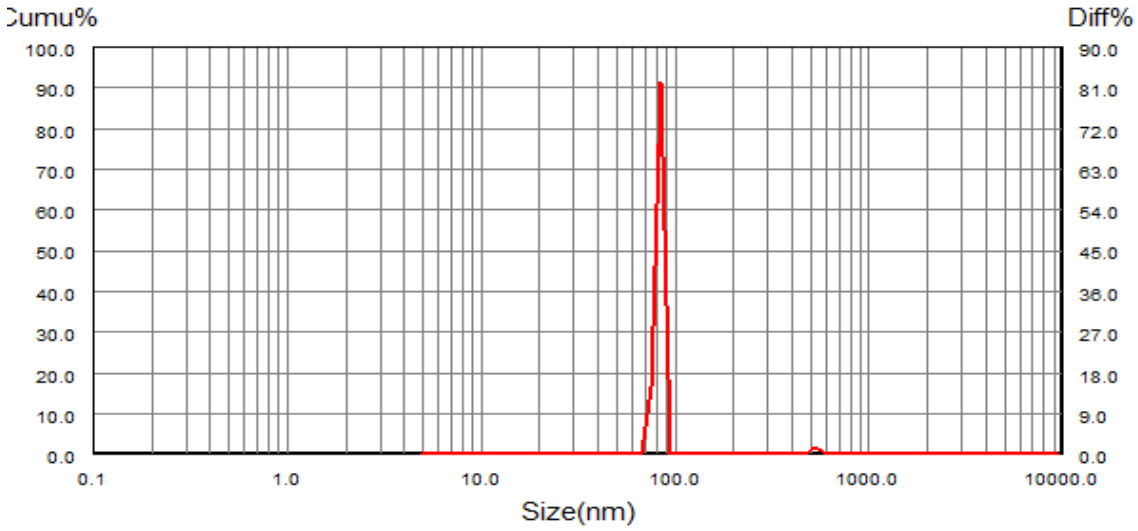


Fig. 6. The particle size of hydrated optimal Proniosomes preparation.

Table 2. Predicted and observed variables of optimal proniosomes preparation.

Independent variable amount	Nominal value (mg)	Predicted values		Observed values		
		Entrapment efficiency %	Particle size (nm)	Batch	Entrapment efficiency %	Particle size (nm)
Span 60	673.60	79.18	106.92	OPF1	77.52	94.7
Tween 80	376.71			OPF2	80.53	96.8
lecithin	414.69			OPF3	78.99	95.1

software. The resulting particle size of the observed optimal preparation ranged from 94.7 nm to 96.8 nm (Fig. 6). The predicted data was 106.92 nm. Furthermore, the EE % of the observed optimal preparation ranged from 77.52 % to 80.53 %, while the predicted data was 79.18 %.

This means the particle size and EE % of observed values were in high agreement with the predicted values. This demonstrated the robustness of the optimization procedure in predicting the effective parameters for the preparation of diclofenac sodium proniosomes. Since the reduced particle size is advantageous to reduce irritation and enhancing the penetration of particles into the skin easily [23,25].

Morphology and surface characteristics

The TEM results of the hydration of the optimal proniosomes preparation revealed that niosome vesicles were successfully formed. The vesicles generally appeared as unilamellar or multilamellar and the shape was predominantly spherical, having sharp boundaries, smooth surfaces and sizes within the nanoscale as shown in Fig. 7. This finding is compatible with the finding of Ghada El-Emam and co-workers, 2020, who reported that hydrated Proniosome found Niosomes vesicles had nearly spherical morphology with a smooth surface and within the nanosize range [26]. Also, it was compatible with the finding of Hiral Shah and her co-workers, 2019, who reported that hydrated proniosome was virtually spherical morphology with nanometer size [27].

Evaluation of the prepared hydrogels

Spreadability Test

The results of the spreadability value for optimal proniosomes preparation and Voltaren® gel were carried out, thus, the optimal proniosomes preparation has 1.27 ± 0.036 and Voltaren® gel 2.29 ± 0.032. This finding is compatible with the finding of Nidhal K. Maraie and co-workers, 2019, as they reported that spreadability studies of the nano-transferosomal gel prepared by adding transfersomes dispersion to the gel base (the gelling agent was carbopol 940) in a ratio (1:1) was around 2 cm ± 0.28 [21].

Viscosity studies

The viscosity test was carried out for optimal

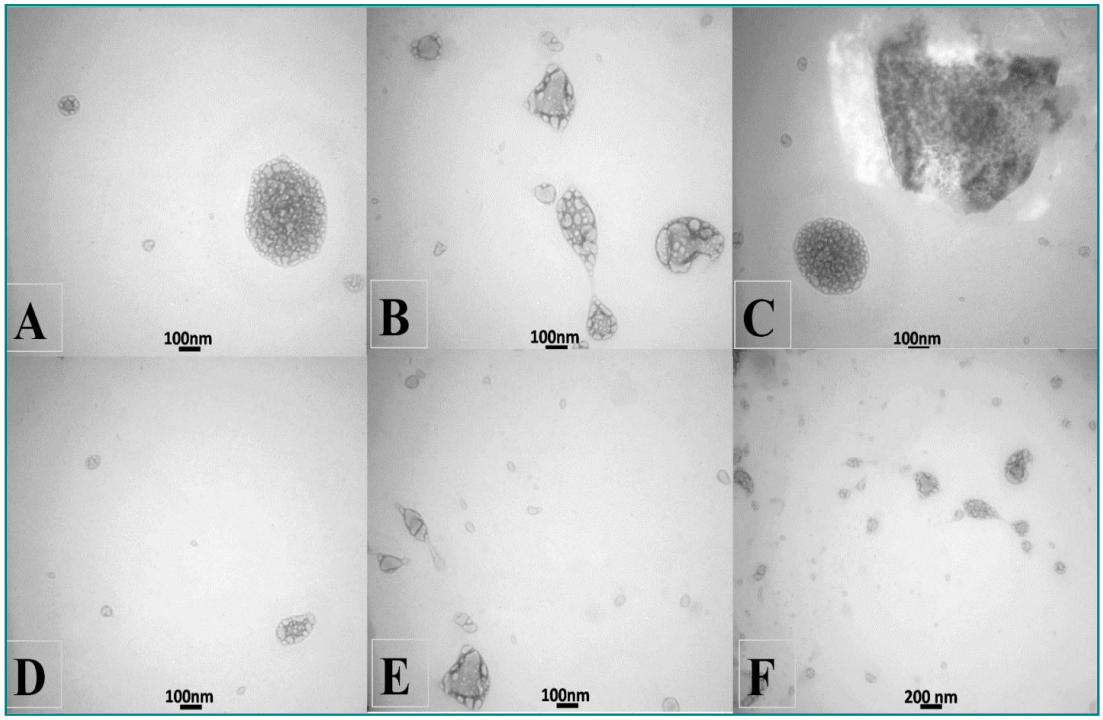


Fig. 7. Transmission electron microscopy micrographs of optimal preparation. (A) Spherical niosome vesicle, (B) unilamellar niosome vesicle (C) smooth surface niosome vesicle, (D) unilamellar and multilamellar, (E) unilamellar and multilamellar and (F) niosome vesicles size within the nanoscale.

proniosomes preparation and Voltaren® gel (as marketing plain gel). The results revealed the viscosity had a negative relationship with shear rate, reflecting a non-Newtonian pseudo-plastic flow behaviour (shear-thinning) as shown in Fig. 8.

Results of the viscosity of optimal proniosomes preparation ranged from 12927 ± 827 cps to 311569 ± 2510 cps and the viscosity of Voltaren® gel ranged from 6806 ± 60 cps to 172654 ± 1132 cps. The non-Newtonian had reduced viscosity when increasing the share rate, which is preferred in topical preparation [28].

This finding is compatible with the finding of Milla Dantas and co-workers, 2016, who reported that gels prepared by using Carbopol 940 have non-Newtonian flow [19]. Also, it was agreed with the finding of Gamal El Maghraby and co-workers, 2015, who reported that proniosomes gels have non-Newtonian flow [26].

Fourier transforms infrared spectroscopy study

Fourier transforms infrared spectroscopy (FTIR) was measured for diclofenac sodium powder to check the purity of the drug powder and drug-excipient interaction (compatibility). The spectra of tested diclofenac sodium powder had peaked at 1284.59 cm^{-1} and 1305.81 cm^{-1} resulting from C-N stretching [29]. The peaks at 3442.94 cm^{-1} resulted from N-H stretching [30]. Peaks at 1504.48 cm^{-1} and 1571.99 cm^{-1} resulted from C=C stretching and C=O stretching, respectively [29]. These peaks of the FTIR spectrum indicated the Diclofenac sodium powder is pure material as shown in Figs. 5 and 6 [31].

The compatibility test was checked by using the FT-IR test to confirm the Diclofenac sodium was compatible with other proniosomes ingredients and to detect any chemical interaction between them. The spectrum of pure diclofenac sodium, cholesterol, Span 60, Tween 80, Lecithin, the physical mixture (of all components) and the optimal proniosomes preparations were tested. Diclofenac sodium pure powder had several main peaks. Infrared spectra of the physical mixture and optimized preparation observed that remained almost unchanged indicated no interaction of

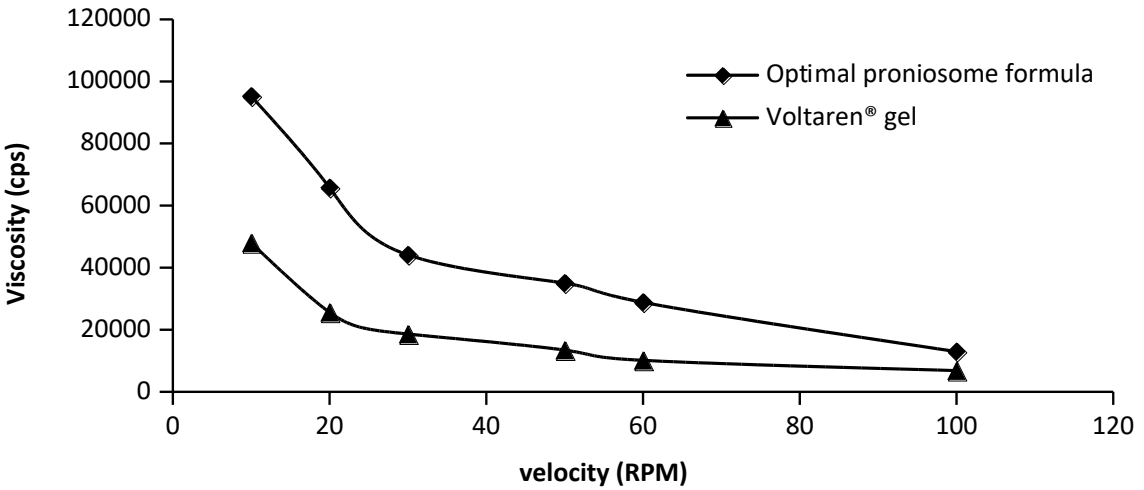


Fig. 8. Viscosity (cps) versus velocity (rpm) revealed a non-Newtonian pseudo-plastic flow behaviour. In the using rotational viscometer apparatus (Fungilab, Spain) with spindle R6, the time interval was 10 seconds and the temperature was 25 °C.

Table 3. Comparison peaks of FTIR of Diclofenac sodium powder, physical mixture and optimal preparation.

Function group	Pure drug	Physical mixture	Optimal preparation
N-H	3442.94 cm^{-1}	3446.79 cm^{-1}	3442.94 cm^{-1}
C-N	1305.81 cm^{-1}	1296.16 cm^{-1}	1296.16 cm^{-1}
C=C	1504.48 cm^{-1}	1456.26 cm^{-1}	1541.12 cm^{-1}
C=O	1571.99 cm^{-1}	1583.56 cm^{-1}	1577.77 cm^{-1}

the drug and another component as illustrated in Table 3 and Fig. 9.

Analysis particles size, surface area and polydispersity index (PDI)

The particle size of all preparations suggested by D-optimal RSM was analysed using ABT-9000 nanolaser particle size analyser apparatus and the sizes were calculated by taking the average of particle diameters. The particle sizes were ranging from small sizes around 50 nm to relatively large sizes up to 4000 nm as shown in Fig. 10A. The total surface area that exposure per unit mass is called

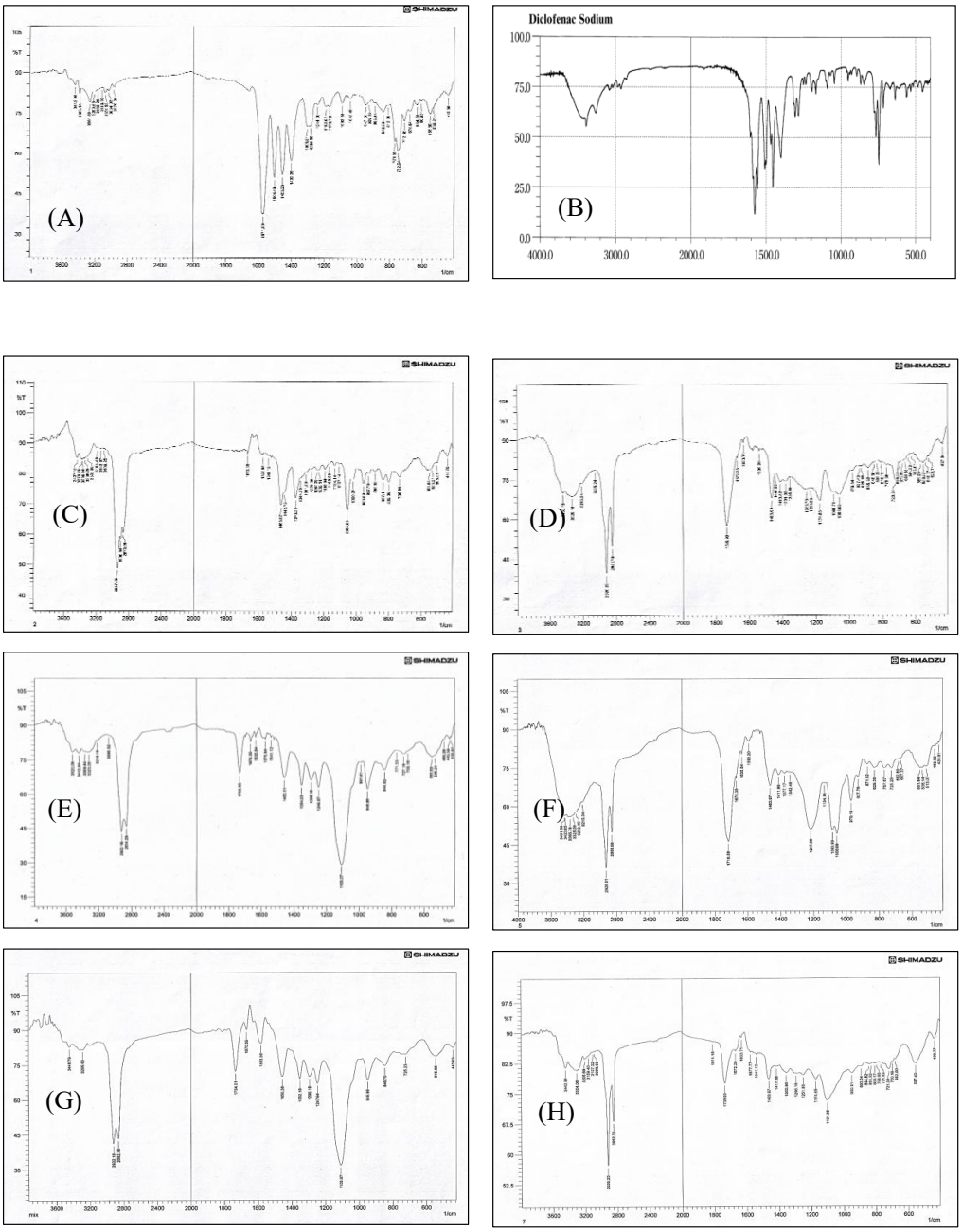


Fig. 9. FTIR spectrum for (A) Diclofenac sodium powder (B) pure Diclofenac sodium (The Japanese Pharmacopoeia, 2016), (C) cholesterol powder (D) Span 60, (E) Tween 80, (F) lecithin, (G) physical mixture of proniosomes component, (H) optimal preparation spectroscopically analysed (Shimadzu, Japan) at 4000 - 400 cm^{-1}.

specific surface area (SSA), which is also measured as shown in Fig. 10B. The polydispersity index (PDI) measures the uniformity of particle size as shown in Fig. 10C. When the PDI value was less than 0.5, reflecting monodispersed (uniform) and homogenous vesicles. However, a PDI value of more than 0.5, reflects polydisperse vesicles [11].

The results of the PDI of preparations ranged from 0.003 to 0.21, which were relatively low PDI values. This means that all preparations were within uniformity size distribution monodispersed and homogenous, which were favours to

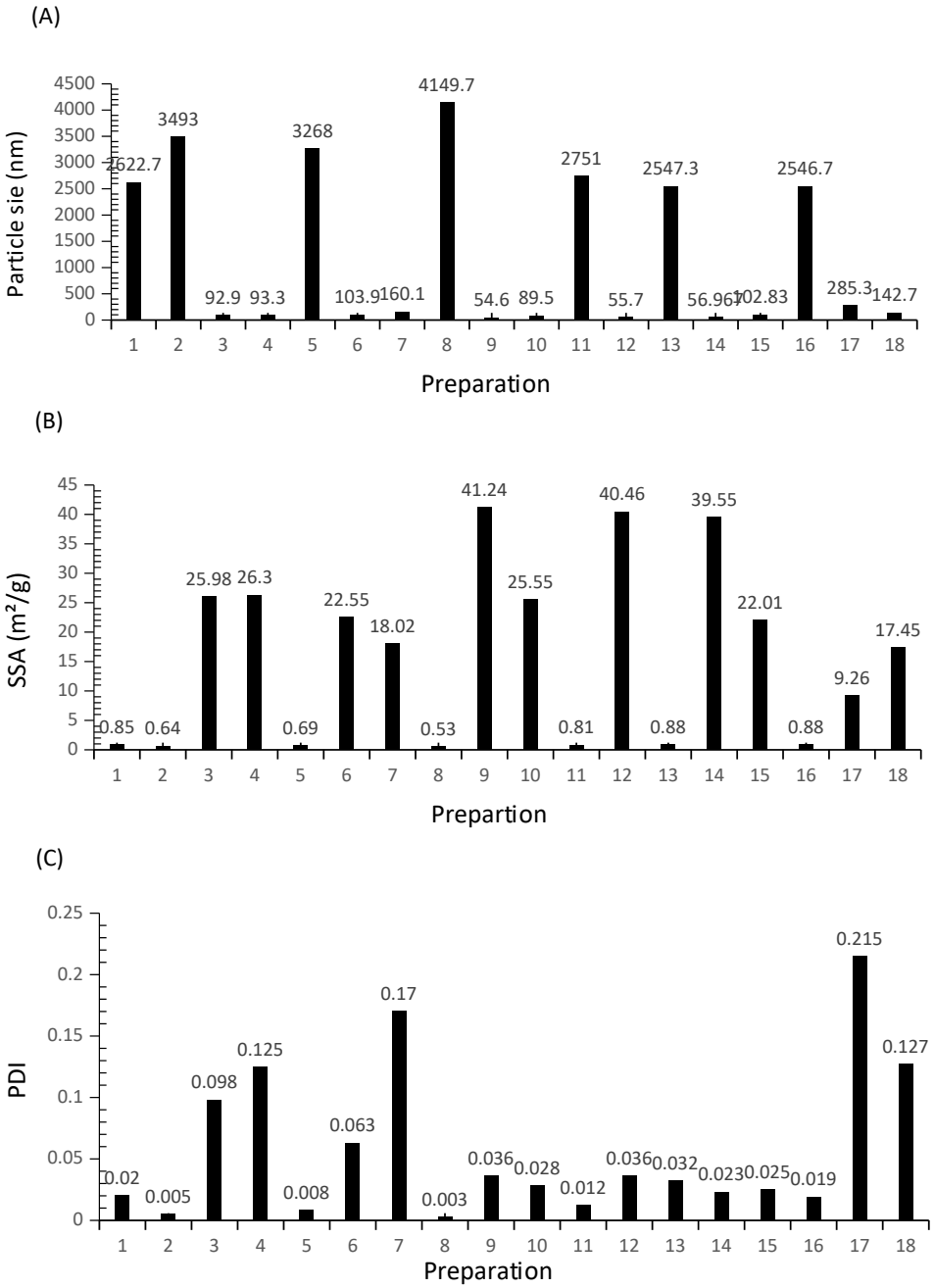


Fig. 10. (A) Effect of different preparations on particles size, (B) Effect of different preparations on the specific surface area, (C) Effect of different preparations on polydispersity index.

transdermal delivery. This finding is compatible with the finding of Ghada El-Emam and co-workers, 2020, who reported that proniosomes prepared by the coacervation phase separation method using Tween 80, Span 60 surfactants had PDI values range of 0.071–0.31, indicating a good homogeneity and uniformity size distribution [32].

Entrapment efficiency

The entrapment efficiency (EE %) is described as a percentage of the number of drug molecules that be entrapped in vesicles. All preparations suggested by D-optimal RSM were analysed against EE % of its payload as shown in Fig. 11. The diclofenac sodium proniosomes preparations were hydrated to be converted to niosome vesicles.

In vitro release study

The *in vitro* release study was done by using a modified Franz diffusion cell and the receptor media was PBS (pH 7.4). The percentage of accumulative drug releases through the dialysis membrane was measured for plain drug solution, plain hydrogel (drug powder in a hydrogel base which is the control reference) and optimal proniosomes preparation as shown in Fig. 12.

The time required for reaching 90 % of accumulative drug release was found in the following order, optimal proniosomes preparation < plain hydrogel preparation < plain drug solution with about 32 h, 4 h, and 1h, respectively. That revealed the proniosomes were successful in a prolonged released time of Diclofenac sodium. The

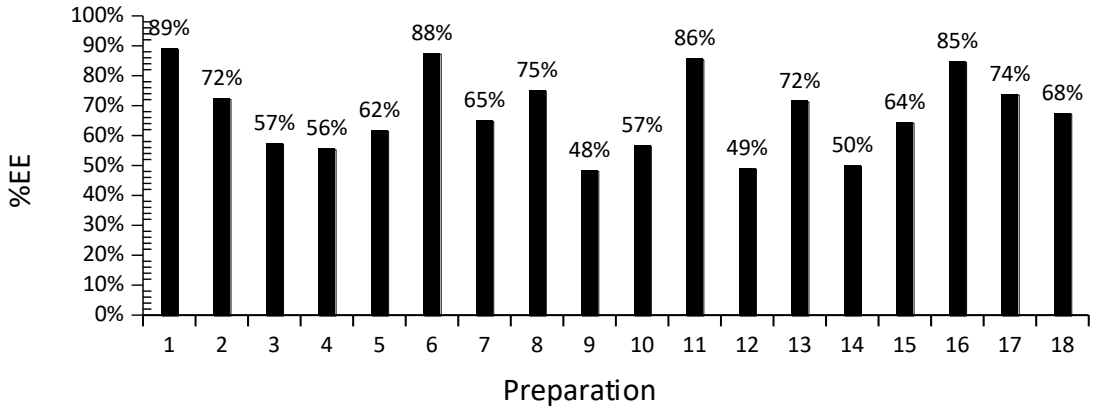


Fig. 11. Effect of different preparations on Entrapment efficiency.

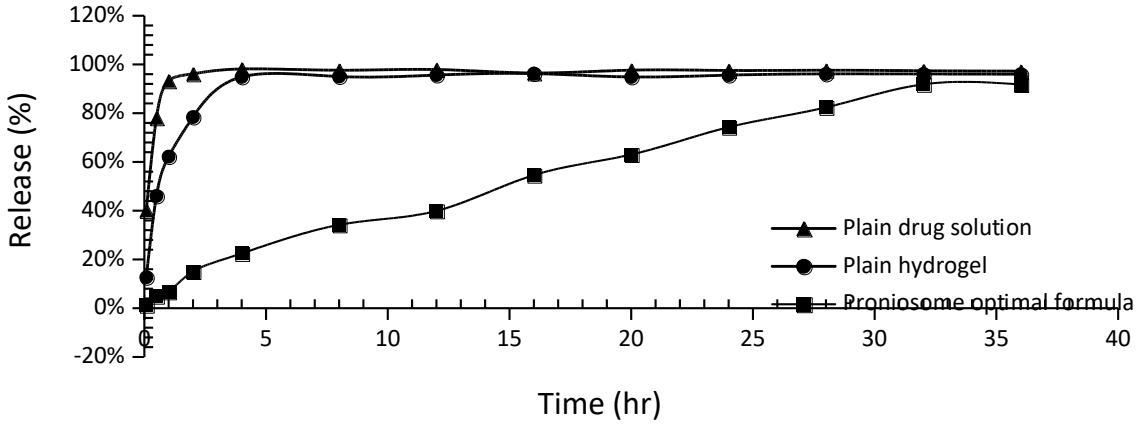


Fig. 12. *In vitro* permeation of Diclofenac sodium from different preparations at pH 7.4 through dialysis membrane in modified Franz diffusion cells at 37 °C.

optimal proniosomes preparation had released in pattern model near to constant released amount per unit time over 24 h that indicated this gel behaviour as the controlled release dosage form. This result is concordant with the previous study of proniosomes and niosome, where researchers Ramkanth and co-workers, 2018, reported that proniosomes act as a sustained drug release system for up to 24 h [25]. Also, the result was in agreement with the finding of Shikha Chauhan and co-workers, 2019, who reported that proniosomes exhibited a sustained release pattern that prolonged more than 24 h, where the percentage cumulative release ranged from 74% to 86% in 24 h [10]. Also, it was in agreement with the finding of Mahmoud M. Ibrahim and his co-workers, 2019, who reported that preparation of proniosomes exhibited prolonged release for more than 24 h [33].

The statistical analysis of *in vitro* release study

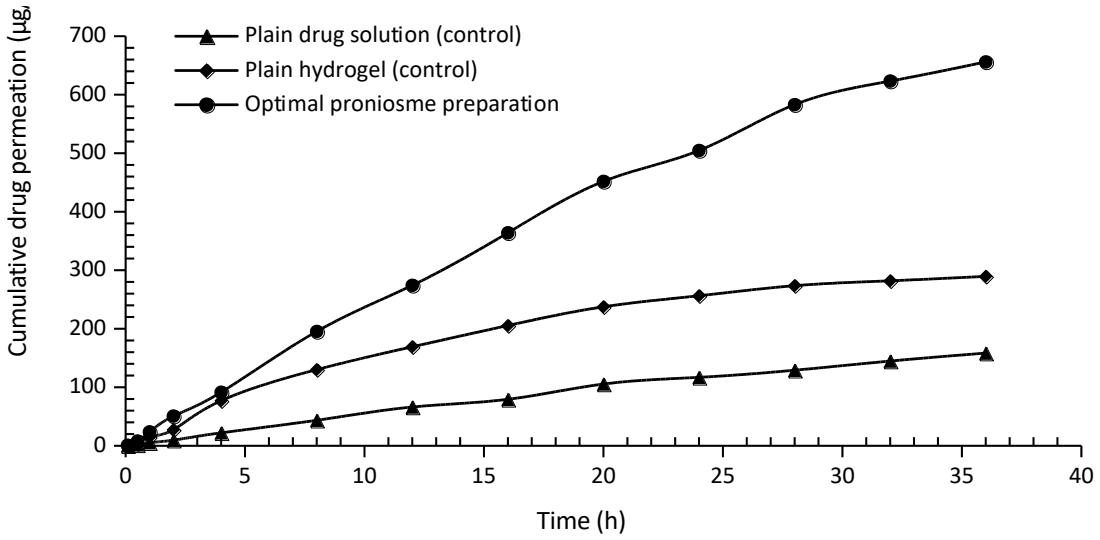


Fig. 13. *Ex-vivo* permeation of Diclofenac sodium from different preparations at pH 7.4 through rat skin in modified Franz diffusion cells at 37 °C.

Table 4. Stability test at different temperatures for three months.

A: Temp. 4 ± 2 °C				
Test	Day 0	Day 30	Day 60	Day 90
Physical appearance	jelly texture	jelly texture	jelly texture	jelly texture
pH value	6.48	6.5	6.51	6.51
Entrapment efficiency	80.53	79.8	81.2	78.7
Particle size	96.8	92.5	111	119
B: Temp. 25 ± 2 °C				
Test	Day 0	Day 30	Day 60	Day 90
Physical appearance	jelly texture	jelly texture	jelly texture	jelly texture
pH value	6.48	6.44	6.37	6.39
Entrapment efficiency	80.53	80.01	77.3	79.23
Particle size	96.8	109	120	148
C: Temp. 32 ± 2 °C				
Test	Day 0	Day 30	Day 60	Day 90
Physical appearance	jelly texture	liquid-like texture	liquid-like texture	liquid-like texture
pH value	6.48	6.37	6.33	6.28
Entrapment efficiency	80.53	81.22	78.2	75.9
Particle size	96.8	131	135	158

was carried out by using the similarity factor ƒ2 test. When the value of Factor ƒ2 was less than 50 that revealed there was a significant difference between releases of the two groups. This comparison was done between the controlled reference gel (which was plain hydrogel) and optimal proniosomes gel. The results of analysis factor ƒ2 were 18.16 which means the significantly different between releases of control plain gel and optimal proniosomes gel.

Ex vivo permeation study

The *ex vivo* release study was carried out by a modified Franz diffusion cell and the receptor media was PBS (pH 7.4) where diclofenac is permeated through rat skin at an effective surface area of 1.4 cm^2 for the plain drug solution and plain hydrogel as control references and optimal proniosomes gel. The permeability was measured by the accumulative drug permeated per unit area ($\mu g.cm^{-2}$) versus the time (h) of different preparations. The permeability in 24 h of preparations in the following ordered, plain drug solution < plain hydrogel < optimal proniosome gel, which was about 116.9, 256.3 and 504.6 $\mu g.cm^{-2}$, respectively, as shown in Fig. 13.

That revealed the optimal proniosomes preparation had a great permeability amount when compared to the plain hydrogel. The statistical analysis was shown the permeability of optimal proniosomes preparation had significantly different (at $p \leq 0.05$) from the permeability of plain hydrogel. This result is concordant with the previous study of proniosomes and niosome, where researchers Yen Tran and his co-workers, 2020, reported the diclofenac niosomal hydrogel that improved the rate and amount of permeation through the skin found the accumulative amount permeated from niosomal hydrogel after 8 hours, was 0.44 $mg.cm^{-2}$ higher than the plain hydrogel was only 0.02 $mg.cm^{-2}$ [30]. Also, the finding of Giuseppina Ioele and co-workers, 2015, reported that diclofenac niosomal hydrogel could potentially improve permeation and performance as a drug reservoir in the skin that gives extended pharmacologic effects, were found the accumulative amount permeated from niosomal hydrogel was 1.3×10^{-7} while from plain hydrogel was only 4.3×10^{-8} moles [34].

Stability study

The stability study was carried out to confirm any possible changes in proniosomes properties with time. Different temperatures were selected to test the semisolid properties versus conditions to evaluate an appropriate storage temperature.

The physical appearance, pH value, entrapment efficiency and particles size were recorded during storage under different temperatures (4 ± 2 °C, 25 ± 2 °C, 32 ± 2 °C) for up to three months. The physical appearance remains unchanged at 4 ± 2 °C and 25 ± 2 °C. However, at 32 ± 2 °C, a noticeable change was recognized, thus the jelly texture became a liquid-like texture. The pH value remains stable with very minimal change at all different temperatures. The entrapment efficiency and particle size revealed very limited change and remain within an acceptable range, reflecting relative stability at different temperatures as shown in Table 4. This finding is compatible with the finding of Ramkanth and his co-workers, 2018, who reported that was not much difference in the properties of Proniosome gel before and after storage in temperatures 4 ± 2 °C and 37 ± 2 °C. This means it was stable and can be stored in both refrigeration and room temperature [25].

CONCLUSION

Diclofenac sodium-loaded Proniosomes were successfully prepared using the coacervation phase separation method. Surface response methodology was a successful way to study and optimize the effect of important variables on particle size and drug entrapment efficiency. The hydration of optimal Diclofenac sodium proniosomes preparation was successful to produce niosomes within nanoscale size and had high entrapment efficiency. The Span 60 had highly affected on both vesicles' particles size and entrapment efficiency, whereas Tween 80 had highly affected on vesicles' particles size with moderated affected on entrapment efficiency and lecithin had moderated affected both vesicles' particles size and entrapment efficiency. The optimal Diclofenac sodium proniosomes had successfully improved both the *in vitro* release and *ex vivo* permeation. In addition, a controlled release formula was prepared to overcome the possible multiple uses of conventional diclofenac topical dosage form.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this

manuscript.

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