

# Fabrication and Characterization of Herbal Drug –Loaded Nonionic Surfactant Based Niosomal Topical Gel

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## Abstract

The aim and objective of the study was to develop herbal drug loaded niosomes as topical gel by using thin film hydration technique. The practicability of herbal drug (CODE: THDC3-2615RD) of niosomes by thin film hydration technique using Span series 20, 60, 80 and cholesterol has been successfully revealed in this study. The evolved herbal niosomes were evaluated for morphology, particle size and *in vitro* release study. The optimized batch F<sub>7</sub> (Span 60 C/S; 1.5:5) showed highest entrapment efficiency 97.12%, prolonged *in vitro* release of 81.56% and the stability studies showed good results at 4°±2°C. Niosomal gel was formulated by dispersing the optimized batch F<sub>7</sub> into Carbopol 940 and HPMC. The *in vitro* release study was carried out for the plain herbal gel and niosomal herbal gel, in which the niosomal gel revealed better results. Furthermore *ex vivo* permeation study was carried out for the niosomal gel.

**Key Words:** Herbal Niosomes; Niosomal gel; Targeted delivery; Bladder stone; Permeation study

## INTRODUCTION

Novel drug deliveries are used in the pharmaceutical formulations with respect to sustained and targeted release, to increase bioavailability, to maintain stability and to reduce the side effects of drug. The main advantage of targeted drug delivery is it releases the drug to the specified tissues, organ (or) location on the body. The drug delivered in the specified location increase the bioavailability by decreasing the volume of distribution of the drug. There is various targeted drug delivery strategies used as carriers such as nanoparticles, liposomes, niosomes, aquasomes, micro emulsions, etc. Among these liposomes and niosomes are well documented as carriers in dermal drug delivery. Niosomes are preferred than the liposomes, since niosomes maintains better stability than liposomes.<sup>[1]</sup>

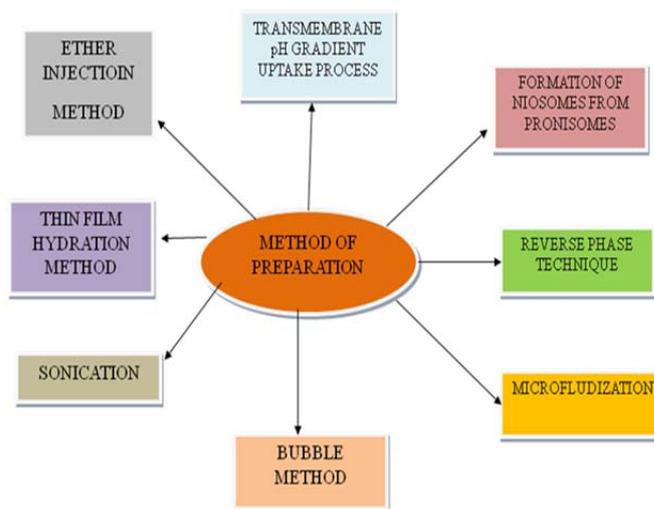
Niosome is a bilayer vesicle carrier, which entraps both hydrophilic and lipophilic drugs. It is composed of lipid layer cholesterol and an aqueous layer non-ionic surfactant. The non-ionic surfactants include alkyl ethers, alkyl esters, crown ethers and series of span and tween.

The lipid layer cholesterol gives the shape and rigidity to the vesicle, also gives membrane properties like permeability, elasticity and mechanical strength.<sup>[2]</sup>

The non-ionic surfactants are used in the formation of vesicle with respect to stability, less toxic, less irritating to the cellular surfaces and maintain physiological pH in solution. The HLB (hydrophilic lipophilic balance) and CPP (critical packing parameter) value of surfactant plays a major role in selection of the surfactant. The HLB value 14-17 is not eligible in the formation of niosomes. Low HLB (HLB<6) value shows good drug entrapment. CPP is the other parameter, important for the shape of vesicles. Surfactant having CPP value between 0.5-1 form spherical vesicles more than 1 forms inverted micelles. The surfactant and lipids are evaluated by the gel liquid phase transition temperature (TC). Phase transition temperature

also effects the entrapment efficiency. Among the surfactants **Span 60** comprises the above characters; HLB value is 4.7, CPP value is 0.5-1 and the phase transition temperature is 53°C. Thus Span 60 forms spherical vesicles and shows highest entrapment efficiency.<sup>[3]</sup>

Niosomes are been prepared by various methods such as



## NIOSOMES AS DRUG CARRIER SYSTEM

- ◆ Retain drugs qualitatively
- ◆ Exhibit long plasma half-lives
- ◆ Have high entrapment efficiencies
- ◆ Retard drug metabolism
- ◆ In the case of site-directed drugs. Allow the attachment of targeting ligands to the vesicle surface and assist in the movement of drugs across membranes<sup>[4]</sup>

In this study, herbal topical niosomal gel has been formulated for the treatment of bladder stones. Drugs when administrated orally have various disadvantages such as absorption, first pass metabolism which reduces the bioavailability of the drug, drug- food interactions and degradation of the drug due to gastric enzymes. Topical administration the drug gives a localized drug delivery through the skin. Stratum corneum is the major barrier of the skin which is the top layer of the epidermis. In novel drug delivery niosomes are mostly used to enhance the permeation of the drug through the skin.<sup>[5]</sup>

#### BLADDER STONE

Urinary bladder is an elastic organ which is located in the pelvic cavity. The function of the bladder is, it acts as a tank which stores the body urine. Urine is produced by the kidney as it flows through the ureters to the urinary bladder. The bladder plays salient role retarding and controlling the urination. The capacity of bladder is around 300-350ml of urine. One of the disorders related to the urinary bladder is bladder stone.<sup>[6]</sup>

**THDC3-2615RD** is a herbal drug used in the treatment of bladder stone traditionally. Bladder stone is a calculus formed in the urinary bladder. The calculus is hard masses of minerals such as calcium, magnesium ammonium and uric acid salts. It also varies in size, shape and texture as small, rigid and spike. The stones are formed when the urine becomes more concentrated (or) due to often dehydration in the body, inflammation in bladder and use of urinary catheters (causes irritation the bladder) and leads to formation of stones. The symptoms are pain in the lower abdomen, burn (or) paining sensation in the urethra at the time of urination. Urine may contain blood and appears cloudy. Initially, bladder stones can be treated by uptake of large amount of fluids, later stage by oral medications. When the size of the stone are found to be large they are broke down using cystolitholapaxy or surgery is an alternative method for the removal of stones.<sup>[7]</sup>

In this study, a novel approach has been carried out to cure the bladder stones. Niosomal topical gel as been formulated as an alternate to conventional formulation to enhance the bioavailability when compared with oral route of administration. It is having challenges that deliver the drug or reach the herbal drug at bladder site.

### MATERIALS AND METHODS

#### Materials

**DRUG CODE: THDC3-2615RD** was a gift sample from The Himalaya Drug Company, surfactants (Span 20, 60, 80), cholesterol. All the solvents used were in analytical grade.

#### Methods

### 1. THDC3-2615RD – EXCIPIENTS COMPATIBILITY STUDIES

FT-IR studies are performed to find compatibility between the herbal drug and the excipients used in the formulation. FTIR was carried out by press pellet technique. Potassium bromide was used to prepare the pellets and was dried under dry condition. The spectra of the drug sample and

that of the drug-excipient mixtures were compared to check the incompatibility, if any.<sup>[8]</sup>

### 2. METHOD OF PREPARATION OF NIOSOMES

Niosome was prepared by **THIN FILM HYDRATION TECHNIQUE**. Constant amount of Cholesterol and different ratios of Spans (20, 60, and 80) as given in table 1 were weighed and dissolved in chloroform and methanol (7:3ml) respectively in a round bottom flask. The organic solvents were evaporated at 20°C under reduced pressure using “Rotary Flash Evaporator”. A thin film of surfactant was found to be formed on the walls of the flask. The film was rehydrated with 10ml of phosphate buffer 7.4 at 45°C for 45mins in which the herbal drug to be loaded (10mg) was dissolved. After rehydration the niosomes were sonicated for 10mins in a bath sonicator.<sup>[9]</sup>

Table 1

FORMULATION	SURFACTANT	RATIO OF		
		DRUG	CHOLESTEROL	SURFACTANT
F <sub>1</sub>	Span20	1	1.5	3
F <sub>2</sub>	Span20	1	1.5	4
F <sub>3</sub>	Span20	1	1.5	5
F <sub>4</sub>	Span20	1	1.5	6
F <sub>5</sub>	Span60	1	1.5	3
F <sub>6</sub>	Span60	1	1.5	4
F <sub>7</sub>	Span60	1	1.5	5
F <sub>8</sub>	Span60	1	1.5	6
F <sub>9</sub>	Span 80	1	1.5	3
F <sub>10</sub>	Span 80	1	1.5	4
F <sub>11</sub>	Span 80	1	1.5	5
F <sub>12</sub>	Span 80	1	1.5	6

### 3. EVALUATION OF NIOSOME

#### 3.1. FORMATION OF VESICLE

##### 3.1.1. PHOTO ELECTRON MICROSCOPY

The vesicles formed were characterized by using photo electron microscopy. Small amount of the dispersion was spread on the glass slide and viewed for the vesicle structure using (Leica) Light Microscope with varied magnification powers (10X and 40X) and the photographs were taken.<sup>[10]</sup>

##### 3.1.2. TRANSMISSION ELECTRON MICROSCOPY

The internal morphology of vesicle formed was examined by using TEM (FEG JAPAN). The sample was prepared by diluting the formulation in the ratio of 1:5 and placed on a copper grid which contains carbon coated at one side. 20µL of sample was placed on the copper grid and dried for overnight.<sup>[11]</sup>

##### 3.2. PARTICLE SIZE DISTRIBUTION

The particle size distribution of the niosome was analyzed using laser diffraction (Malvern Particle size Analyzer) and the mean of vesicular diameter was calculated.<sup>[12]</sup>

### 3.3. ENTRAPMENT EFFICEINCY

The entrapment efficiency of niosomes was determined by centrifugation method. 2 ml of the suspension was centrifuged at 14,000 rpm for 60 minutes maintaining a temperature of 4°C, in order to separate niosomes from untrapped drug. The free drug concentration in the supernatant liquid was determined at 265nm using UV-Visible Spectrophotometer. The percentage of drug entrapment in niosomes was calculated by using the following formula<sup>[13]</sup>

$$\% \text{ Drug entrapment} = \frac{(\text{Total drug} - \text{unentrapped drug})}{\text{Total drug}} \times 100$$

### 3.4. DRUG CONTENT

The drug content of niosomes was determined by taking niosomal dispersion equivalent to 10mg in a standard flask and making the volume up to required volume using phosphate buffer pH 7.4. After that 1ml of the solution was withdrawn and diluted up to 10ml using phosphate buffer pH7.4, the amount of drug present was measured at 265nm using UV spectrophotometer.<sup>[14]</sup>

### 3.5. IN VITRO RELEASE STUDIES

*In vitro release* of niosomes was carried out using dialysis bag. Prepared niosomes were placed in a dialysis bag length of 5 cm which acts as a donor compartment. Dialysis bag was placed at one end of the open end cylinder and placed in a beaker containing 100 ml of phosphate buffer 7.4, which acts as a receptor compartment. The temperature of the receptor medium was maintained at 37±1°C and the medium was agitated at a speed of 50 rpm using a magnetic stirrer. 5 ml of the sample was collected at regular time intervals and replaced immediately with the same volume of phosphate buffer pH 7.4. The sink condition was maintained throughout the experiment. The collected samples were analyzed spectrophotometrically at 265 nm using UV-Visible Spectrophotometer.<sup>[15]</sup>

### 3.6. STABILITY STUDIES

Leaching of drug from niosomes was investigated by carrying out stability studies. Niosomal dispersion was stored in two different conditions freeze condition (4±2°C) and room temperature (25±2°C) for 10 weeks in sealed transparent glass vial. Drug content of the niosomal dispersion was checked periodically.<sup>[16]</sup>

## 4. FORMULATION OF GEL

Placebo gel (G<sub>1</sub>) and Herbal drug loaded gel (G<sub>2</sub>) were prepared by "Cold Mechanism Method" and physical characterization such as appearance, pH and viscosity studies have been performed for the prepared for the gels.<sup>[17]</sup>

## 5. EVALUATION OF GEL

### 5.1 IN VITRO DIFFUSION STUDY

The release study for the gel was carried out by diffusion method using Franz-diffusion cell through a cellophane membrane. 1g of a plain herbal gel and herbal drug loaded niosomal gel were weighed and placed on the cellophane membrane. The diffusion study was carried out separately maintaining the temperature at 37±1°C using 100ml of phosphate buffer 7.4 as medium. 5ml of sample was carried

out at regular time intervals for 8hrs. Sink condition was maintained throughout the study. The samples were analyzed spectrophotometrically at 265nm using UV – visible spectrophotometer.<sup>[18]</sup>

### 5.2 DRUG RELEASE KINETICS

The results obtained from *in vitro* drug release studies were attempted to fit into various mathematical models such as Zero order release kinetics, First order release kinetics, Higuchi classical diffusion equation, Koresmeyer-Peppas exponential equation and Hixson-Crowell erosion equation to know the mechanism of drug release. The equation with high regression coefficient (r<sup>2</sup>) for formulation will be the best fit of release data. For Koresmeyer-Peppas equation, if n = 0.5 indicating pure fickian diffusion, n = 0.5-1 indicating anomalous non-fickian diffusion and n=1 indicates zero order release.<sup>[19]</sup>

### 5.3. EX VIVO STUDIES

Permeation capacity of the gel was evaluated by carrying out *ex-vivo* permeation study using Goat skin purchased from the slaughter house, Chennai. Skin is stored at -20°C. Skin hairs were trimmed and cleaned triplicate using scissors and the skin contents were removed. Franz diffusion cell was used to carry out the study. The goat dorsal skin was placed between the compartments of the diffusion cell, stratum corneum facing the donor compartment. 12ml of phosphate buffer saline (PBS) pH 7.4 acts as the receiver phase. It was stirred at 500 rpm on a magnetic stirrer, maintaining the temperature at 37±0.5°C. The study was carried out for 8hrs and the amount of drug permeated was found by removing sample (2ml) at regular time intervals. Sink condition was maintained throughout the study. The absorbance was measured at 265nm spectrophotometrically.<sup>[20]</sup>

## RESULTS AND DISCUSSION

Herbal drug loaded niosomes were prepared using various non-ionic surfactants (Span 20,60,80) along with cholesterol in different proportions as shown in Table.1 by thin film hydration technique. The prepared herbal drug loaded niosomes were examined for various parameters like vesicle size, shape, particle size, entrapment efficiency and *in vitro* release study. The prepared optimized niosomal formulation was incorporated in to the gel to formulate the Topical Niosomal Gel.

### FTIR

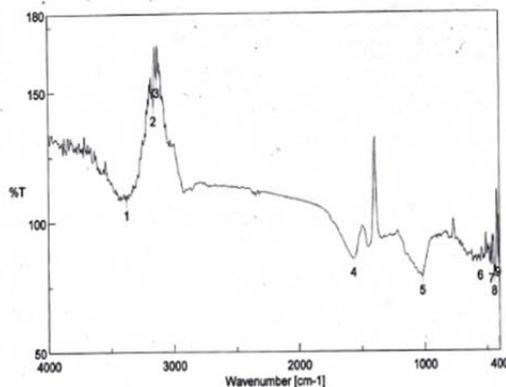


Fig:1 FTIR spectra of drug(code: THDC3-2615RD)

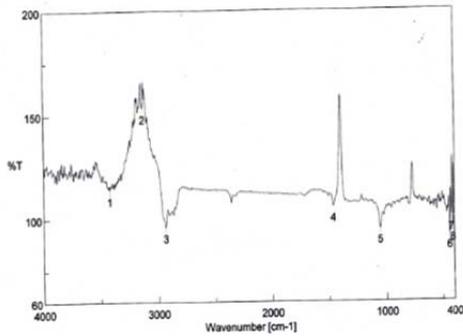


Fig:2 FTIR spectra of cholesterol,

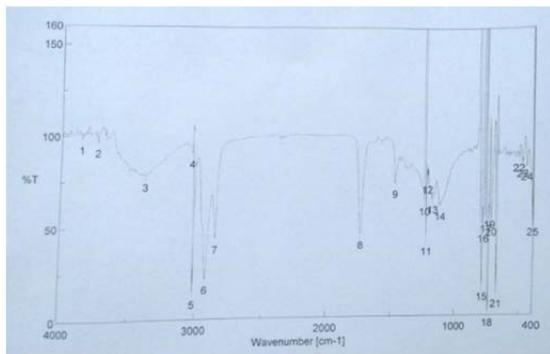


Fig:3 FTIR spectra of Span20,

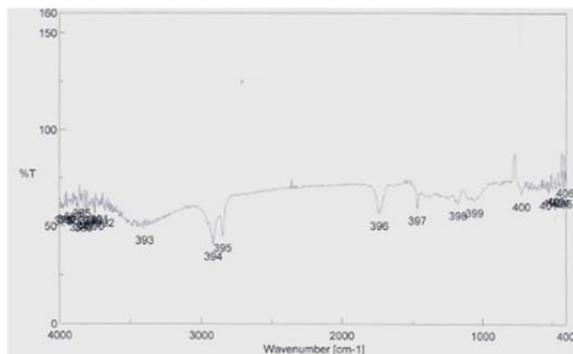


Fig:4 FTIR spectra of Span60,

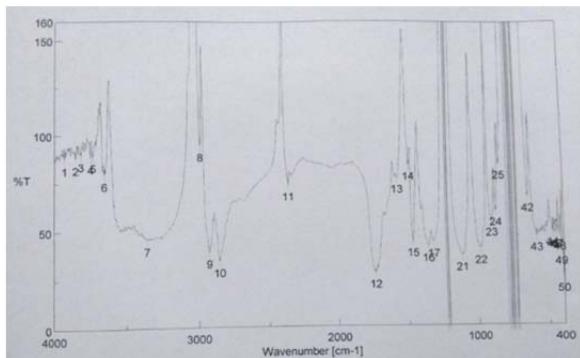


Fig:5 FTIR spectra of Span80

Drug and excipients compatibility studies were performed using FTIR. Results showed that there were no physical and chemical reaction between the herbal drug and excipients. Thus, development of studies have been carried out. Further it confirms that the physical and chemical nature of the prepared formulations would not get changed during the shelf life also.

**EVALUATION OF NIOSOME VESICLE FORMATION**

**(a) PHOTO ELECTRON MICROSCOPY**

The optimized formulation (F<sub>7</sub>) vesicle was characterized studied using optical microscopy under × 40 magnifications to observe the formation of vesicles. The shape of niosomes was found to be spherical due to the less CPP.



Fig 6: Photo electron microscopy of F7

**(b) TRANSMISSION ELECTRON MICROSCOPY**

The optimized formulation F7 shows the particle size uniform and discrete in shape and size. The particle sizes exhibit in the range of 50-100nm.

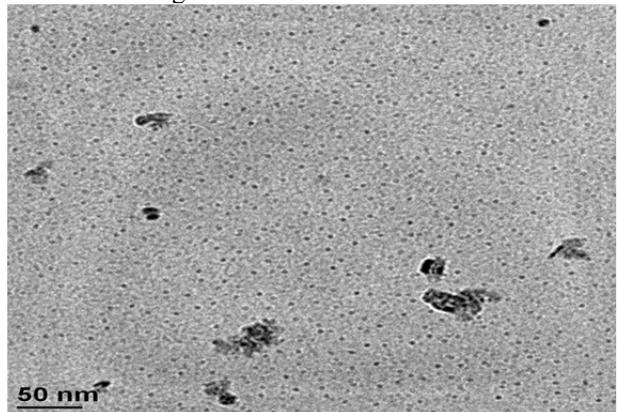


Fig 7: TEM image of F7

**DRUG CONTENT**

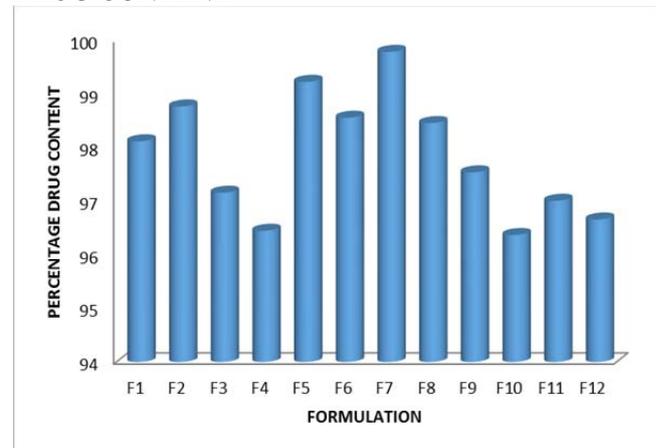


Fig 8

**ENTRAPMENT EFFICIENCY**

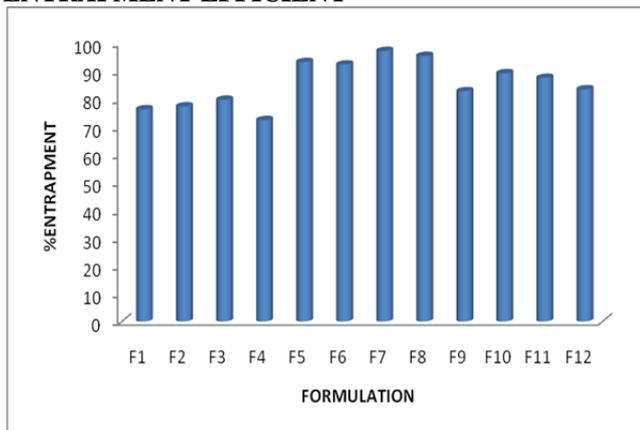


Fig 9 : Entrapment efficiency of formulations

The entrapment efficiency of the niosomal formulations were measured by centrifugation method and determined by subtracting the amount of drug in supernatant liquid from the total amount of drug in the formulation. In all the formulations, the impact of surfactant concentration on entrapment efficiency is significant.

The HLB value and the Phase transition temperature of the surfactant affect the entrapment efficiency. Low HLB and high transition temperature increase the Entrapment Efficiency

Among all the formulations **F<sub>7</sub> (Span 60 C/S; 1.5: 5)** showed the maximum entrapment efficiency compared with other formulations due to its low HLB value and high transition temperature. The entrapment efficiency increases in order of Span 60 > Span 80 > Span 20. Though Span 80 has the low HLB value, Span 60 showed high entrapment efficiency because Span 80 has unsaturated alkyl chain compared to Span 60.

Cholesterol has the ability to cement the leaking space in the bilayer membranes. Increase in cholesterol content increases beyond a certain level, it starts disrupting the regular bilayer structure thereby decreases the drug entrapment. The trial preparation was carried out and the cholesterol ratio was fixed to be **15mg** to give good entrapment efficiency.

**PARTICLE SIZE DISTRIBUTION**

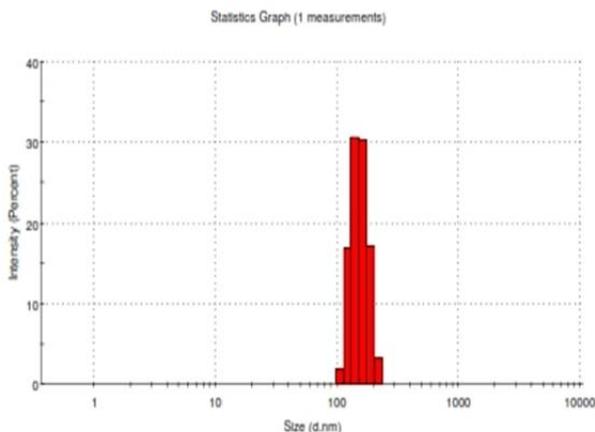


Fig 10: Particle size distribution of F7

Niosomes particle size distribution was measured by light scattering method using Malvern size analyzer. From this study the particle size distribution of the optimized (F<sub>7</sub>) niosomal dispersion was found to be on average of 181.6 nm.

**IN VITRO RELEASE OF NIOSOME**

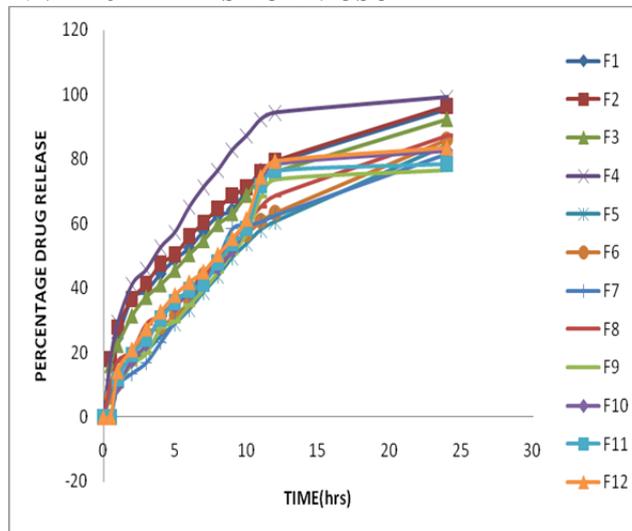


Fig 11: In vitro release of all niosomal formulations

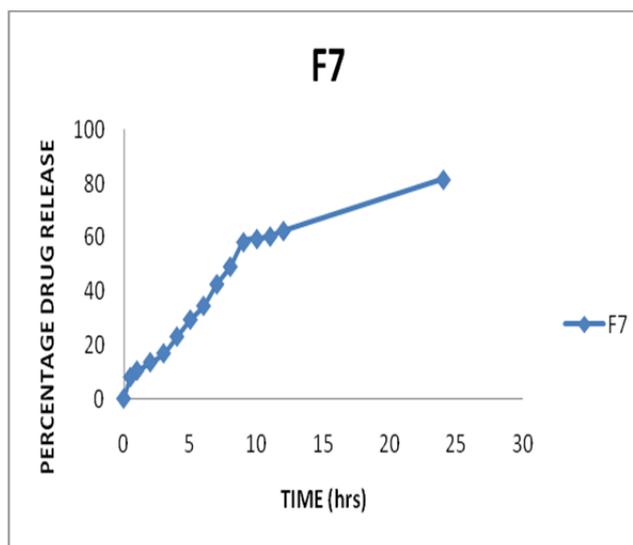


Fig 12: In vitro release of optimized formulation (F7)

Since the herbal drug is insoluble in water, it is needed to develop an appropriate medium that can provide sufficient solubility for the drug to maintain the required sink condition during diffusion studies. PBS pH 7.4 was selected, which the drug was found to be soluble. The volume of the receptor medium used was 100 ml. The in vitro release of all span niosomal formulations were carried out by diffusion method. In niosomal formulations the experimental studies showed that the rate of drug release depends on the percentage of drug entrapment efficiency. In Span 60 niosomes **F<sub>7</sub> (Span 60, C/S; 1.5: 5- 81.56%)** showed prolonged release than the other spans. This is due to higher alkyl chain length of Span 60 which lowers the release rate.

**STABILITY STUDIES**

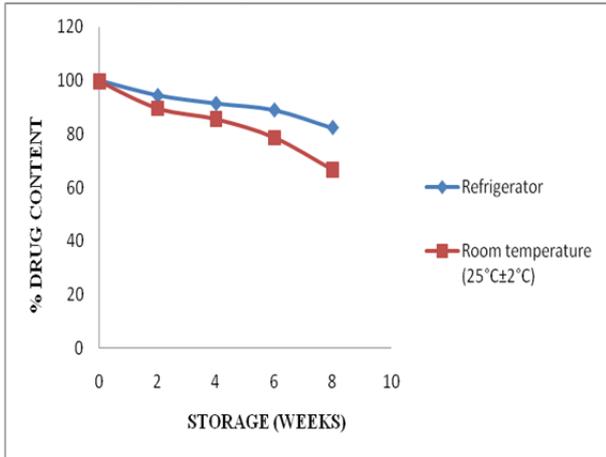


Fig 13: Stability studies of optimized formulation (F7)

The stability study of the optimized formulation (F<sub>7</sub>) was carried out by storing at 4°C±2°C (refrigeration temperature) and 25°C±2°C (room temperature) for 10 weeks. The percentage of drug retention in the niosomal preparation at various time intervals was determined. The results showed that the drug retention capacity was more with niosomal preparation stored at 4°C±2°C 82.28% and 66.52% in (25°C±2°C). Increase in temperature decreases the drug retention capacity due to degradation of polymers. This may be attributed to phase transition of surfactant and lipid causing vesicles leakage at higher temperature during storage. Hence, it is concluded from the obtained data the optimum storage condition for niosomes was found to be 4°C±2°C.

**NIOSOMAL GEL**

Niosomal gel was prepared using Carbopol and HPMC. Carbopol is hydrophilic in nature and bioadhesive it increases the residence time of the drug. The optimized niosomal formulation (F<sub>7</sub>) was incorporated into gel base to formulate niosomal topical gel.

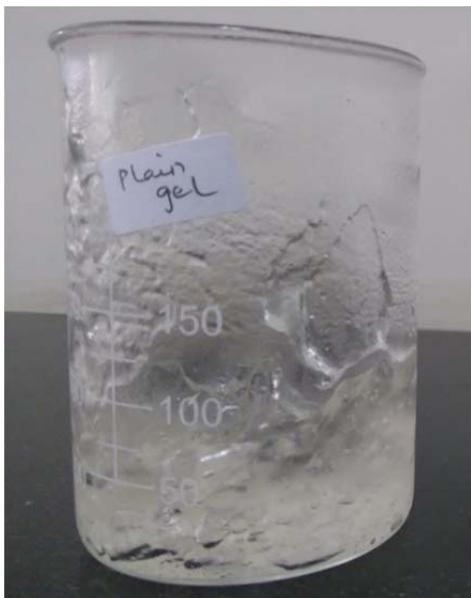


Fig 14: Plain gel

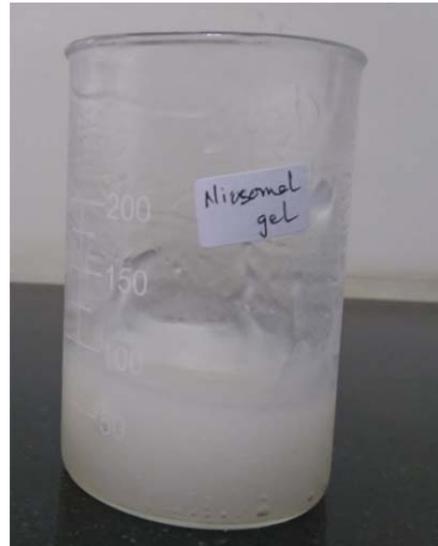


Fig 14: Niosomal gel

**VISCOSITY OF GEL**

S.NO	RPM	VISCOSITY(cps)	
		G <sub>1</sub>	G <sub>2</sub>
1	5	25.80	19.80
2	10	11.10	25.80
3	15	10.80	9.40

The viscosity of prepared gel formulation was carried out by Brookfield viscometer to determine the flow behavior of gels. The formulations exhibited pseudo plastic rheology and showed high viscosity under low shear rate condition and low viscosity under the high shear rate conditions.

**IN VITRO DRUG RELEASE STUDY OF GEL**

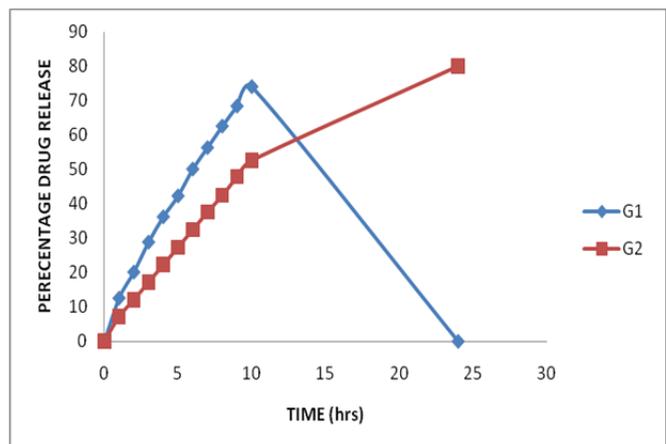


Fig 15: In vitro release of gels

Prepared gels were found to be transparent(G<sub>1</sub>) and milk white in appearance(G<sub>2</sub>). Gels are pleasant in appearance and having compatible pH with skin. The release of drug from the gel is also excellent. The drug release of G<sub>2</sub> shows 52% at 12hrs and 85% at 24hrs.Hence, it proves that, slow release from the gel would happened.

**DRUG RELEASE KINETICS**

FORMULATION	ZERO ORDER	FIRST ORDER	HIGUCHI	KORSMEYER-PEPPAS		HIXSON-CROWELLS
	R <sup>2</sup>	R <sup>2</sup>	R <sup>2</sup>	R <sup>2</sup>	N	R <sup>2</sup>
G <sub>1</sub>	0.9907	0.9890	0.9616	0.9982	0.782	0.9977
G <sub>2</sub>	0.9716	0.9096	0.9587	0.9879	0.738	0.9740

The model with r<sup>2</sup> value nearest one was considered as the best fit model for the formulation. The maximum n value was formed to be for Kosmeyer Peppas Model. As per the Kosmeyer Peppas Model the n value should be the range of 0.45-0.89 of standard values. The G<sub>2</sub> gel formulations showed 0.738 of n value which is in-between 0.45-0.89. Hence the formulation G<sub>2</sub> showed the mechanism of anomalous Non-fickian diffusion model. It states that the drug is released by swell able polymer device.

**EX VIVO PERMEATION STUDY**

TIME(hrs)	PERCENTAGE DRUG RELEASE
0	0
1	12.15
2	15.24
3	22.58
4	27.85
5	36.82
6	48.33
7	53.12
8	57.89

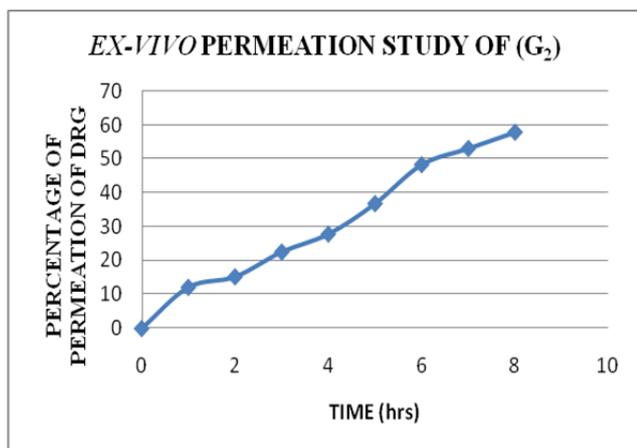


Fig 15: *Ex vivo* permeation of G<sub>2</sub>

The *ex-vivo* permeation study was carried out for the optimized (F<sub>7</sub>) formulation. The results show that 57.89% drug has been permeated at the time of 8 hrs. It reveals that the niosomal gel has permeation through the skin.

**CONCLUSION**

The approach of incorporating herbal drug into niosomes for a better to target the drug at appropriate tissues destination as a drug carrier is a novel drug delivery.

Niosomes containing herbal drug (**THDC3-2615RD**) were formulated using different surfactants such as Span 20, 60, 80 and was evaluated for various parameters. From the obtained result it is concluded that the optimized niosomal formulation F<sub>7</sub> showed maximum entrapment efficiency and sustained release. Gel formulation containing niosomes loaded with herbal drug showed prolonged action than the plain placebo gel. Thus, niosomes represent a promising drug delivery module for transdermal drug delivery. An increase in penetration rate has been achieved to help in the localized delivery of drug and then improved availability of drug at their site of action which in turn will reduce the dose and also the dose dependent side effects like irritation.

**CONFLICT OF INTEREST:**

The authors have described no conflict of interest.

**ACKNOWLEDGEMENT:**

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