RESEARCH ARTICLE

Formulation and Characterization of Hydrogel of Proniosomes Loaded Diclofenac Sodium

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ABSTRACT

Diclofenac Sodium is a non-steroidal anti-inflammatory drug (NSAID), which has analgesic, anti-inflammatory, and antipyretic effects. Diclofenac sodium topically (gel 1%) has mild site reactions, with a relatively short duration of action and low permeability need for multiple uses. This study aimed to produce hydrogel of proniosomes to enhancing solubility and permeation of diclofenac topical used. Proniosome was prepared by using the coacervation phase separation method using Span 60 and Tween 80, lecithin and cholesterol. The hydrogel base was prepared from carbopol 940, then Proniosome was mixed in ratio one to one (1:1). Viscosity, release, permeation and stability were assayed. Results revealed a successfully prepared hydrogel loaded with proniosomes, which was consequently loaded with its payload. The release study carried out *in vitro* revealed a control-released behaviour over 28 hours which was 6 fold higher than the ordinary preparation i.e. lack of proniosomes. To sum up, the hydrogel of Proniosome preparation loading with Diclofenac Sodium was approved to overcome controlled release and skin permeation problems.

Keywords: Coacervation phase separation method, Controlled-release, Diclofenac sodium, *Ex vivo* permeation, Hydrogel, Proniosomes, *In vitro* release.

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INTRODUCTION

Diclofenac sodium was synthesized in 1973 as a phenylacetic acid derivative of a non-steroidal anti-inflammatory drug (NSAID), which acted as the most widely prescribed NSAID worldwide.[1] Diclofenac sodium has inhibited both forms of COX (COX-1 and COX-2) enzymes lead to prevent the conversion of arachidonic acid into prostaglandins with relatively nonselective as a COX inhibitor.[2] Diclofenac sodium acts as an anti-inflammatory and analgesic by blocked COX-2 sub-type enzyme, while the most adverse effect special gastrointestinal side effects appear when blocked COX-1 sub-type enzyme. The inhibits COX-2 is also able to inhibit tumour angiogenesis.[3]

Proniosome is the dehydrated form of Niosome. And are composed of non-ionic surfactants that can be hydrated to produce a dispersion of the aqueous Niosome.[4] Proniosomes could be administered by numerous routes like intravenous, oral, buccal, transdermal, topical, etc. Proniosomal gels exhibit great attention towards topical drug delivery. Proniosomal gels have resistance to stress produced by skin flexion, mucociliary movement and enhance percutaneous absorption due to the fact they are composed of non-ionic surfactants. Proniosomes have high stability, better percutaneous absorption and ease of application they are widely employing for different categories of drugs such as NSAIDs, antifungals, antihypertensive, etc.[5,6]

Hydrogel gel is the most common type used. It consists of a suitable hydrophilic gelling agent dispersed in an aqueous dispersion medium forming polymeric networks in three-dimensional configuration with a high capacity of absorbed large amounts of aqueous fluids. The hydrophilic groups such as: OH, SO_3H, CONH and $CONH_2$, in polymers give this affinity to absorb water and depending on the polymer composition and nature of the aqueous environment will be hydrated to different degrees.[7]

The aim of the following research was to prepare a hydrogel of proniosomes to overcome multiple uses of topical application, though enhancing solubility and permeation.

MATERIALS AND METHODS

Materials

Construction of Calibration Curve

The calibration curve of Diclofenac sodium in PBS pH 7.4 was generated by formulation serial dilutions of the working

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Diclofenac sodium solution to produce 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 30 µg/mL. The absorbance of these diluted solutions was determined spectrophotometrically (at λ_{max} 275 nm) and plotted against concentrations to get the calibration curve. The regression coefficient (R^2) value and calibration curve equation were obtained.[8]

Preparation of the Hydrogel Base

The hydrogel base was prepared by employing carbopol 940 as gelling agents. A specific amount of carbopol 940 was dispersed in distilled water to get (1% w/v) and stirred for 1 hour (at 1000 rpm) using a magnetic stirrer (R LABNCO, USA) to get a uniform dispersion. Also, the spatula may be needed to disperse any formation of indispensable lumps. The hydrogel base was neutralized by adding few drops of triethanolamine to adjust pH about (6–6.5). The hydrogel left for 24 hours at room temperature for swelling.[9]

Preparation of Diclofenac Sodium Proniosomes

The Proniosomes were prepared by coacervation phase separation method accurately weighed amounts of the surfactant, lecithin, cholesterol, and drug were mixed with 1-mL of absolute ethanol in a wide-mouthed cup. The amount of each component was listed in Table 1. the cup was tightly closed and put in a shaker water bath (GLF, Germany) at 60–70°C for about 5–10 minutes with frequent shaking until all the ingredients were completely dissolved and a clear dispersion was formed. After that, the aqueous phase (PBS pH 7.4 with 0.1% glycerol) was gradually added and left on the water bath until a clear dispersion was formed. Finally, the dispersion was leave to maturation at a temperature 25 ± 2°C for 24 hours.[10]

Then, the final dosage form was obtained by mixing of hydrogel base with Proniosome preparation in the ratio one to one (1:1) using a magnetic stirrer and spatula for 1-hour until getting uniform dispersion.

Characterization Hydrogel of Proniosomes

Physical Appearance of the Hydrogel of Proniosomes

The physical properties (colour, homogeneity, clarity and matter texture) for the hydrogel dosage form were done visually.

Measurement of pH

The electronic pH meter (WTW inoLab, Germany) was used to determine the pH value of a sample. A 100 mg of gel was dissolved in a beaker containing distilled water (10 mL) and the electrode of the device was immersed in this beaker. After two minutes, results were recorded.[11] The hydrogel of proniosomes and Voltaren® gel was determined pH value.

Table 1: Composition of proniosome.

Name	*Quantity*
Span 60	673 mg
Tween 80	376 mg
lecithin	414 mg
Cholesterol	100 mg
Ethanol	1-mL
PBS with 1% glycerin	0.8 mL

Spreadability Test

The spreadability of the hydrogel dosage form 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 on the upper glass plate until there was no further spreading. Finally, the new diameter of the circle in (cm) was recorded.[12] The hydrogel of proniosomes and Voltaren® gel has measured the spreadability.

***In vitro* Drug Release**

In vitro hydrogel release study was carried out by employing modified Franz diffusion cell (The area of diffusion was 1.4 cm^2 and the receptor volume was 15 mL full with PBS pH 7.4 and maintain at 37 ± 2°C and shaking by using a hot plate magnetic stirrer, the dialysis membrane cut-off 8000-14000 Dalton was used). Gel samples (equivalent to 1-mg of Diclofenac Sodium) were placed on one side of the dialysis membrane. 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. plain hydrogel preparation (contain only pure Diclofenac powder in hydrogel base) and hydrogel of proniosomes was studied.[13]

***Ex vivo* Permeation Study**

The modified Franz diffusion cell was employed to study the *ex-vivo* permeation above with minor modification. Except if, rat's skin was used instead of the synthetic membrane. Albino rat weighing 268 g were employed in these permeation experiments. The skin was mounted on a modified Franz diffusion cell with the stratum corneum facing up to the donor compartment. Specific amounts (equivalent to 1-mg of Diclofenac sodium) of plain hydrogel preparation and hydrogel of proniosomes were studied.

Drug Release Mechanisms

The cumulative amount of Diclofenac *in-vitro* released was fitted at the graphical interface using several kinetic mechanisms including: Zero-order kinetics, first-order kinetics, Higuchi and Korsmeyer-Peppas mechanisms to characterize the mechanism of drug release. The model that has the highest regression coefficient (R^2) would be referred to as the best mechanism to represent the kinetic release. The exponent (n) of Korsmeyer-Peppas mechanisms determined the mechanism of release, Where Diclofenac had Fickian diffusion when n values are 0.5 or near it, and non-Fickian diffusion when n values 1.0 or near it.[4]

Viscosity Study

The viscosity study was performed by using rotational viscometer apparatus (Fungilab, Spain) with spindle R6.[14] A sample of hydrogel was transferred to a small cup and the spindle was immersed in it. The rotation speed of the

spindle was increased from 3 to 100 rpm, the temperature was 25°C.[15]

Statistical Analysis

Microsoft Excel software (version 2016) was used to analyse the data and produce the required mean, standard deviation, t-test and similarity *f*2 tests.

RESULT AND DISCUSSION

Construction of Calibration Curve

The calibration curve was carried out by drawing a line between serious concentrations versus absorption as shown in Figure 1. It was a linear line over concentration rang 2 to 30 µg/mL. The regression coefficient (R^2), the intercept and the slope were 0.9996, 0.0056 and 0.0317. This result was corresponding to the finding of Sankha Bhattacharya and his co-workers, 2020, who reported that Diclofenac sodium has a linear line in PBS (pH 7.4) in concentration range 0.5 to 25 µg/mL and regression coefficient (R^2) was 0.997.[8]

Physical Appearance of Preparation

The physical appearance was inspected virtually. The appearance of the hydrogel of proniosomes has appeared as homogenous thin jelly texture and the color became yellowish-brown.

Measurement of pH

The Carbopol 940 was used as a polymer that forming a hydrogel base have acidic pH nature and when it dispersed in an aqueous solution that was formed hydrogel with no good matrix gel. Therefore, few drops of triethanolamine were added to neutralized hydrogel dosage and adjusting the pH to 6–6.5. Also, triethanolamine plays a role in forming a stable, good matrix gel and safe gel base.[16] Results of the pH measurement for gels were listed in Table 2, revealed a pH within requirements of topical preparation. this finding of hydrogel was compatible with the finding of Karade Preeti and his co-workers, 2012, who reported that hydrogel preparation using carbopol 940, were found the pH value was range from 6.35 ± 0.04 to 6.70 ± 0.03.[17]

Spreadability Test

The spreadability test is one of the most important characteristic tests of topical preparation and it is related to the complaints of patients.[18] It referred to the preparation have easy to be spread on the skin when applied a small force, also it is indicated to the behaviour of preparation when taking it out of the tube. Results of the spreadability test for gels were listed in Table 2. This finding is compatible with the finding of Nidhal K. Maraie and his co-workers, 2019, who 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.[12]

In-vitro Release Study

The *in vitro* release study revealed that the time required to reach 90% of accumulative drug release of the hydrogel of proniosome and plain hydrogel were 28 hours, 4 hours, respectively, as shown in Figure 2. That means the hydrogel of proniosome was successful in a prolonged released time of Diclofenac sodium. The hydrogel of proniosome had released in pattern model near to constant released amount per unit time over 24 hours that indicated this gel behaviour as the controlled release dosage form. The statistical analysis of *in vitro* release study was carried out by using the similarity factor *f*2 test. When the value of the factor *f*2 was less than 50 that revealed there was a significant difference between releases of the two groups. The result of analysis factor *f*2 was 20.47 that means there is a significant difference between releases of plain gel and tests gel. the result was in agreement with the finding of Shikha Chauhan and his co-workers, 2019, who reported that the proniosome gel was exhibited a sustained release pattern that prolonged more than 24 hours, where

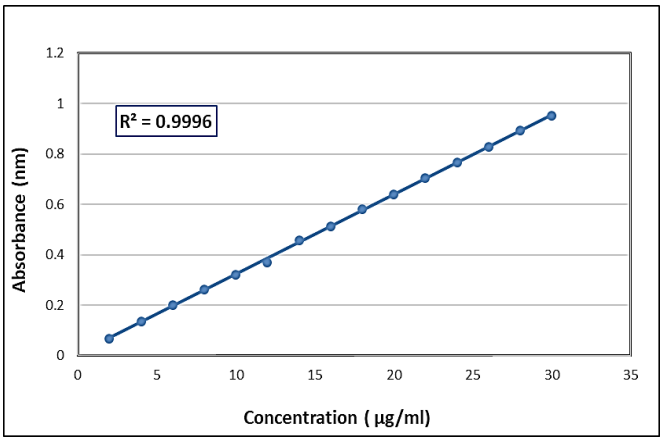

Figure 1: Calibration curve of Diclofenac sodium in PBS (pH 7.4), the linear line over concentration ranged from 2 to 30 µg/mL. The regression coefficient (R^2) was 0.9996.

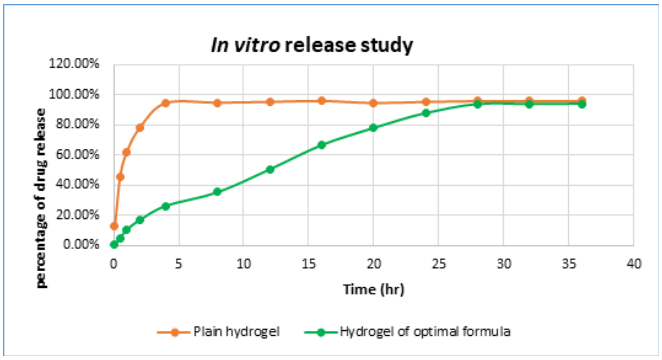

Figure 2: *In vitro* permeation of Diclofenac sodium from different preparations at pH 7.4 through dialysis membrane in modified Franz diffusion cells at 37°C. Where the orange line is plain hydrogel (drug powder in hydrogel as control gel); the green line is hydrogel of proniosome.

Table 2: The pH value and spreadability value of the different gels.

	pH test		*Spreadability test*	
Preparation name	*Value*	*SD*	*Value (cm)*	*SD*
Hydrogel of proniosomes	6.48	0.124±	1.97	0.045±
Voltaren® gel	6.79	0.02±	2.29	0.032±

Table 3: Correlation coefficient R^2 of different release models.

Model name	*R^2 of Zero order*	*R^2 of First order*	*R^2 of Higuchi*	*n value of Korsmeyer-Peppas*
Hydrogel of Proniosome	0.9422	0.6785	0.9841	0.7196

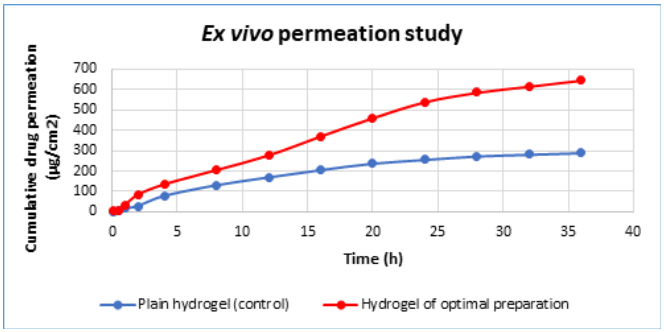

Figure 3: *Ex-vivo* permeation of Diclofenac sodium from different preparations at pH 7.4 through rat skin in modified Franz diffusion cells at 37ºC. Where the blue line is plain hydrogel (drug powder in hydrogel as control gel); and the red line is hydrogel of proniosome preparation.

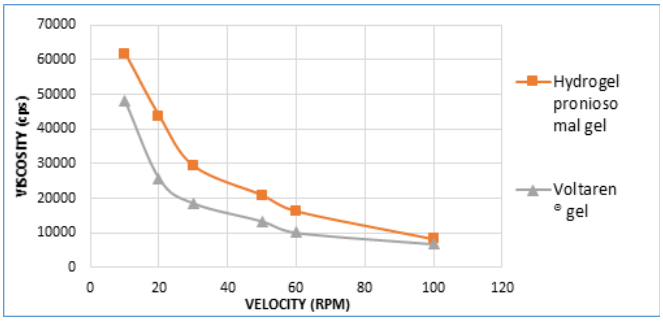

Figure 4: Viscosity (cps) versus velocity (rpm) shown a non-Newtonian pseudo-plastic flow behaviour, where the orange line is hydrogel of the optimal Proniosome preparation; Grey line is Voltaren® gel. The using rotational viscometer apparatus (Fungilab, Spain) with spindle R6, the time interval was 10 seconds and the temperature was 25ºC.

percentage cumulative release was ranging 74% to 86% in 24 hours.[10]

Drug Release Mechanisms

The best fit model has a higher correlation coefficient R^2 value of zero-order, first-order kinetics and Higuchi models. Korsmeyer-Peppas model was used to measure the release exponent values (n) that interpretation which better drug release mechanism. The R^2 value results were found the hydrogel of proniosome is best fitted to the Higuchi model where R^2 was 0.9841, as shown in Table 3. These results are in agreement with the finding of Maryam Khatoon and his co-workers, 2019, they reported that proniosome gel preparation was best fitted to the Higuchi model based on the R^2 value.[19]

The n value is the exponential release of the Korsmeyer-Peppas model that was used to determine the mechanism of release drugs from the matrix of the dosage form. The result n value of hydrogel of proniosome was 0.7196. That revealed these gels had a non-Fickian release mechanism, this mechanism was anomalous transports were combined diffusion and erosion mechanisms. This finding is agreed with the finding of Tamer M. Shehata and his co-workers, 2021, who reported that Proniosome gel had a non-Fickian release mechanism.[20]

Table 4: The viscosity values (cps) of the hydrogel of Proniosome, Voltaren® gel.

Speed (rpm)	*Hydrogel Proniosome*		*Voltaren® gel*	
	mean	*SD ±*	*mean*	*SD ±*
2	248205	4393±	172654	1132±
3	184835	2822±	123676	2010±
5	113639	1218±	91467	628±
10	61804	2839±	47982	401±
20	43692	1745±	25604	402±
30	29360	2128±	18586	387±
50	20915	692±	13417	518±
60	16300	333±	10108	944±
100	8243	127±	6806	60±

Ex-vivo Permeation Study

The permeability was measured by the accumulative drug permeated per unit area (µg/cm^2) versus the time (h) of plain hydrogel and hydrogel of proniosome. The permeability in 24 hours of preparations was shown the plain hydrogel and hydrogel of proniosome which are about 256.30 and 540.56 µg/cm^2, respectively, as shown in Figure 3. That revealed the hydrogel of proniosome had a great permeability amount when compared to the plain hydrogel. The statistical analysis was shown the permeability of hydrogel of proniosome had significantly different (at $p < 0.05$) from the permeability of plain hydrogel. This result is concordant with the previous study of Yen Tran and his co-workers, 2020, reported the Diclofenac niosomal hydrogel that improved the rate and amount permeation through the skin were 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.[21]

Viscosity Studies

The viscosity test was carried out for the hydrogel of Proniosome 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 Figure 4. The non-Newtonian had reduced viscosity when increasing share rate, which is preferred in topical preparation.[7] Results of the viscosity of gels shown the hydrogel Proniosome preparation < Voltaren® gel, where the viscosity of hydrogel Proniosome preparation ranged from 8243 ± 127 cps to 248205 ± 4393 cps and the viscosity of Voltaren® gel ranged from 6806 ± 60 cps to 172654 ± 1132 cps as listed in Table 4. This result was agreed with the finding of Gamal El Maghraby and his

co-workers, 2015, who was reported that Proniosome gels have non-Newtonian flow.[22]

CONCLUSION

The formulation of Diclofenac sodium as hydrogel of proniosomes was successfully produced a controlled release formula that improved the *in vitro* release and *ex vivo* permeation.

The used carbopol 940 hydrogel-based was successfully produced as a good final dosage form with acceptable appearance, pH and viscosity.

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