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FORMULATION AND EVALUATION OF PHARMACOSOMAL GEL LOADED WITH NSAID

Kolar Kusuma*, D. Priyanka and Dr. J. Sundaraseelan

Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tiruchanoor-517503.

***Corresponding Author: Kolar Kusuma**

Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tiruchanoor-517503.

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ABSTRACT

The design of the present research work is to formulate and Evaluate Naproxen loaded pharmacosomal gel. Naproxen loaded pharmacosomes were formulated by Ether injection method. Formulations of pharmacosomes from NF1 to NF9 are formulated by using various concentrations of phospholipid and organic solvents. The prepared formulations were evaluated for Drug Entrapment efficiency, Drug release, Drug content, Drug release kinetics and characterised for Zeta potential, Particle size and Scanning Electron Microscopy. Among all the formulations NF2 exhibited better drug release of 86.13% for 7hrs; better Entrapment efficiency of 83.2%; Drug content was found to be 92.36%. The formulation that showed better Entrapment efficiency, Drug content was selected and incorporated into carbopol gel for transdermal delivery of the drug. The prepared delivery system was evaluated for In-vitro drug diffusion studies and the drug release was found to be 84.5% at the end of 7 hours.

KEYWORDS: Pharmacosomes, Naproxen sodium, Soya lecithin, Ether injection method.

INTRODUCTION

Naproxen,6-methoxy-α-methyl-2-napthalenen acetic acid, is a NSAID with analgesic and antipyretic effects which is used in the treatment of musculoskeletal disorders like osteoarthritis, rheumatoid arthritis, dysmenorrheal and traumatic contusions. As other NSAID'S, oral therapy of naproxen is very effective, but the clinical use is often limited because of the gastritis and peptic ulceration.[1] In order to avoid the irritation and gastrointestinal tract, minimize systemic toxicity and achieve a better therapeutic effect, one promising method is to deliver drug via skin. Topical administration may help to maintain consistent plasma levels for long-term therapy from a single dose. To improve skin permeability of naproxen, several research attempts have been made using different formulation strategies, including use of penetration enhancers, liposome, prodrug, microemulsion.[2] However, there is very less literature about pharmacosomes for transdermal delivery of naproxen, although it has been used in pharmaceutics as a transdermal delivery carrier.

Pharmacosomes are colloidal dispersions of drug covalently bound to lipids and may exist as ultrafine vesicular, micellar or hexagonal aggregates, depending on the chemical structure of the drug-lipid complex. They are amphiphilic phospholipid complexes of drugs bear active hydrogen that bind to phospholipids.[3] Both hydrophilic and hydrophobic substances can be embedded in pharmacosome vesicles, thus, it is known that sparingly soluble drugs can entrapped in vesicles. Based on these considerations and to overcome all problems associated with poorly water soluble drug, naproxen the current work has aimed to develop a novel naproxen pharmacosomal controlled transdermal strategy.[4]

MATERIALS AND METHODS

Naproxen was received as a gift sample from TCI chemicals, Chennai, India. Soya lecithin, Diethyl ether, Ethanol and Acetone were purchased from Bros scientifics, Tirupati.

Preparation of Naproxen Pharmacosomal vesicles

Pharmacosomal formulation was prepared by Ether injection method. Phospholipid (soya lecithin) and Naproxen was dissolved in ether in a covered vessel at room temperature by vigorous stirring. This mixture was injected slowly drop wise in the preheated distilled water at 55-60^{0}C, vesicles of pharmacosomes are formed.[5]

Preparation of Naproxen Pharmacosomal Gel

Based on the results of pharmacosomal vesicle evaluation, the formulation which showed better Entrapment efficiency, Drug content i.e., NF2 was incorporated into gel. sufficient quantity of water taken and carbopol 934 was added little by little on continuous stirring, to this gel base methyl paraben and propyl paraben dissolved in water was added with continuous stirring. To this gel mixture 5ml of pharmacosomes was dispersed followed by triethanolamine with continuous stirring until stiff gel was formed.[6]

Table No. 1: Composition of Naproxen loaded pharmacosomes.

Pharmacosomal Formulation	NF1	NF2	NF3	NF4	NF5	NF6	NF7	NF8	NF9
Soya lecithin (mg)	100	100	100	150	150	150	200	200	200
Naproxen (mg)	100	100	100	100	100	100	100	100	100
Ethanol (ml)	5	-	-	5	-	-	5	-	-
Diethyl ether (ml)	-	5	-	-	5	-	-	5	-
Acetone (ml)	-	-	5	-	-	5	-	-	5
Distilled water (ml)	10	10	10	10	10	10	10	10	10

Table No. 2: Composition of Naproxen Pharmacosomal gel formulation.

Pharmacosomal Dispersion (ml)	Carbopol 934 (%)	Methyl paraben (g)	Propyl paraben (g)	Triethanolamine
5	1	0.020	0.002	2.0

EVALUATION OF PHARMACOSOMES

i) Entrapment efficiency

10ml of the prepared pharmacosomes were taken and diluted with 10ml of PBS pH 7.4. The resulting solution was sonicated for 10min in bath sonicator. The sonicated mixture was centrifuged at 10,000rpm for 30 min in a cooling centrifuge at a temperature of 4^0C. The entire supernatant liquid was separated and 10ml of PBS was added to it. The resulting dilution was assayed using UV-spectrophotometer at 232nm. The drug concentration was calculated using calibration curve of naproxen and entrapment efficiency was calculated using the formula.[7]

$$EP\ (\%) = [(C_t - C_r)/C_t] \times 100,$$

Where

EP is the encapsulation percentage,

C_t is the concentration of total drug.

C_r is the concentration of free drug.

ii) In vitro drug release studies

The in-vitro drug release studies of Naproxen Sodium and pharmacosomes dispersion formulations was determined by containing phosphate buffer pH 7.4 by using dialysis membrane was constantly stirred at 50 rpm and the temperature was maintained at 37±2° C. The aliquots were withdrawn at 1, 2, 3, 4, 5, 6, 7 and 7 hours. Soon after withdrawal of the aliquots, the receptor compartment was replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed by using a UV-visible spectrophotometer at 232nm.[8]

iii) Drug content

A specific quantity of pharmacosomal dispersion which is equivalent to drug was taken and dissolved in 100ml of phosphate buffer of pH 7.4. The volumetric flask containing dispersion was shaken for 2hr in bath sonicator in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically at 232 nm using phosphate buffer (pH 7.4) as blank.[9]

iv) Drug release kinetics

As a model-independent approach, comparison of the time taken for the given proportion of the active drug to be dissolved in the dissolution medium and figures such as T_{50} and T_{90} calculated by taking the time points of 50% and 90% of the drug dissolved and another parameter dissolution efficiency (DE) suggested by Khan were employed. DE is defined as the area under the dissolution curve up to the time 't' expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

$$\text{Dissolution Efficiency (DE)} = \left(\frac{\int_0^t y.dt}{y_{100}.t} \right) 100$$

The dissolution efficiency can have a range of values depending on the time interval chosen. In any case constant time intervals should be chosen for comparison. For example, the index DE_{30} would relate to the dissolution of the drug from a particular formulation after 30 minutes could only be compared with DE_{30} of other formulations. Summation of the drug dissolution data into a single figure DE enables ready comparison to be made between a large numbers of formulations.

As a model-dependent approach to describe the mechanisms and also the release kinetics, dissolution data were fitted to popular release models.[10]

CHARACTERISATION

i) Microscopic images

A drop of the pharmacosomes dispersion was placed onto the slide and examined under the microscope. At 45× magnification, circular single layered vesicle bodies were observed with uniform small size.

ii) Zeta potential measurements

Zeta potential measurements were performed by PCS using the Malvern Zetasizer Nano ZS (Malvern Instruments). Before measurement, pharmacosomes formulations were hydrated and samples were appropriately diluted with distilled water.

iii) SEM (Scanning electron microscopy)
Scanning electron microscopy (SEM) was conducted to characterize the surface morphology of the pharmacosomes including the controls (empty vesicles). One drop of pharmacosomal system was mounted on clear glass stub, air dried and sputter coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope JSM-5510 (Jeol Ltd., Tokyo, Japan) equipped with a digital camera, at 15 or 20 kV accelerating voltage.

EVALUATION STUDIES OF PHARMACOSOMAL GEL

i) Physical appearance and Homogeneity
The physical appearance and homogeneity of the prepared gels were tested by visual observations after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

ii) Clarity
The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows; turbid: +, clear: ++, very clear (glassy): +++ [11]

iii) pH Determination
1.0 g gel was accurately weighed and dispersed in 100 ml purified water. The *p*H of the dispersion was measured using digital pH meter, which was calibrated before use with standard buffer solution at 4.0, 7.0 and 9.0. The measurements of *p*H were done in triplicate and average values were calculated.

iv) Viscosity
Brookfield digital viscometer was used for the determination of viscosity and rheological properties of Naproxen pharmacosomal gel using spindle noT-96. The viscosity of gel was measured at different angular velocities at a temperature of 37°C.A typical run comprised changing of the angular velocity from 0.3 to 2.5 rpm. The averages of two readings were used to calculate the viscosity [12]

v) Spreadability
To determine the spreadability of formulation 0.5g gel was placed within a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gels was noted.

vi) Extrudability
To determine extrudability a closed collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 sec was determined. The average extrusion pressure in g was reported [13]

vii) In Vitro drug diffusion studies
In vitro release studies were performed using modified Franz diffusion cell. Pharmacosomal gel of different formulations was placed in the donor compartment and the top of the donor compartment was covered with paraffin film. 200 ml of phosphate buffer pH 7.4 was used as receptor medium to ensure a sink condition. The receptor compartment was maintained at 37°C and stirred by a magnetic bar at 600 rpm. The donor compartment was separated from the receptor compartment by cellulose dialyzing membrane which was soaked in the receptor medium overnight. At predetermined time intervals (15, 30, 45, 60,120,180,240,300,360,420 min.)1 ml aliquots were withdrawn from the sampling port and were replaced with an equal volume of fresh solvent mixture to maintain constant volume. The samples were analyzed spectrophotometrically at 232 nm. In reference with the constructed calibration curve (0.999). For each formula, drug release was studied in triplicate and the cumulative amount of drug released was determined [14]

RESULTS AND DISCUSSION

i) Calibration curve of Naproxen sodium

Table No.3: Calibration curve data of Naproxen sodium using phosphate buffer pH 7.4.

S. No.	Concentration(µg/ml)	Absorbance
1.	0	0
2.	2	0.071±0.007
3.	4	0.152±0.001
4.	6	0.226±0.001
5.	8	0.299±0.002
6.	10	0.378±0.001
7.	12	0.445±0.001

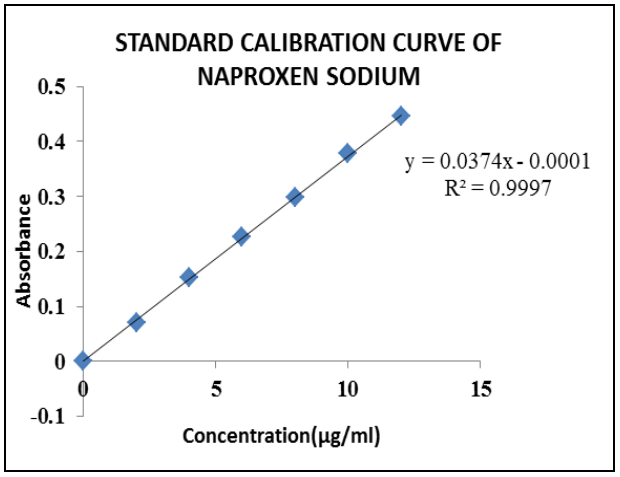


Figure No.1: Standard curve of Naproxen using phosphate buffer pH 7.4.

ii) Melting Point
Melting point of the pure Naproxen was found to be 153.3 ºC, thus indicating purity of the drug sample.

iii) FT-IR Spectroscopy

Table No. 4: FT-IR Interpretation of pure drug and excipients.

S. no.	Functional group	Characteristic Peaks	Observed peaks		
			Naproxen Sodium	Soya Lecithin	Physical Mixture
1.	C=C	1450-1600	1569	1525	1557
2.	C-H	2850-2975	2914	2921	_
3.	C-N	1342-1266	1278	_	1262
4.	C-O	1075-1020	1091	1051	1027
5.	N-H	3300-3350	_	3269	3323
6.	O-H	1320-1210	1219	1213	1211

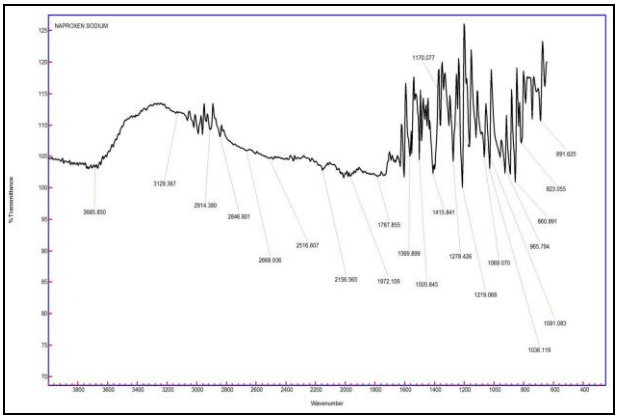


Figure No. 2: FT-IR Spectroscopy of Naproxen sodium.

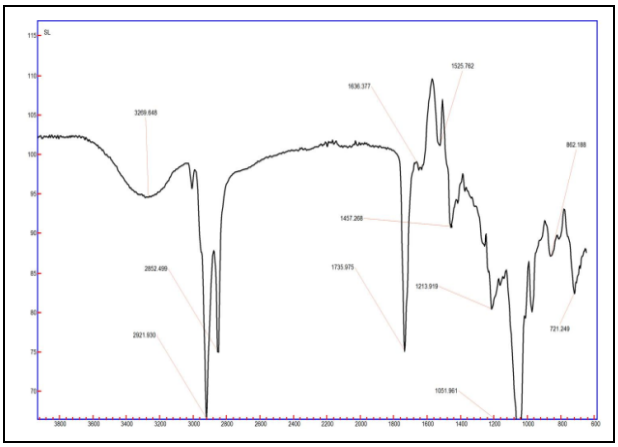


Figure No.3. FT-IR Spectroscopy of Soya lecithin.

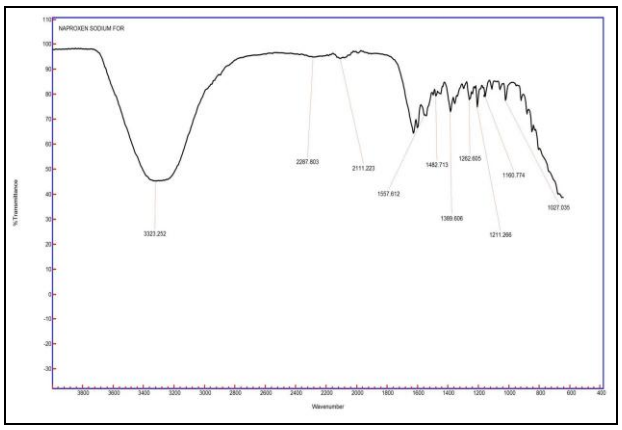


Figure No.4: FT-IR Spectroscopy of Mixture (Naproxen sodium, Soya lecithin).

FT-IR studies reveals that there is no physical interaction between drug and physical mixture.

EVALUATION OF PHARMACOSOMES

i) Entrapment Efficiency

Table No.5: Entrapment efficiency of Naproxen Pharmacosomes formulations.

S. No	formulation codes	%Entrapment Efficiency (n=3)
1.	NF_1	80.6±0.967
2.	NF_2	83.2±0.308
3.	NF_3	78.48±0.785
4.	NF_4	75.95±0.560
5.	NF_5	77.7±0.654
6.	NF_6	74.91±0.377
7.	NF_7	72.4±0.946
8.	NF_8	73.7±0.528
9.	NF_9	71.98±0.559

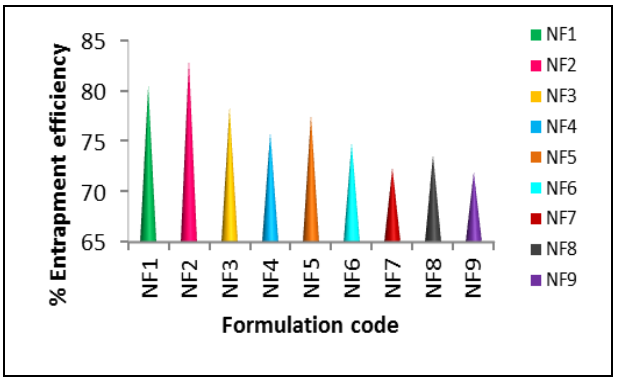


Fig. No. 5: %Drug Entrapment efficiency of Naproxen loaded pharmacosomes.

ii) In-Vitro Dissolution Studies

The maximum percentage drug release was found to be **86.13%** which was exhibited by formulation NF2.

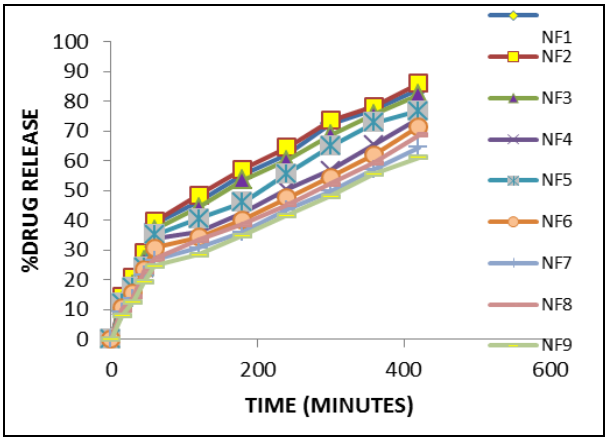


Fig. no. 6: In-vitro dissolution studies of Naproxen Pharmacosomal formulations.

iii) Drug Content

Table No.6: % Drug content of Naproxen sodium loaded pharmacosomal formulations.

S.No	Formulation Code	% Drug Content (n=3)
1.	NF1	87.1±0.184
2.	NF2	92.36±0.645
3.	NF3	82.89±0.381
4.	NF4	78.15±0.784
5.	NF5	80.78±0.432
6.	NF6	76.84±0.590
7.	NF7	67.1±0.741
8.	NF8	75.26±0.339
9.	NF9	65.52±0.826

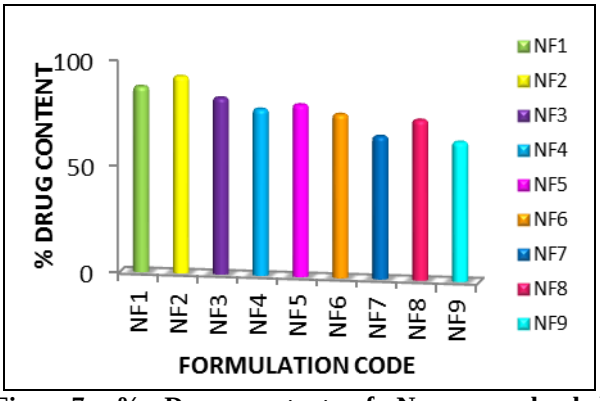


Fig.no.7: % Drug content of Naproxen loaded pharmacosomal formulations

The drug content of the prepared pharmacosomes was performed and the maximum drug content was shown by NF2 formulation i.e. 92.36% when compared to other eight formulations (NF1=87.1%, NF3=82.89%, NF4=78.15%, NF5=80.75%, NF6=76.84%, NF7=67.1%, NF8=75.26%, NF9=65.52%) this may be due to less loss of drug during preparation of pharmacosomes.

DRUG RELEASE KINETICS

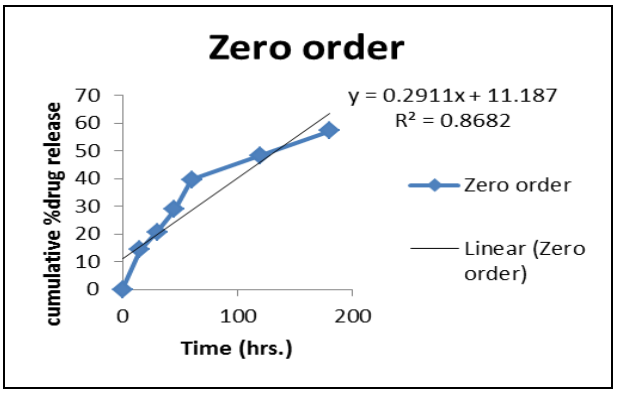


Fig no.8: Drug release kinetics of Zero order.

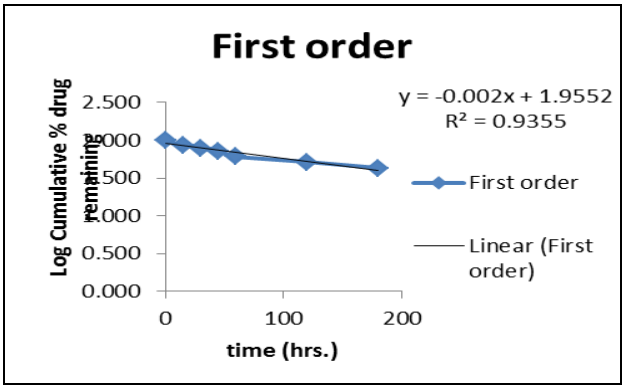


Fig. No.9: Drug release kinetics of first order.

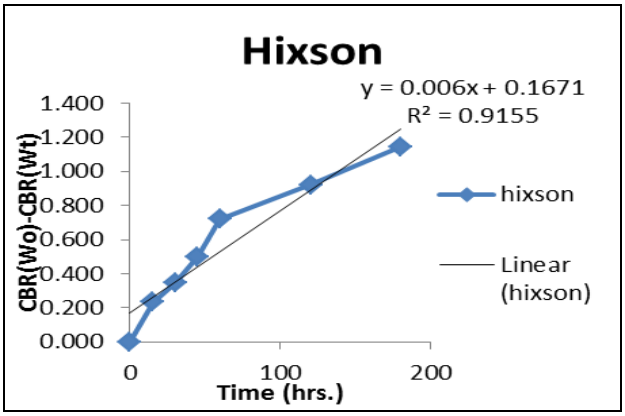


Fig. No.10: Drug release kinetics of Hixon crowell model.

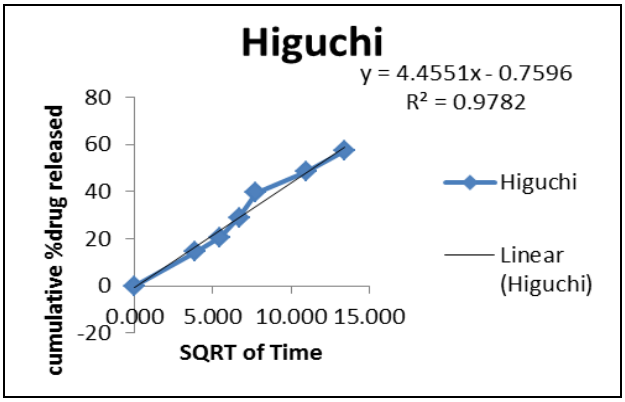


Fig. No. 11: Drug release kinetics of Higuchi model.

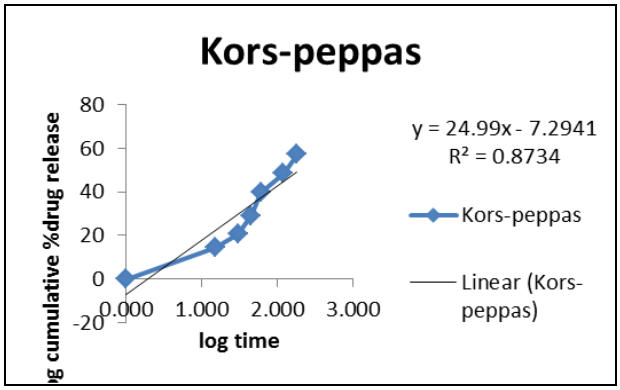


Fig No.12: Drug release kinetics of kors-peppas model.

Table no. 7: R^2 values of NF2 formulation.

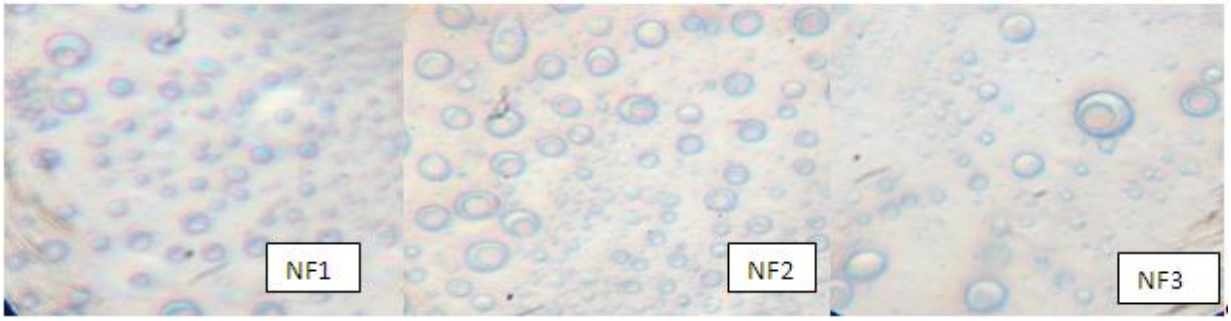
Formulation	Zero order plot	First order plot	Higuchi plot	Peppas plot	Hixon plot
NF2	0.868	0.935	0.978	0.873	0.915

Above all the Drug release kinetics of optimized formulation i.e.NF2 follows the Higuchi model with Correlation coefficient **r** value **0.978**. And thus indicating drug release kinetics in Higuchi which is the best and fit model for Naproxen pharmacosomal formulations.

CHARACTERISATION OF PHARMACOSOMES

i) Microscopic images

Microscopic images were taken to find the vesicle morphology. From the images it was evident that the vesicles in the pharmacosomes were discrete and almost spherical in shape.



Microscopic images of Pharmacosomes.

ii) Zeta Potential

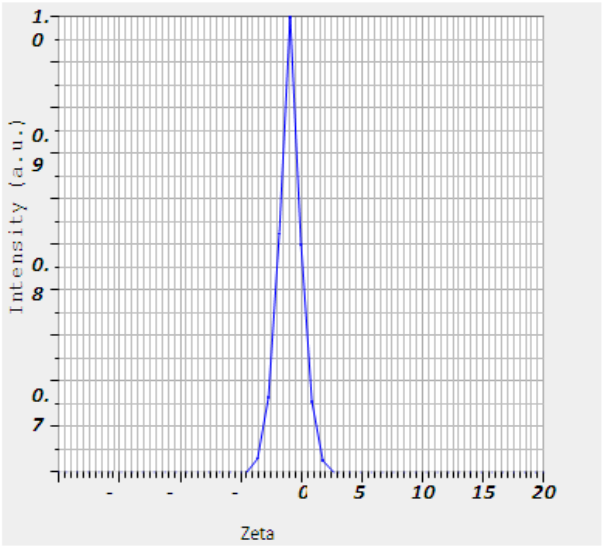


Fig. No. 13: A graphical representation of Zeta potential of NF2 Formulation

iii) Particle Size

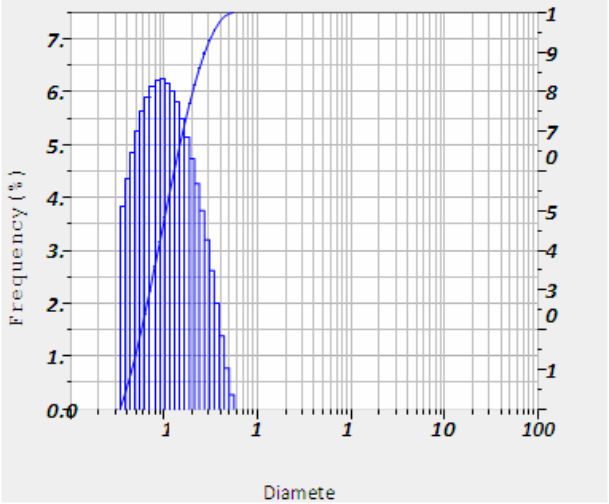


Fig. No.14: A graphical representation of Particle size of NF2 Formulation

The zeta potential of Naproxen loaded pharmacosome formulation (NF2) was found to be **-9.5mV.**

The mean particle size of Naproxen loaded pharmacosome formulation (NF2) was found to be **1.4nm.**

iv) SCANNING ELECTRON MICROSCOPY

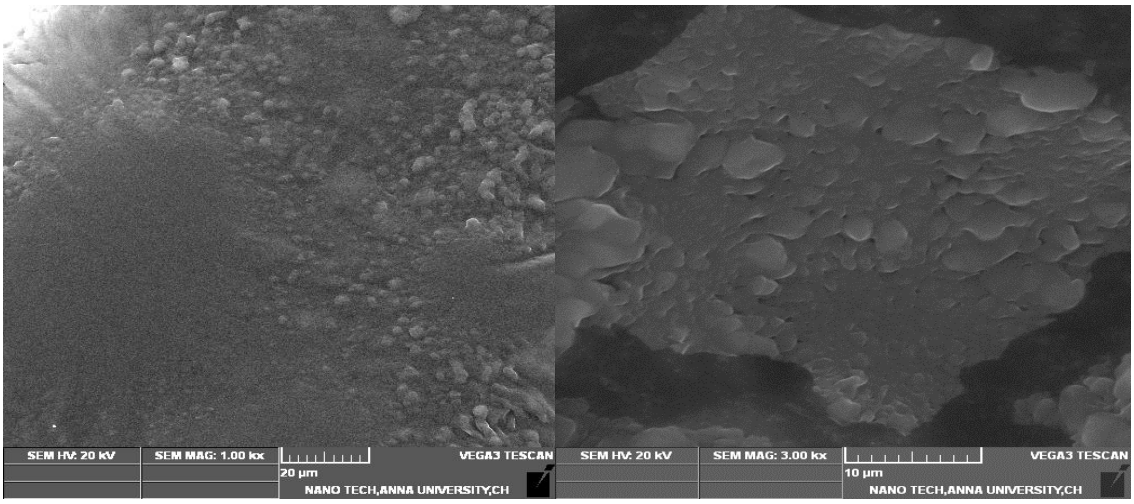


Fig. no. 15: Scanning electron microscopic images of optimized formulation (NF2).

The surface morphology of Pharmacosomes were studied by Scanning electron microscopy. Pharmacosomes of Naproxen sodium were found to be of irregular shape. The surface was found to be sticky in the pharmacosomes complexes prepared with low purity grades(30%) of phospholipids. As the phospholipids are natural components, their different purity grades may have different effects in shape and surface morphology.

EVALUATION OF PHARMACOSOMAL GEL

Table no.8: Physical evaluation of Naproxen Pharmacosomal gel.

Formulation	Color	Homogeneity	Phase separation	Occlusiveness	Washability
NF2	White	Homogenous	No	Yes	Washable

The Naproxen pharmacosomal gel formulation has showed no phase separation, good Occlusiveness and washability. And white color and homogeneity due to more amount of unentrapped drug.

Table No. 9: Evaluation parameters of Naproxen pharmacosomal gel.

Formulation	pH	Viscosity (c.ps)	Extrudability	Spreadability (g.cm/sec)
NF2	5.7±0.10	237	Good	0.106±0.02
	6.5±0.21	253	Good	0.342±0.104
	6.6±0.134	276	Good	0.521±0.219

vi) In-Vitro Drug Diffusion Studies

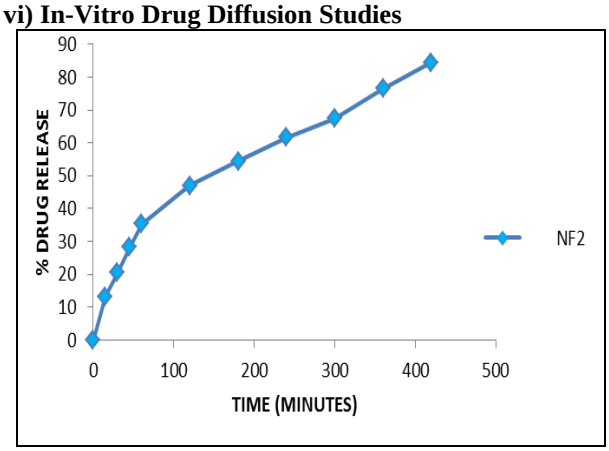


Figure No. 16: In-vitro drug release of Naproxen loaded pharmacosomal gel.

The permeation of drug from pharmacosomal gel prepared using NF2 formulation is high compared to other formulations. Hence, fast drug release was observed in case of Naproxen pharmacosomal gel which is prepared using NF2 formulation. In addition, lecithin acted as penetration enhancer. Sustained drug release pattern was observed with Naproxen pharmacosomal gel. The percent drug release at the end of 7 hours was found to be **84.5%**.

CONCLUSION

Naproxen sodium was successfully formulated as Pharmacosomes by using the phospholipid i.e., soya lecithin acts as membrane stabilizer at different concentrations and various organic solvents such as Ethanol, Diethyl ether and Acetone acts as permeation enhancer by Ether injection method. Amongst all the prepared formulations from NF1 to NF9, the formulation NF2 possessing Drug, Soya lecithin and Diethyl ether at a concentration of 100 mg, 100 mg and 5ml considered as optimized formulation on the basis of % drug release,

% drug content, % entrapment efficiency, particle size, zeta potential and surface morphology. The optimized formulation NF2 released **86.13 %** of drug by its dissolution within 7 hours. The Entrapment efficiency is also high for the optimum formulations NF2 (83.2%) and has clear surface morphology. The drug content of NF2 was showed as 92.36% and particle size and zeta potential of NF2 is 1.4nm and -9.5mV. FTIR studies revealed that the absence chemical interactions between drug and polymer.

The optimized pharmacosomal formulation NF2 is incorporated into gel prepared by using cabopol 934 as a gel base. The prepared pharmacosomal gel was evaluated for organoleptic characters, pH, viscosity, spreadability, extrudability and drug diffusion studies. The prepared gel is homogenous, white with good occlusive and washability properties. The pH of the NF2 formulation is 6.6, spreadability and extrudability were found to be effective i.e. they showed best results for spreadability and extrudability. **84.5 %** Naproxen drug was diffused at the end of the 7 hours from the pharmacosomal gel.

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