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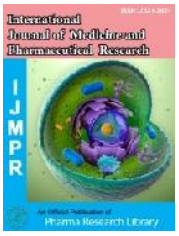

Research Article **Open Access**

Formulation, *In-Vitro* Evaluation & Characterization of Naproxen Loaded Proliposomal Gels

Yeduru Krishna Reddy*[1], Dr. Shailesh Sharma[2]

[1]*Pharmaceutical Sciences, NIMS Institute of Phrmacy, NIMS University, Shobhanagar, Jaipur, Rajasthan*
[2]*Associate Professor, NIMS Institute of Phrmacy, NIMS University, Shobhanagar, Jaipur, Rajasthan*

A B S T R A C T

Non-ionic surfactant based Proliposomal Gels of naproxen sodium, an cox II inhibitor, were prepared by coacervation phase separation method. The prepared systems were characterized for encapsulation efficiency, shape, size and in vitro drug releas e. Stability study was carried out to investigate the leaching of drug from the Proliposomal 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 Proliposomal were observed upon varying the type of surfactant and cholesterol content. The encapsulation efficiency of Proliposomal prepared with Span 40:60 was superior to that prepared with all Span preparation. A preparation with Span 40:60, cholesterol and lecithin gave maximum encapsulation efficiency (84.61%) and release results (Q12h= 96%) as compared to other compositions. Proliposomal formulations showed fairly high retention of naproxen inside the vesicles at refrigerated temperature (4-8^0 C) up to 1 month.

Keywords: Naproxen, Proliposomal, encapsulation efficiency, drug delivery.

A R T I C L E I N F O

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***Corresponding Author**
Yeduru Krishna Reddy
Pharmaceutical Sciences, NIMS Institute of Phrmacy, NIMS University, Shobhanagar, Jaipur, Rajasthan
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1. 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 [1]. 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 [2].

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. Proliposomal 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 [3].

2. Materials and Methods

Naproxen Sodium was procured as a gift sample from Macleod's Pvt. Ltd, Mumbai, India. span 20, span 40 & span 60, Lecithin, Cholesterol was obtained from Paxmy, Chennai. All other reagents used were of analytical grade.

Method of Preparation of Naproxen Loaded Proliposomal Gel Using Coacervation-Phase Separation Technique:

Proliposomal 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 (10 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 [4]. 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 Proliposomal g e l on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization.

Characterization of Proliposomal Gel

Morphological Evaluation

Physical Apperance: The Prepared gels was viewed by naked eye to characterize color and physical state of gel [5]. The appearance for each formula was checked such as color, consistency and fluidity and comparison of each one with the other.

Optical Microscopic Examination:

Hydration of Proliposomal gel (100mg) was done by adding PBS 7.4 (5 ml) in a small glass vial with occasional shaking for 10 min [6]. An optical microscope with a camera attachment was used to observe the shape of the prepared liposomal vesicles.

Vesicle Size Analysis: Size and size distribution studies were done for Proliposomal prepared from Proliposomal hydration with agitation (shaking) and without agitation size Analysis was done by adding saline solution (0.9% solution) to the Proliposomal gel (100mg) in a small glass vial with occasional shaking for 10 min. After hydration, the dispersion of Proliposomal 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 [7].

Surface Morphology:

Electron micrographs were obtained using scanning electron microscope. The surface morphology (roundness, smoothness and formation of aggregates) of Proliposomal gel was studied by Scanning Electron Microscopy .Hydration of Proliposomal gel was done similarly as optical microscopy [8]. One drop of liposomal 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:

The pH of prolipomal 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 [9]. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

% Encapsulation Efficiency:

The concentration of drug entrapped was determined by taking 0.2 g of Proliposomal gel, weighed in a glass tube and added to 10ml of pH 7.4 phosphate buffer. The aqueous suspension was sonicated in a sonicator bath. The drug-containing Proliposomal were separated from untrapped drug by centrifugation at 18000 rpm at 5°C for 40 min [10]. 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:

$$\text{Entrapment efficiency (\%)} = \frac{Ct-Cf}{Ct} \times 100$$

Where, Ct = total concentration of drug,

Cf = Concentration of free drug.

Percent amount of drug release from semi permeable membrane: Franz diffusion cell was used for the in vitro drug release studies. Semi permeable membrane was placed between donar and receptor chamber of diffusin cell. Receptor chamber was filled with freshly prepared 30ml 7.4 PH phosphate buffer. proliposomal gel equivalent to 1gm was placed on semi permeable membrane [11]. The franz diffusion cell was placed over magnetic stirrer (REMI 1ML) with 500rpm and temperature was maintained at 37 ± 1^0C. 5ml of samples were withdrawn periodically and replaced with fresh buffer. The withdrawn samples were periodically diluted and analysed for drug content using UV visible spectrophotometer (Lab India 3200) at 230nm.

Data Analysis via Drug Release Kinetics study:

The results of in-vitro release profile obtained for all the formulations were plotted in kinetic models as follows, Cumulative of drug released versus time (zero order kinetic

model) [12]. Log cumulative percent drug remaining to be absorbed versus time (First order model). Cumulative amount of drug release versus square root of time (Higuchi model). Log cumulative drug released versus log time (Korsmeyer-Peppas model).

3. Results and Discussion

Characterization of Proliposomal Gel Morphological Evaluation [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 NP2 & NP3 gave a white semisolid appearance whereas NP1 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 NP4, NP5 and NP6 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.

Optical Microscopic Examination:

Hydration of Proliposomal 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 liposomal vesicles.

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.

Surface Morphology:

The morphology of Proliposomal derived from Proliposomal gel was studied using Scanning Electron Microcopy. SEM revealed that the Proliposomal formed were nearly spherical and homogenous.

Determination of pH:

The pH of each formula was determined in order to investigate the possibility of any may irritate skin .The pH was found between 6.4 and 7.5, this range is within the physiologically skin surface pH. 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.

% Encapsulation Efficiency:

Table.4 shows the effect of various sorbitan fatty acid esters and their ratio on the encapsulation of Naproxen in Proliposomal gel. Naproxen was best encapsulated by Proliposomal 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]

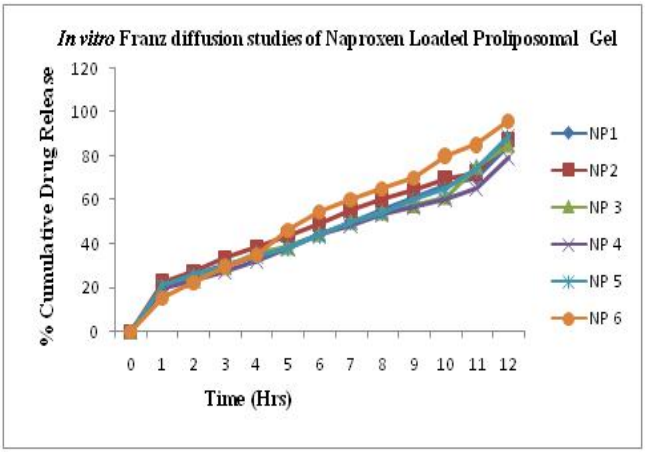

Figure 1: *In-vitro* Franz diffusion studies of Naproxen loaded proliposmal gel

Drug Release Kinetics with Model Fitting:

Calculated regression coefficient 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 best fit model for NP1, NP2, and NP3 was Zero order and for NP4, NP5, and NP6 was Peppas. Further Korsmeyer and Peppas equation resulted into the values of n >1, which appears to indicate the drug release.

Table 1: Formulation Design of Naproxen Loaded Proliposomal Gel

Formulation code	Drug (mg)	Non ionic surfactants	Ratio(mg)	Lecithin (mg)	Cholesterol (mg)	Ethanol (ml)
NP1	10	Span 20	1000	150	150	10
NP2	10	Span 40	1000	150	150	10
NP3	10	Span 60	1000	150	150	10
NP4	10	Span 20:Span 40	600:600	150	150	10
NP5	10	Span 40: Span 60	600:600	150	150	10
NP6	10	Span 20: Span 60	600:600	150	150	10

Table 2: Physical Appearance of Naproxen Loaded Proliposomal Gel

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

Table 3: Vesicle size Analysis of Naproxen Loaded Proliposomal Gel vesicle

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

Table 4: Determination of pH for Naproxen Loaded Proliposomal gels

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

Table 5: % Encapsulation Efficiency for Naproxen Loaded Proliposomal gels

Formulation code	pH
PN1	65.22
PN2	77.56
PN3	78.81
PN4	71.16
PN5	84.61
PN6	74

Table 6: *In-vitro* Dissolution studies Naproxen Loaded Proliposomal gels

S.No	Time (hrs)	% Cumulative Amount of Drug Release					
		NP1	NP2	NP 3	NP 4	NP 5	NP 6
1	1	21	22	20	19	21	15
2	2	26	27	25	23	25	22
3	3	30	33	29	27	30	29
4	4	34	38	35	32	34	35
5	5	38	43	38	38	38	46
6	6	44	48	44	44	43	54
7	7	50	55	50	48	49	60
8	8	55	60	54	53	54	65
9	9	61	64	57	56	60	70
10	10	66	69	61	60	64	80
11	11	70	72	75	65	74	85
12	12	85	87	85	79	89	96

Table 7: Drug Release Kinetics with Model Fitting

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

4. Conclusion

In the present study, an attempt will be made to prepare and evaluate Naproxen sodium Proliposomal 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, The Proliposomal Gels showed controlled drug release properties. The results of the present study indicated that Naproxen Proliposomal 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 Proliposomal gel could be an effective alternative vehicle for delivering the drug through trans dermal route to avoid side effects associate with oral route.

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