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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF NAPROXEN PRONIOSOMAL GEL FOR THE TREATMENT OF INFLAMMATORY AND DEGENERATIVE DISORDERS OF THE MUSCULOSKELETAL SYSTEM

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ABSTRACT

Non-ionic surfactant based Proniosome Gels of naproxen sodium, an cox II inhibitor, were prepared by coacervation phase separation method. The prepared systems were characterised for encapsulation efficiency, shape, size and in vitro drug release. Stability study was carried out to investigate the leaching of drug from the proniosomal system during storage. The results showed that naproxen in all the formulations was successfully entrapped and a substantial change in release rate and an alteration in the encapsulation efficiency of naproxen from proniosomes were observed upon varying the type of surfactant and cholesterol content. The encapsulation efficiency of proniosomes prepared with Span 40:60 was superior to that prepared with all Span prepration . A preparation with Span 40: 60, cholesterol and lecithin gave maximum encapsulation efficiency (84.61%) and release results (Q24h= 81%) as compared to other compositions. Proniosomal formulations showed fairly high retention of naproxen inside the vesicles at refrigerated temperature (4-8^{o}C) up to 1 month.

Keywords: Naproxen, proniosomes, Niosomes, encapsulation efficiency, drug delivery.

INTRODUCTION:

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the drugs most commonly used to reduce inflammation and pain. NSAIDs inhibit cyclooxygenase-2 enzyme system results in anti-inflammatory action, while inhibition of the Cox-1 enzyme system results in anti-inflammatory action as well as gastric irritation. The main factor limiting the oral use of NSAIDs is the development of gastrointestinal (GI) adverse events, ranging from dyspepsia to serious life-threatening events. Several studies have shown the effectiveness of topical NSAIDs in treating acute and chronic soft tissue conditions. Naproxen sodium [(S)-6-methoxy-alpha-methyl-2naphthaleneacetic acid sodium salt] is an NSAID with analgesic and antipyretic properties used for the treatment of musculoskeletal disorders with non-optimal characteristics to be delivered through the skin. The advantage of a NSAID gel over its oral equivalent is that therapeutic benefit can be achieved, while significantly reducing any potential systemic side effects. The plasma concentration achieved via topical delivery is 1 - 10% of that attained by oral medication and therefore has a significantly reduced risk of potentially serious side effects. Proniosomal Gels can resist the physiological stress caused by skin flexion, mucociliary movement, adopting to the shape of the applied area and for controlling drug release. Proniosome, a dry product in gel form may avoid many of the problems associated with aqueous noisome dispersions and minimize problems of physical stability (aggregation, fusion or leaking).

MATERIALS AND METHODS:

Materials

Drug: Naproxen Sodium

Excipients: Non-ionic surfactants such as span 20, span 40 & span 60, Lecithin, Cholesterol

Methods

Method of Preparation of Naproxen Loaded Proniosomal Gel Using Coacervation-Phase Separation Technique[17,18,50,51]

Proniosomal gel was prepared by a coacervation-phase separation technique. Precisely weighed amounts of surfactant, lecithin, cholesterol and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (1.0 ml) was added to it. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (phosphate buffer saline pH 7.4) was added and warmed on a water bath till a clear solution was formed which was converted into Proniosomal gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization. Compositions of proniosomal gel formulations are given in Table 1.

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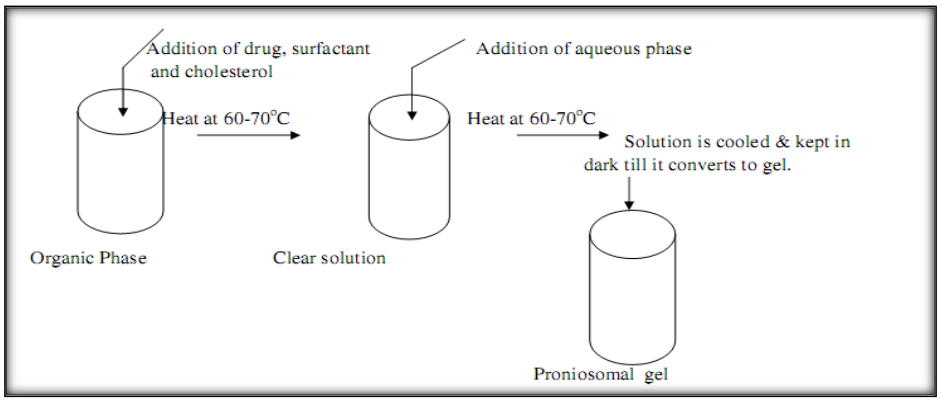


Figure 1: Method of Preparation of Proniosomal Gel

Table 1: Formulation Design

Formulation code	Drug (mg)	Non-ionic surfactants	Ratio (mg)	Lecithin (mg)	Cholestrol (mg)	Ethanol (ml)	PBS pH 7.4 (ml)
PN1	10	Span 20	1000	100	100	1.0	0.5
PN2	10	Span 40	1000	100	100	1.0	0.5
PN3	10	Span 60	1000	100	100	1.0	0.5
PN4	10	Span 20:Span 40	500:500	100	100	1.0	0.5
PN5	10	Span 40: Span 60	500:500	100	100	1.0	0.5
PN6	10	Span 20: Span 60	500:500	100	100	1.0	0.5

Characterization of Proniosomal Gel

Morphological Evaluation

A. **Physical Appearance**[50] : The prepared gel was viewed by naked eye to characterize color and physical state of gel. The appearance for each formula was checked such as color, consistency and fluidity and comparison of each one with the other.

B. **Optical Microscopic Examination:** Hydration of proniosomal gel (100mg) was done by adding PBS 7.4 (5 ml) in a small glass vial with occasional shaking for 10 min. An optical microscope with a camera attachment was used to observe the shape of the prepared niosomal vesicles.

C. **Vesicle Size Analysis**[51] :Size and size distribution studies were done for niosomes prepared from proniosomes hydration with agitation (shaking) and without agitation size Analysis was done by adding saline solution (0.9% solution) to the proniosomal gel (100mg) in a small glass vial with occasional shaking for 10 min. After hydration, the dispersion of niosomes was observed under optical microscope (Olympus) at 100, 40 and 10x magnification. The sizes of 150-200 vesicles were measured using a calibrated ocular and stage micrometer fitted in the optical microscope

D. Surface Morphology[17,60] **:** Electron micrographs were obtained using scanning electron microscope. The surface morphology (roundness, smoothness and formation of aggregates) of proniosomal gel was studied by Scanning Electron Microscopy .Hydration of proniosomal gel was done similarly as optical microscopy. One drop of niosomal suspension was mounted on clear glass slab, air dried and sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope equipped with a digital camera, at 15 or 20 kV accelerating voltage.

Determination of pH[14,50,80]: The Ph of the Proniosomal gels were determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

% Encapsulation Efficiency[50] : The concentration of drug entrapped was determined by taking 0.2 g of proniosomal gel, weighed in a glass tube and added to 10 ml of pH 7.4 phosphate buffer. The aqueous suspension was sonicated in a sonicator bath. The drug-containing Niosomes were separated from untrapped drug by centrifugation at 18000 rpm at 5°C for 40 min. The supernatant clear fraction was used for the determination of free drug and assayed for drug content. The percentage of drug encapsulation (% EE) was calculated by the following equation:

$$\% \text{ EE} = \frac{C_t - C_f}{C_t} \times 100$$

Where, Ct = total concentration of drug, Cf = concentration of free drug.

In Vitro Skin Permeation Studies [50,51]

Preparation of Human cadaver skin: The skin was stored at 0-4°C after collection. The excised human abdomen skin was treated to remove hair and subdermal tissue. The subdermal fat was removed with help of scalpel and swapped with isopropyl alcohol. The treated skin was stored at 0°C in deep freezer for not more than 2 days. To actually mimic the in vitro permeation study, the permeation studies were performed using excised cadaver skin mounted on Franz cell. The capacity of

receptor compartment was 20 ml. The area of donor compartment exposed to receptor compartment was 2.0 cm^2. The cadaver skin was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel equivalent to 10 mg of naproxen was placed on one side of the membrane. The receptor medium was saline phosphate buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1ºC. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, (1 ml) were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 230 nm.

Data Analysis via Drug Release Kinetics study[17] :The results of in-vitro release profile obtained for all the formulations were plotted in kinetic models as follows, 1.Cumulative of drug released versus time (zero order kinetic model). 2. Log cumulative percent drug remaining to be absorbed versus time (First order model) 3. Cumulative amount of drug release versus square root of time (Higuchi model) 4. Log cumulative drug released versus log time (Korsmeyer-Peppas model)

Skin Irritation Test[18]:Skin irritation study was performed by using control, standard skin irritant, placebo and test placebo niosomal gel. Primary skin irritation test was performed since skin is a vital organ through which drug is transported. Skin irritation studies were performed on healthy rabbits (average weight: 1.5 to 2.25 kg). The dorsal surface (50 cm^2) of the rabbits was cleaned, and the hair was removed by shaving. The skin was cleansed with rectified spirit. The best formulation was placed over the skin and was removed after 24 hrs. The resulting skin reaction was evaluated according to score as per Table.

RESULTS AND DISCUSSION:

CHARACTERIZATION OF PRONIOSOMAL GEL

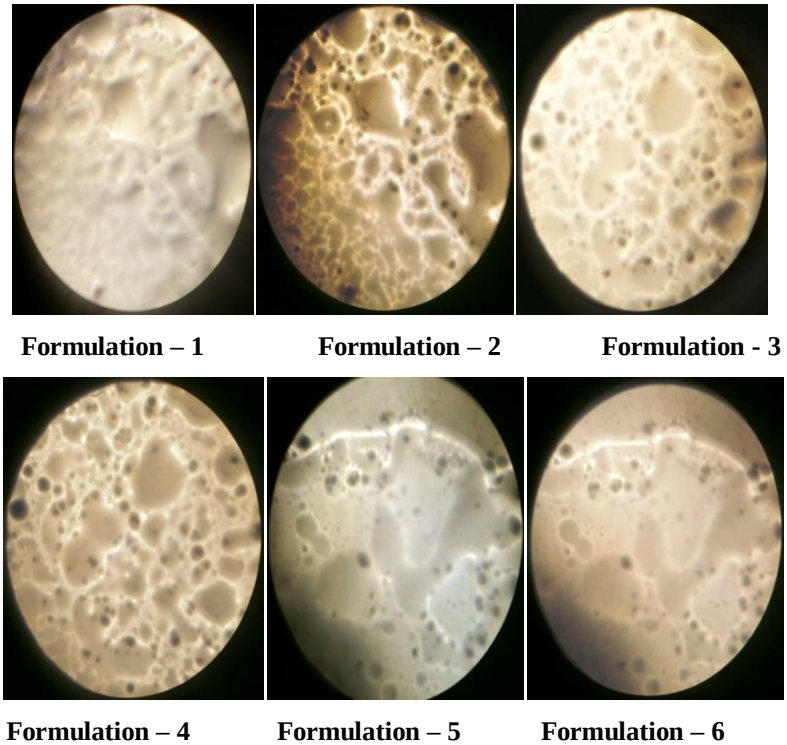
Morphological Evaluation

A. Physical Appearance: Table shows the color and physical state for each formula, these properties are differ from each other since they depend on the composition. For example, formula PN2 & PN3 gave a white semisolid appearance whereas PN1 showed a brown liquid, this is due to the property of span 40, span 60 and span 20 for each formula respectively. The inspection of formula PN4, PN5 and PN6 offered the light brownish color with gel state at 37ºC which represents the combination of the surfactants cause a change in the physical properties of surfactant after mixing and addition of alcohol with a few drops of water.

Table 2: Physical Appearance of Proniosomal formulation

Formulation Code	Colour	Physical State
PN1	Brown	Liquid
PN2	White	Semi-solid
PN3	White	Semi-solid
PN4	Light-brown	Gel
PN5	Light brown	Gel
PN6	Light-brown	Gel

B. Optical Microscopic Examination: Hydration of proniosomal gel (100mg) was done by adding PBS 7.4 (5 ml) in a small glass vial with occasional shaking for 10 min. An optical microscope with a camera attachment was used to observe the shape of the prepared niosomal vesicles.



Formulation – 1 **Formulation – 2** **Formulation - 3**

Formulation – 4 **Formulation – 5** **Formulation – 6**

Figure 2: Optical Microscopic Examination of different Proniosomal formulation

Vesicle Size Analysis: Determination of vesicle size is important for the topical application of vesicles. Size was reduced when the dispersion was agitated. The reason for this is the energy applied in the agitation which results in the breakage of the larger vesicles to smaller vesicles.

Table 3: Vesicle Size Analysis

Formulation code	Mean vesicles size before shaking (µm)	Mean vesicles size after shaking (µm)
PN1	4.23	1.91
PN2	3.87	1.75
PN3	3.05	1.43
PN4	4.09	1.97
PN5	3.20	1.66
PN6	4.15	2.12

Surface Morphology: The morphology of niosomes derived from proniosomal gel was studied using Scanning Electron Microcopy. SEM revealed that the niosomes formed were nearly spherical and homogenous.

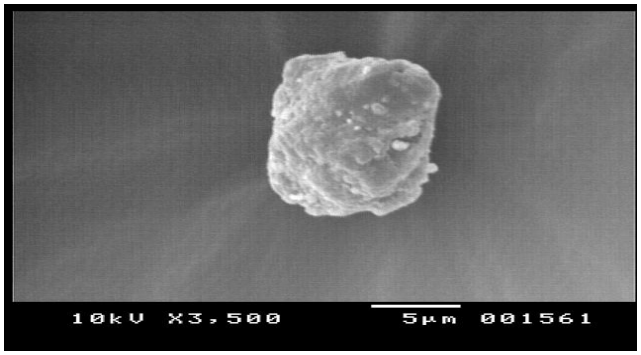


Figure 3: Scanning electron image of hydrated proniosomal formulation PN5 (magnification 1,000 X)

Determination of pH: The pH of each formula was determined in order to investigate the possibility of any side effects in vivo. Due to acidic or alkaline pH which may irritate skin .The pH was found between 6.4 and 7.5, this range is within the physiologically skin surface pH. Changes in the pH are reported to play a role in the pathogenesis of skin diseases like irritant contact dermatitis and atopic dermatitis. Maintaining the skin's pH factor helps maintain a proper balance of the "acid mantle" which aids in protecting the body from bacteria and helps prevent moisture loss.

Table 4: pH Determination

FORMULATION	pH
PN1	6.4
PN2	6.9
PN3	7.3
PN4	6.8
PN5	7.5
PN6	6.7

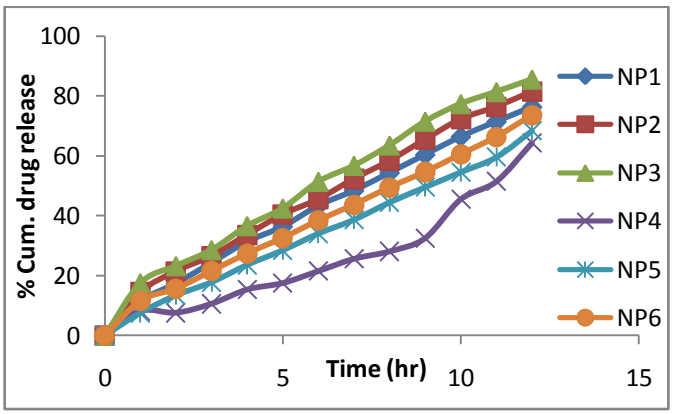
% Encapsulation Efficiency: Table.4 shows the effect of various sorbitan fatty acid esters and their ratio on the encapsulation of Naproxen in proniosomal gel. Naproxen was best encapsulated by proniosomal prepared using Spans 40 and 60. This might be attributed to fact that Spans 40 and 60 are solid at room temperature and showed a higher phase transition temperatures [Tc]

Table 5: % Encapsulation Efficiency

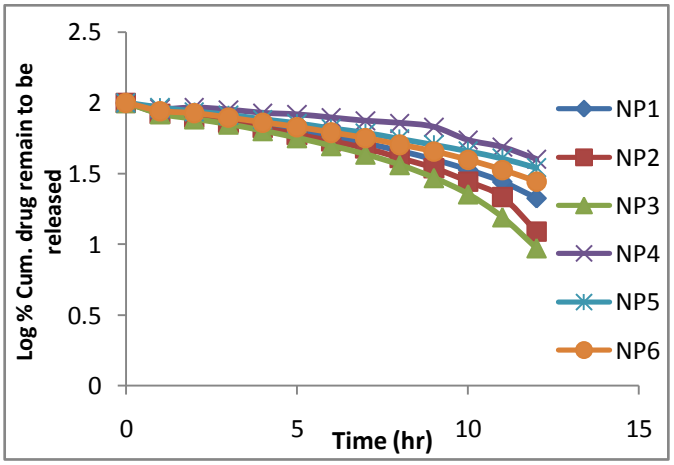
Formulation	% Encapsulation Efficiency
PN1	65.22
PN2	77.56
PN3	78.81
PN4	71.16
PN5	84.61
PN6	74.11

(F) In-Vitro Release Study

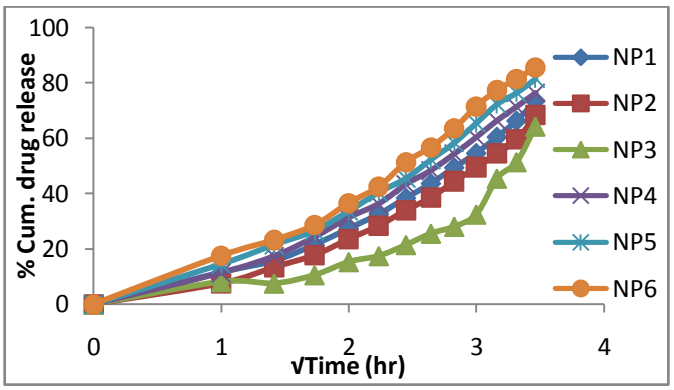
Graph 1: Zero order release kinetics Data



Graph 2: First Order Release Kinetics Data



Graph 3: Higuchi matrix release kinetics data



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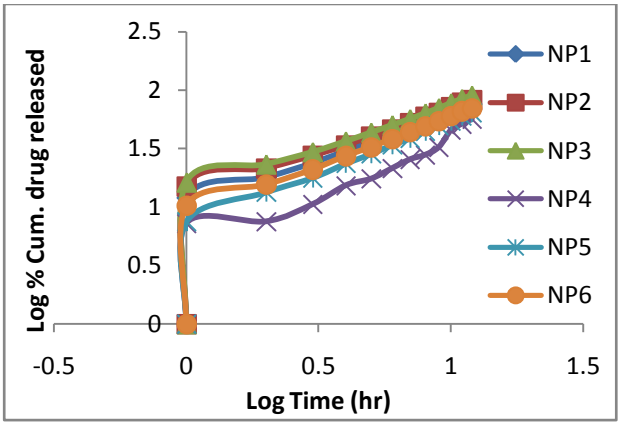
Graph 4: Peppas release kinetics data



Drug Release Kinetics with Model Fitting: Calculated regression co-efficient for different formulations are shown in Table. These values of in-vitro release were attempted to fit into various mathematical models, plot of zero order, first order, higuchi matrix and peppas. These values were compared with each other for model fitting equation. Based on the highest regression values (r), the bestfit model for PN1, PN2, and PN3 was Zero order and for PN4, PN5, and PN6 was Peppas. Further Korsmeyer and Peppas equation resulted into the values of n > 1, which appears to indicate that the release from the prepared microspheres was by Super Case II transport.

Table 6: Drug Release Kinetics with Model Fitting

Formulation code	Correlation coefficient of Model fitting (R^2)				'n' values for Peppas	Best fit model
	Zero order	First order	Higuchi matrix	Peppas kinetics		
NP1	0.9903	0.9627	0.9508	0.9759	2.2538	Zero Order
NP2	0.9814	0.8855	0.9298	0.9797	2.2386	Zero Order
NP3	0.9676	0.8844	0.9044	0.9673	2.2864	Zero Order
NP4	0.9544	0.8968	0.8822	0.9701	2.2167	Peppas Model
NP5	0.9497	0.8921	0.8754	0.9668	2.2109	Peppas Model
NP6	0.9186	0.8467	0.8317	0.9666	2.1168	Peppas Model

Skin irritation studies: The optimized formulation PN5 showed irritation potential of '0', thus proving to be non - irritant. The '0' value in an irritancy test indicates that the applied formulations are generally non irritant to human skin. No obvious erythema and edema were observed on rabbit skin after 24 hr of application of the optimized formulation. Moreover, the optimized formulation is composed of phospholipids, a natural component of the cell membranes in skin; they act as nonirritating moisturizing agents.

Table 7: Possible score for skin irritation

TEST	SKIN REACTION	SCORE
Erythema	Very slight erythema	0
	Well defined erythema	0
	Moderate to severe erythema	0
	Severe edema	0
Edema	Very slight erythema	0
	Well defined erythema	0
	Moderate to severe erythema	0
	Severe edema	0

CONCLUSION:

In the present study, an attempt will be made to prepare and evaluate Naproxen sodium proniosomal gels by coacervation-phase separation method for the treatment of inflammatory and degenerative disorders of the musculoskeletal system. The exhaustive literature survey has been done on Vesicle system, Pro-vesicle system, their method of preparations, various excipients so that a stable Proniosomal formulation of Naproxen sodium can be formulated which can release drug for number of hours and exhibit good anti- inflammatory activity.The Transdermal Proniosomal Gels showed controlled drug release properties. The results of the present study indicated that Naproxen proniosomal gel containing lecithin, cholesterol and in combination of surfactants like span 20, 40 and 60 produce sustained release of drug over a period of 12 hrs for the treatment of inflammatory and degenerative disorders of the musculoskeletal system. The proniosomal gel could be an effective alternative vehicle for delivering the drug through transdermal route to avoid side effects associate with oral route.

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