WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 17, 828-846. Research Article ISSN 2277– 7105

PREPARATION OF THYMOQUINONE NIOSOMAL GEL AND EVALUATION OF ANTI INFLAMMATORY ACTIVITY IN RATS

Syeda Aiman Faheem[1]* and Dr. Abdul Mannan[2]

[1]M. Pharmacy*, [2]Professor

Dept. of Pharmaceutics

Article Received on
29 October 2022,

Revised on 19 Nov. 2022,
Accepted on 09 Dec. 2022

DOI: 10.20959/wjpr202217-26277

***Corresponding Author**
Syeda Aiman Faheem
M. Pharmacy Dept. of Pharmaceutics

ABSTRACT

Thymoquinone (TQ) is the major active constituent in Nigella sativa and has shown to possess beneficial activities like anti histaminic, anti inflammatory and immunopentiating. Thymoquinone topical application is restricted due to its hydrophobicity, reduced aqueous solubility and permeability. So to overcome these disadvantages thymoquinone niosomes are formulated which helps in enhanced solubility and permeability. The aim of this work is the formulation and development of Thymoquinone niosomal gel and evaluation of AntiInflammatory Activity in rats. Preformulation studies shows high solubility of Thymoquinone in methanol and FTIR shows no interaction between drug and excipients, Absorption maxima of Thymoquinone in methanol was found to be 255 nm. Niosomes were prepared by thin film hydration method using different surfactants (Spans40, Span60, Span 80) with different ratio each. SEM of optimized Thymoquinone niosomes appeared as spherical, well identified, unilamellar nanovesicles. The drug content and entrapment efficiency was found to be 87.89 % & 89.58% respectively. The zeta potential of the optimised formulation was found to be -24mV and the particle size of the optimised formulation was found to be in the range of 145-150nm., The gel prepared was of good homogenecity and the evaluation parameters like spreadibility was 79.5±0.26cm, viscosity 19521±0.75 pa/s, pH =6.5, drug content was 87.89% and invitro drug release was found to be 96.78±0.29%. When release kinetics is applied it follows zero order and Higuchi model. Finally, stability studies showed that Niosomal gel prepared is more stable at 4^{o}C when compared to room temperature. Thymoquinone Niosomal gel showed good release kinetics along with good stability. Invivo studies of the 0.4% thymoquinone gel was performed and the results showed that there was a significant decrease in the paw edema when treated with

F6 formulation, initially the volume of the was 9.93±0.04mm on the 1st day and then gradually decreased to 6.56±0.04mm on the 9th day.

KEYWORDS: Thymoquinone, Niosomes, Anti-inflammatory, Topical gel etc.

INTRODUCTION

The largest of the body organs is the skin, the surface area of an average adult's skin is about $2m^2$.[1] It is one of the most easily accessible organs of the human body.[2] There are two types of human skin; one that is of the soles of foot and palms of hand without hair, and the other which bears hair and sebaceous glands such as arms and face.[3] It also varies in thickness, color, and structure.

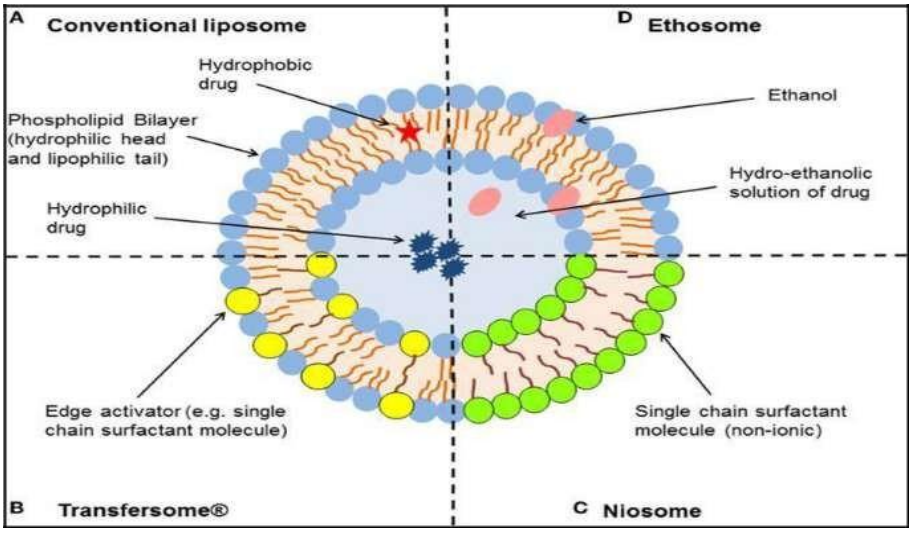
Histological classification of the skin

Histologically, the skin is categorized into three layers, namely, the epidermis, the dermis, and the hypodermis; which collectively forms a cover against an external agent and loss of water from the body.

Vesicular systems

Vesicular systems are prepared by the self-assembly of the lipids/surfactants to form the bilayers where an aqueous space is present in the core. It was first reported by Bingham et al in 1965. Vesicular systems include liposomes, niosomes, transferosomes, ethosomes etc. Drug delivery via vesicular system offer many advantages like increased solubility, high permeability, acts as a carrier for various drugs which exhibits different solubility. It also acts as drug reservoirs, allows drug targeting and control release. It prolongs the residence time of drug in the body, reduces toxicity (if selective uptake is achieved), improves bioavailability and reduces cost of therapy, delays elimination of rapidly metabolizable drug. These systems are widely used in different fields of science like immunology, membrane biology, diagnostic techniques and genetic engineering.

Different types of vesicular system like conventional liposomes, transferosomes, ethosomes and niosomes have been shown in fig.



Niosomes

Definition

Niosomes are type of vesicular drug delivery systems, where an aqueous space is entrapped within bilayer structures formed by the self assembly of non-ionic surfactants, along with the cholesterol and charge inducer. They are similar to liposome structurally and show similar *in-vitro* and *in-vivo* behavior.[53] The aqueous space is present in the core and polar groups are in contact with them, whereas the bilayer assembly of hydrophobic tail is shielded away from that.

Inflammation of the skin

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is the body's defense reaction to eliminate or reduce the spread of injurious agent, followed by removal of the necroses cells[66] and tissues. Inflammation is specified in acute phaseby increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes and inflammatory mediators such as cytokines.

In the sub acute/chronic phase it is characterized by the development of specific humoral and cellular immune responses to pathogenspresent at the site of tissue injury.

Causes of an inflammation of the skin

Many different things can cause inflammations. These are the most common:

- Pathogens (germs) like bacteria, viruses or fungi.
- External injuries like scrapes or damage through foreign
- Effects of chemicals or radiation.

Treatment

Inflammation is a natural part of the healing process. But when it becomes chronic, it's importantto get it under control to reduce your risk of long-term damage. Some of the options that have been explored for managing inflammation include

Nonsteroidal anti-inflammatory drugs (NSAIDs)

Over-the-counter NSAIDs, such as aspirin, ibuprofen (Advil), and naproxen (Aleve), effectively reduce inflammation and pain. **Steroids:** Corticosteroids are a type of steroid hormone. They decrease inflammation and suppress the immune system, which is helpful when it starts attacking healthy tissue. Butlong-term use of corticosteroids can lead to vision problems, high blood pressure, and osteoporosis. When prescribing corticosteroids, your doctor will weigh the benefitsand risks with you. **Thymoquinone from Nigella sativa was found to possess anti inflammatory and wound healing properties and reported no such side effects till date.**

Nigella sativa

The black seed (Scientific name: *Nigella sativa*; Urdu: Kalonji; Arabic: Habba-tu sawda/ Habba Al-Barakah; English: Black cumin/ Black seed; Persian: Shonaiz; Bengali: Kalajira; Hindi/Nepali: Mangrail) and its oil have an old history of traditional usage in the Indian and Arabian culture as food and medicine and have been used in the treatment of different health conditions of the digestive tract, respiratory system, kidney and liver functions, cardiovascular system, and immune system support. The Nigella sativa seed and its oil have an old history of traditional usage in the Indian and Arabian culture as food and medicine and have been used in the treatment of different health conditions of the digestive tract, respiratory system, kidney and liver functions, cardiovascular system, and immune system support. Studies reveal that the major bioactive principal constituent of Nigella sativa is Thymoquinone with a range of therapeutic benefits including antioxidant, anti-inflammatory, anti-cancer, antibacterial, antifungal activity and anticonvulsant activity. Immunomodulatory effects of N. sativa have also been reported.

Also, several investigations reported the antiviral effect of the black seed.

Thymoquinone

Thymoquinone is the major bioactive principal constituent of Nigella sativa. Thymoquinone (TQ), a principal constituent of the volatile oil of Nigella sativa (NS) seeds, has been shown

to produce multiple health beneficial activities, which include antihistaminic, antibacterial, antihypertensive, hypoglycaemic, antiinflammatory, immunopotentiating and antiarthritic actions. Recently, Nader, El-Agamy, and Suddek (2010) showed hypolipidemic and antioxidantproperties of TQ in cholesterol fed rabbits. Woo et al. (2011) reported that TQ has anticarcinogenic activity by increasing PPAR-c activity and down-regulating the expression of the genes for Bcl-2, Bcl-xL and survivin in breast cancer cells. Regarding dermato-pharmacological actions, TQ exerts antifungal, antibacterial, antineoplastic, and anti-inflammatory actions. The significant anti-inflammatory effect of TQ may be attributedto the in vivo attenuation of the expression of cyclooxygenase-2 (COX-2) enzyme and the invivo induction of several cytoprotective enzymes like hemeoxygenase-1, glutathione-Stransferase, and glutamate cysteine ligase in mouse skin. TQ can inhibit 5- lipooxygenase (LOX)enzyme and COX-2 enzyme-induced arachidonic acid metabolism in rat peritoneal leukocytes. TQ application is restricted due to its hydrophobicity, reduced aqueous solubility and thermal and photo degradation.

List of materials

Thymoquionone, cholesterol, span 40,span 60, span 80, chloroform, methanol, sodium chloride, potassium chloride, Di- basic sodium phosphate, Monobasic sodium phosphate, Carbopol 934.

List of equipments

Rotatry evaporator, digital balance, digital Ph meter, UV/Visible Spectrophotometer, FTIR Spectrophotometer, Magnetic stirrer, Centrifuge, Brookfield Viscometer, Probe Sonicator.

UV Spectroscopic analysis of the drug

Determination of absorption maxima of thymoquinone

100 mg of Thymoquinone was dissolved in 100 ml of methanol to produce stock solution of 1mg/ml (1000 µg/ml). From the stock solution, 10 ml was taken and further diluted to 100 ml with methanol. The prepared solution was scanned in a wavelength range of 190-400 nm, to findthe maximum absorbance.[110]

Preparation of calibration curve of thymoquinone in methanol

Preparation ofstock solution

Accurately weighed amount of 100 mg of drug should be transferred to 100 ml volumetric flask, and the volume is made up to 100 ml with methanol. The resultant solution had the

concentrationof 1000mcg/ml which is labeled as stock.

Preparation of working standard solution

From this stock solution, 1ml is taken and diluted to 100ml with methanol which has given thesolution having the concentration of 100 µg/ml.

Preparation of serial dilutions for standard calibration curve

Required dilutions are to be made by using the second solution to obtain the different concentrations of Thymoquinone (2,4,6,8,10 and 12 mcg/ml) solutions. The absorbances of above solutions are recorded at λmax (255 nm) of the drug using double beam UV Visible Spectrophotometer. Standard graph is plotted between the concentration (x-axis) and absorbance(y-axis)

Method of preparation of thymoquinone niosomes

Thin-Film hydration method

Thin film hydration method is one of the most widely used methods for the preparation of liposomes. This method can also be used in the formulation of niosomes. It is a simple method which involves dissolving the membrane –forming materials in an organic solvent in a flask. Then after removing the organic solvent by vaccum evaporation, a layer of dried thin filmforms inside the flask. Then the drug is dissolved in aq. solution such as water or buffer and then added to hydrate the dry film. It is incubated above the transition temperature ofthe surfactant in a water bath to form niosomes. Niosomes prepared by this method are multilamellar vesicles.

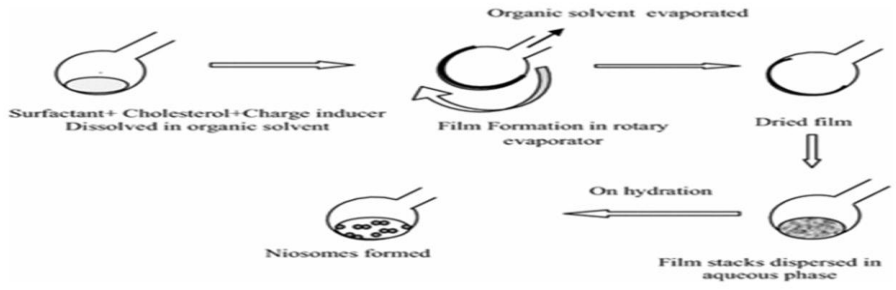


Fig. no. 3.11: Preparation of niosomes by thin film hyration technique.

The following is the formulation chart of the preparation.

Table 3.3: Formulation chart for thymoquinone niosomes.

Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)	F7 (mg)	F8 (mg)	F9 (mg)
Drug	100	100	100	100	100	100	100	100	100
Cholesterol	50	50	50	50	50	50	50	50	50
Span 40	50	75	100	-	-	-	-	-	-
Span 60	-	-	-	50	75	100	-	-	-
Span 80	-	-	-	-	-	-	50	75	100
Methanol(ml)	20	20	20	20	20	20	20	20	20
Phosphate buffer (6.8pH)	20	20	20	20	20	20	20	20	20

Preparation of niosomes

Formation of thin film using Rotary evaporator

Sonication of formulation

Method of preparation of niosomal gel

The niosomes were formulated into gel for the ease in application. Carbopol 934 was dispersed in water to prepare 1% w/w dispersion. The dispersion was mechanically stirred and then neutralized with 0.5% v/v triethanolamine solution (0.5%). The neutralized dispersion was kept overnight to remove any entrapped air. Finally, Niosomes were then added to thedispersion.

Evaluations parameters of niosomal gel

Homogeneity

Homogeneity of the developed gel was tested for by visual inspection by pressing smallquantity of the gel between the thumb and the index finger. The gel was tested for its appearanceand presence of any aggregates. The consistency was determined as homogeneous or not.

Spreadability

Two glass slides of 20 cm × 20 cm were selected. A small amount of sample was sandwiched between the two glass slides. A 100 g weight was placed on the upper slide so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed andthen fixed to a stand without slightest disturbance in such a way that the upper slide slides off freely, to the force of weight tied to it. The time taken for the upper slide to separate away from the lower one was noted using a stop clock. This parallel plate method is the most widely used method for determining and quantifying the spreadability of semisolid preparations. Simplicity and relative lack of expense are the advantages of this method. The following equation was usedfor this purpose:

$S = m \times L/T$

Where,

S - Spreadability

m - Weight tied to the upper slide l - Length of the glasst - Time taken in seconds

Viscosity

V**i**scosity of the niosomal gel was measured using Brookfield viscometer (Brookfield DV-E viscometer) using spindle number S64 rotated at a speed of 12 rpm for a 10-s run time at 37°C.

Measurement of pH

One gram of gel was dispersed in 20 mL of distilled water, and a digital pH meter (Systronics Digital- 335) was used to determine the pH value. The measurement was performed three timesand the mean ± SD was calculated

Drug content

1 gm of gel was dissolved in a 100 ml of phosphate buffer pH 6.8. The resultant solution was filtered and drug content was analyzed spectrophotometrically.

In vitro drug release studies

A diffusion study of Thymoquinone loaded Niosomal gel was carried out using Franz diffusion cell through the dialysis membrane. Dialysis membrane was soaked in distilled water for 24 hours. The receptor compartment was filled with 6.8 pH and donor compartment contain 1 g of Niosomal gel (equivalent to 5 mg) on dialysis membrane with exposure area of

2cm^{2}to receptor.

Medium and whole assembly was kept on magnetic stirrer at 600 rpm for a period of 10 hours and samples were withdrawn at specified time interval of 1 hr and replaced with equal volume of buffer. Samples were appropriately diluted with buffer and analyzed using UV spectrophotometer at 255 nm. Steady state Flux (Jss) was calculated from the slope of the linear part of the cumulative amount of drug permeated per unit area (µg/cm2) against a time (h) plot. Permeability coefficient (Kp) =Jss/Co, (Co = initial Thymoquinone concentration).

In vivo animal studies

a) Species and strain: Albino wister rats
b) Age and weight: 150- 200 grams
c) Gender: Either gender
d) Number to be used – 12
e) Number of days each animal will be housed: 2 months.

Carageenan induced rat paw edema

- Animals are fasted for 24 hrs, before the experiment with free access of water. Approximately 50 µl of a 1% suspension of carrageenan in saline is prepared 1hr before each experiment and is injected into the plantar side of the right hind paw of the rat.
- 0.2g of herbal gel containing 1% Niosomal extract is applied to the plantar surface of the hind paw by gently rubbing 50 times with the index finger.
- Rats of the control received the plain gel base and 0.2g 1% Aceclofenac gel applied in the same way as the standard.
- Drugs or placebo were applied 1h before the carrageenan injection.
- Paw volume was measured immediately after carrageenan injection at 1,2,3 and 4 hrs intervals after the administration of the noxious agent by using plethysmometer.

RESULTS AND DISCUSSIONS

Physicochemical properties of the thymoquinone

- Color: Pale yellow
- Melting point: 46˚C

Melting point study: The reference melting point of Thymoquinone was in the range of 44-47˚C and practicallyobserved melting point was 46˚C.

Solubility study: The solubility of pure Thymoquinone was checked, it was found to be

soluble inethanol and methanol

FT-IR Spectroscopic analysis

The FTIR spectra observed that the characteristic absorption peaks of pure Thymoquinone were obtained at 2854, 1658, 1058 cm-1 corresponding to C-H, C=C, C=O. Following the obtained spectrum given above, it was observed that there were no considerable changes in the characteristic peaks observed in the pure drug spectrum whenmixed with excipients. This indicates that the drug was molecularly dispersed in the excipients thereby indicating the absence of any interaction.

Interpretation of FTIR of Pure drug

Table 4.3

S. No	Functional group	Frequency	Observed group
01.	C-H	3000-2800	2968
02.	C=C	1600-1650	1643
03.	C=O	1500-1000	1249

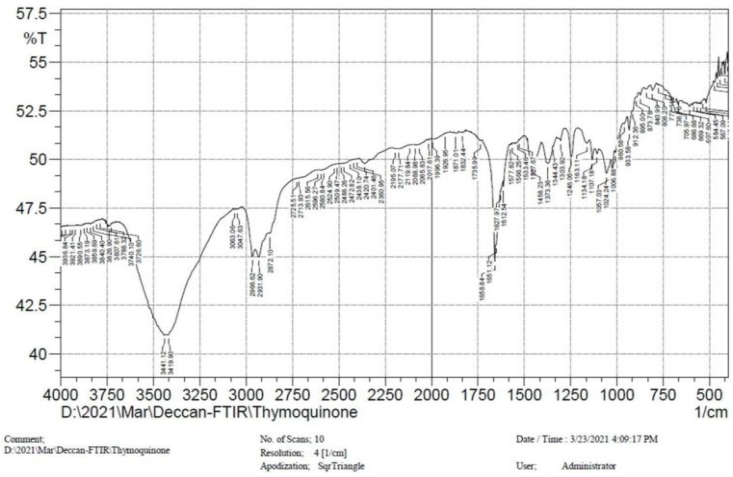


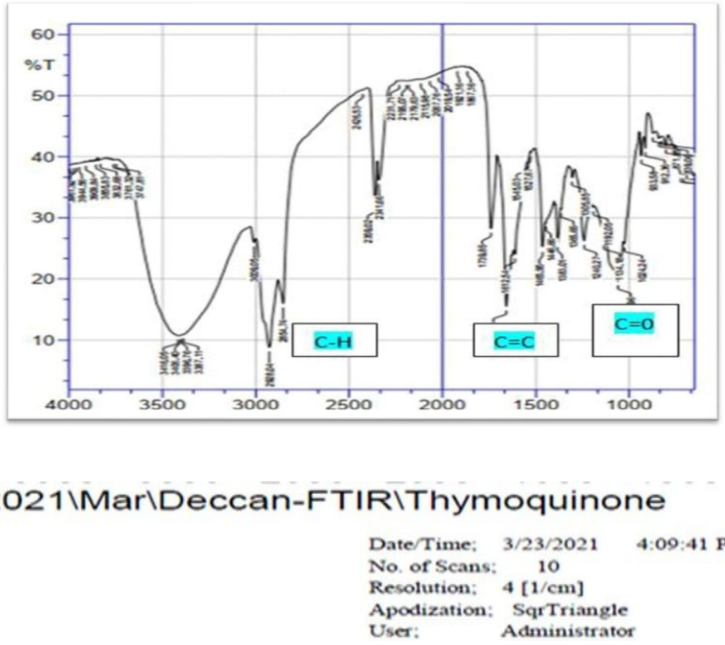
Figure 4.1 FTIR of pure drug.

The FTIR spectra observed that the characteristic absorption peaks of pure Thymoquinone were obtained at 2968, 1643, 1249 cm-1 corresponding to C-H, C=C, C=O.

Interpretation of FTIR Pure drug with excipients

Table 4.4

S. No.	Functional group	Frequency	Observed group
01.	C-H	3000-2750	2854
02.	C=C	1750-1500	1658
03.	C=O	1250-900	2854



UV-Spectroscopy - Analysis of drug

Determination of ƛmax of thymoquinone in methanol by uv.

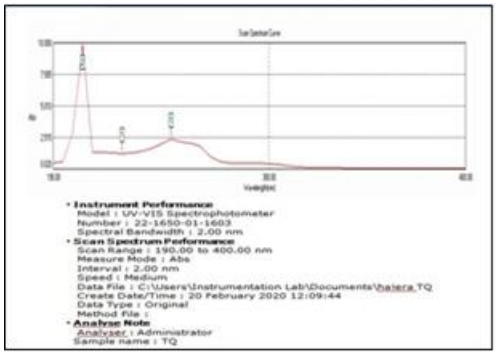
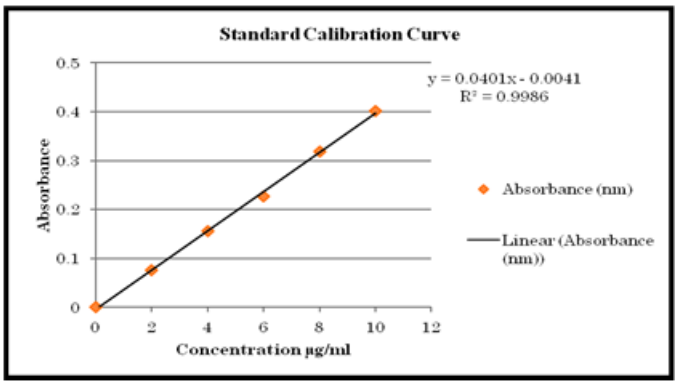


Fig. 4.3: ƛ max of Thymoquinone in methanol.

Standard calibration curve of thymoquinone

Table 4.5: Standard calibration curve of thymoquinone.

S. No	Concentration (µg/ml)	Absorbance at 255nm
1.	0	0
2.	2	0.076 ± 0.01
3.	4	0.156 ± 0.01
4.	6	0.226 ± 0.03
5.	8	0.318 ± 0.01
6.	10	0.402 ± 0.02



Preparation of thymoquinone niosomes

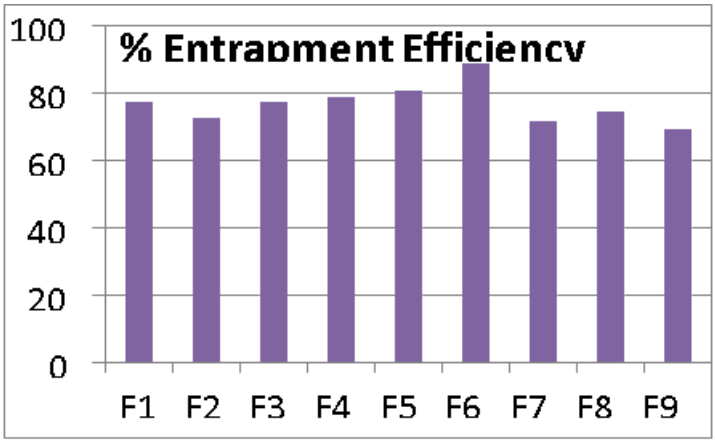
The Niosomes of Thymoquinone were prepared using thin film method. Accurate amounts of surfactant, cholesterol and drug were dissolved in a mixture of organic solvents consisting chloroform: methanol (2:1 v/v) in a dry, round bottom flask. The organic solvent was allowed to evaporate in the rotary evaporator adjusted to 60 rpm, at 40˚C for 15 mins under low pressure to prepare a thin film on the wall of the round-bottom flask.

The film was subjected to hydration with phosphate buffer pH 6.8 by rotation for 1 hr at 60 rpmat room temperature. The multilamellar lipid vesicles (MLVs) were then sonicated using the ultrasonic probe Sonicator for 30 min to reduce the vesicle size and stored at 4°C for further investigation.

Characterisation of thymoquinone phytosomes

Table 4.6

Formulationcode	% entrapmentefficiency (% w/w)
F1	77.36
F2	72.57
F3	77.12
F4	78.38
F5	80.67
F6	89.58
F7	71.43
F8	74.27
F9	69.15



Invitro drug release

Table no. 4.7

Time (hr)	F1% CDR	F2% CDR	F3% CDR	F4% CDR	F5% CDR	F6% CDR	F7% CDR	F8% CDR	F9% CDR
0	0	0	0	0	0	0	0	0	0
1	13.2± 0.45	15.24± 0.24	17.43± 0.32	18.45± 0.45	19.34± 0.45	21.03± 0.13	12.11± 0.04	14.75± 0.45	16.89± 0.76
2	21..32± 0.70	28.27± 0.46	23.42± 0.60	22.45± 0.23	24.67± 0.34	29.56± 0.45	20.50± 0.56	25.67± 0.78	27.67± 0.45
3	34.41± 0.65	37.38± 0.28	33.51± 0.57	38.21± 0.41	33.78± 0.90	38.56± 0.67	34.23± 0.78	31.27± 0.90	32.43± 0.34
4	40.63± 0.37	42.76± 0.54	42.21± 0.43	41.52± 0.43	41.80± 0.34	50.78± 0.86	44.26± 0.57	46.43± 0.34	46.± 0.23
6	49.58± 0.58	56.74± 0.74	49.35± 0.56	59.91± 0.18	53.67± 0.43	69.23± 0.34	58.78± 0.35	58.45± 0.21	57.90± 0.45
8	54.43± 0.76	67.71± 0.48	54.67± 0.45	67.50± 0.26	67.79± 0.56	72.45± 0.57	64.34± 0.23	63.63± 0.45	64.± 0.56
10	63.70± 0.17	72.13± 0.13	67.80± 0.89	74.89± 0.69	78.35± 0.35	88.48± 0.23	76.69± 0.12	71.23± 0.34	78.45± 0.78
12	78.46± 0.21	81.12± 0.62	88.54± 0.41	80.46± 0.34	84.89± 0.67	96.78± 0.29	82.49± 0.67	89.78± 0.12	90.34± 0.89
0	0	0	0	0	0	0	0	0	0
1	13.2± 0.45	15.24± 0.24	17.43± 0.32	18.45± 0.45	19.34± 0.45	21.03± 0.13	12.11± 0.04	14.75± 0.45	16.89± 0.76
2	21..32± 0.70	28.27± 0.46	23.42± 0.60	22.45± 0.23	24.67± 0.34	29.56± 0.45	20.50± 0.56	25.67± 0.78	27.67± 0.45
3	34.41± 0.65	37.38± 0.28	33.51± 0.57	38.21± 0.41	33.78± 0.90	38.56± 0.67	34.23± 0.78	31.27± 0.90	32.43± 0.34

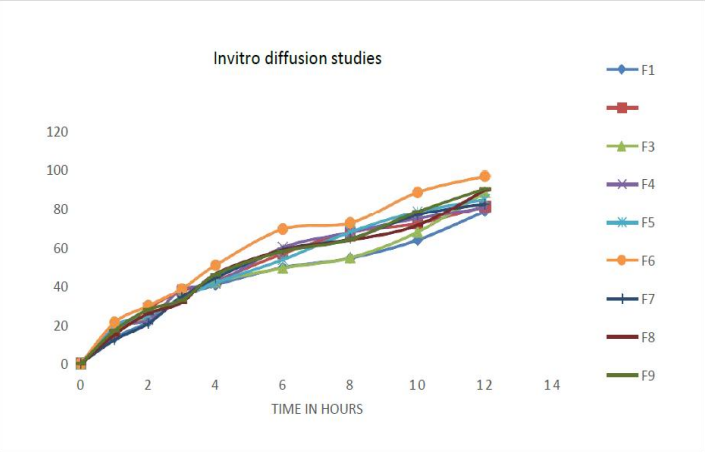


Figure 4.6: Invitro diffusuion studies.

Particle size Distribution and Zeta potential determination

202103230427003.nsz

Measurement Results

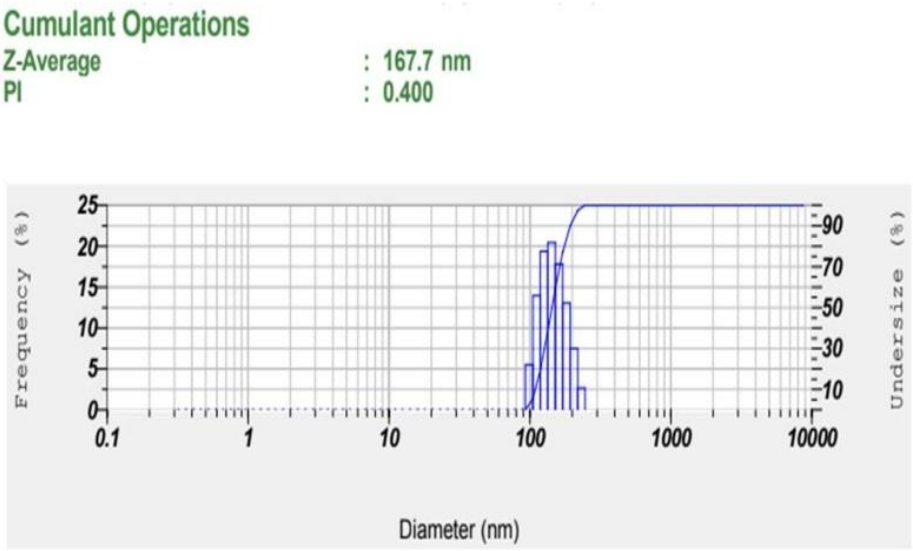
Date	:	23 March 2021 04:27:07
Measurement Type	:	Particle Size
Sample Name	:	NF1-Size
Scattering Angle	:	173
Temperature of the holder	:	25.0 deg. C
T% before meas.	:	7
Viscosity of the dispersion medium	:	0.894 mPa.s
Form Of Distribution	:	|Standard|
Representation of result	:	Scattering Light Intensity
Count rate	:	3347 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	148.2 nm	32.1 nm	141.9 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	148.2 nm	32.1 nm	141.9 nm

Histogram Operations

Size (Median)	:	143.5 nm
Mode	:	141.9 nm
% Cumulative (1)	:	10.0 (%) - 109.4 (nm)
% Cumulative (2)	:	50.0 (%) - 143.5 (nm)
% Cumulative (3)	:	90.0 (%) - 193.8 (nm)
% Cumulative (4)	:	30.0 (%) - 127.0 (nm)
% Cumulative (5)	:	40.0 (%) - 135.2 (nm)
% Cumulative (6)	:	50.0 (%) - 143.5 (nm)
% Cumulative (7)	:	20.0 (%) - 119.2 (nm)
% Cumulative (8)	:	70.0 (%) - 163.3 (nm)
% Cumulative (9)	:	95.0 (%) - 210.3 (nm)
% Cumulative (10)	:	100.0 (%) - 8510.6 (nm)



Zeta potential of Thymoquinone loaded niosomes of formulation showed good stability.

Formulation of thymoquinone niosomal gel

The thymoquinone niosomal gel was prepared using 1% Carbapol 934 as a gelling agent. Theconcentration of Thymoquinone in the prepared Niosomal gels was 0.4% w/w.

Evaluations of thymoquinone niosomal gel

0.4% thymoquinone niosomal gel:

Evaluations	Results
Homogenecity	Good
Spreadibility (cm)	79.5±0.26
Viscosity (pa/s)	19521±0.75
pH measurements	6.5±0.47
Drug content %	94.4±1.66 %,
Invitro drug release	95.4 ±0.36

Invivo studies

S. no.	Group	No. Ofrats	Treatment
1	Standard	6	Standard gel
2	Test	6	Test gel

Grouping of animals

The body weight of the animals which received the treatment are given in the table below:

Grouping of animals	Body weight with paw (gm)
F1	183±0.04
F2	188±0.05
F3	203±0.08
F4	182±0.06
F5	194±0.03

F6	193±0.05
F7	185±0.04
F8	193±0.08
F9	186±0.04

Changes in the measurements of Rat's paw edema

Table 4.16: Changes in the measurements of Rat's paw edema.

Groups	1st day	2nd day	4th day	6th day	8th day	9th day
F1	9.66±0.04	8.81±0.04	8.23±0.04	7.75±0.04	7.73±0.04	7.5±0.04
F2	9.54±0.04	9.48±0.04	9.43±0.04	9.3±0.04	9.02±0.04	8.08±0.04
F3	9.31±0.04	9.05±0.04	8.99±0.04	8.8±0.04	8.67±0.04	8.39±0.04
F4	9.93±0.04	9.6±0.04	9.27±0.04	8.75±0.04	8.11±0.04	7.84±0.04
F5	9.81±0.04	9.12±0.04	9.1±0.04	8.61±0.04	8.29±0.04	8.05±0.04
F6	9.93±0.04	8.8±0.04	8.77±0.04	8.58±0.04	6.89±0.04	6.56±0.04
F7	9.58±0.04	9.54±0.04	9.01±0.04	7.96±0.04	7.92±0.04	7.67±0.04
F8	9.96±0.04	9.08±0.04	7.85±0.04	7.83±0.04	7.74±0.04	7.73±0.04
F9	9.71±0.04	9.57±0.04	9.5±0.04	9.14±0.04	8.92±0.04	8.16±0.04
SD	0.2172	0.3239	0.5484	0.5610	0.6732	0.5320
SE	0.0724	0.1079	0.1828	0.1870	0.2244	0.1773

From the above instances we have concluded that the test group receiving the thymoquinone niosomal gel showed 70-80% healing where as the standard group receiving the treatment with the Aceclofenac standard gel of the showed almost 100% healing of the rat paw.

4. CONCLUSION

The aim of the study is to formulation of Thymoquinone Niosomal gel and evaluation of its Anti inflammatory activity in rats. Thymoquinone has numerous therapeutic effects including the anti- inflammatory effect. Preformulation studies shows high solubility of Thymoquinone in methanol and FTIR shows no interaction between drug and excipients, Absorption maxima of Thymoquinone in methanol was found to be 255 nm.

Niosomes were prepared by thin film hydration method using different surfactants (Spans40, Span60, Span 80) with different ratio each. SEM of optimized Thymoquinone niosomes appeared as spherical, well identified, unilamellar nanovesicles. The drug content and entrapment efficiency was found to be 87.89 % & 89.58% respectively. The zeta potential of the optimised formulation was found to be -24mV and the particle size of the optimised formulation was found to be in the range of 145-150nm., The gel prepared was of good homogenecity and the evaluation parameters like spreadibility was 79.5±0.26cm, viscosity 19521±0.75 pa/s, pH =6.5, drug content was 87.89%and invitro drug release was found to be 96.78±0.29%.

When release kinetics is applied it follows zero order and Higuchi model, it was found that drug release follows Quasi fickian mechanism. Finally, stability studies showed that Niosomal gel prepared is more stable at $4^{o}C$ when compared to room temperature. Thymoquinone Niosomal gelshowed good release kinetics along with good stability.

Invivo studies of the 0.4% thymoquinone gel was performed and the results showed that there was a significant decrease in the paw edema when treated with F6 formulation, initially the volume of the was 9.93±0.04mm on the 1^{st} day and then gradually decreased to 6.56±0.04mm on the 9^{th} day.

REFERENCES

1. Watkinson A. 'A commentary on transdermal drug delivery systems in clinical trials', J. Pharm. Sci, 2013; 102: 3082-3088.
2. Keleb E, Sharma RK, Mosa E and Aljahwi A. 'Transdermal drug delivery system- design and evaluation', Int. J. Adv. Pharm. Biol. Sci, 2010; 1: 201-211.
3. McGrath JA, Eady RAJ and Pope FM. 'Anatomy and organization of human skin', Rook's textbook of Dermatology, Blackwell Science Ltd, Oxford, 2008; 8: 3.1-3.84
4. Andrews SN, Jeong E and Prausnitz MR. 'Transdermal delivery of molecules is limited byfull epidermis, not just stratum corneum', Pharm. Res, 2013; 30: 1099-1109.
5. Pathan IB and Setty CM. 'Chemical penetration enhancers for transdermal drug delivery system', Trop J Pharm Res, 2009; 8: 173-179.
6. Ansel HC, Popovich NG and Allen LV. Pharmaceutical dosage forms and drug delivery systems. Lea & Febiger, Philadelphia, 199.
7. Sajid A, Maryam S, Nabeel S. 'The Structure of Skin and Transdermal Drug Delivery System- A Review', Research J. Pharm. and Tech, 2015; 8: 103-109.
8. Igarashi T, Nishino K and Nayar SK. 'The appearance of human skin: a survey', Foundations and Trends in Computer Graphics and Vision, 2007; 3: 1-85.
9. Elias PM. 'Epidermal lipids, barrier function, and desquamation', J Invest Dermatol, 1983; 80: 44S – 9S.
10. Elias PM, Menon GK. 'Structural and lipid biochemical correlates of the epidermal permeability barrier', Adv Lipid Res, 1991; 24: 1 – 26.
11. Prausnitz MR, Elias PM, Franz TJ, Schmuth M, Tsai J, Menon GK, Holleran WM and Feingold KR. 'Skin Barrier and Transdermal Drug Delivery', 2012; 2065-2073.
12. Kolarsick P, Kolarsick M, and Goodwin C. 'Anatomy and Physiology of the Skin' J

Dermatol Nurses Assoc, 2011; 3.

13. Derrickson T. Principles of Anatomy and Physiology, Chapter 5, Atlas and Registration Card, 2005; 11: 157-158.
14. Alkilani AZ, McCrudden MT and Donnelly RF. 'Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the stratumcorneum', Pharmaceutics, 2015; 7: 438-470.
    Shirsand SB, Ladhane GM, Prathap S and Prakash PV. 'Design and evaluation of matrix transdermal patches of meloxicam', J Pharm Sci, 2012; 2(4): 58-65.
15. Chien YW and Banga AK. 'Iontophoretic (transdermal) delivery of drugs: overview of historical development'. J Pharm Sci, 1989; 78: 353-354.
16. Shingade GM, Quazi A, Sabale PM, Grampurohit ND, Gadhave MV, Jadhav SL, and Gaikwad D. 'Review on: Recent trend on Transdermal drug delivery system' J. drug deliv. Ther, 2012; 2: 66-75.
17. Bendas ER and Tadros MI. 'Enhanced transdermal delivery of salbutamol sulfate via ethosomes', Pharm Sci Tech, 2007; 8: 107.
18. Arora N, Singh K, Garg T. Areas of nanomedicine applications. Int J Univ Pharm Life Sci, 2012; 2: 216–27. [Google Scholar]
19. Barker DJ, Bercovicz D, Servilio LC, et al. Rat ultrasonic vocalizations demonstrate that the motivation to contextually reinstate cocaine-seeking behavior does not necessarily involve a hedonic response. Addict Biol.[Epub ahead of print], 2013. doi: 10.1111/adb.12044 [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
20. Basnet P, Hussain H, Tho I, Skalko-Basnet N. Liposomal delivery system enhances anti-inflammatory properties of curcumin. J Pharm Sci, 2012; 101: 598–609. [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
21. Bei D, Meng J, Youan BB. Engineering nanomedicines for improved melanoma therapy: progress and promises. Nanomedicine (Lond), 2010; 5: 1385–99. [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
22. Brown L, Langer R. Transdermal delivery of drugs. Annu Rev Med, 1988; 39: 221–9. [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
23. Cao J, Guo LH, Wan B, Wei Y. In vitro fluorescence displacement investigation of thyroxine transport disruption by bisphenol A. J Environ Sci (China), 2011; 23: 315– 21. [Crossref], [PubMed], [Web of Science ®], [Google Scholar]
24. Chen M, Gupta V, Anselmo AC, et al. Topical delivery of hyaluronic acid into skin using SPACE-peptide carriers. J Control Release, 2014; 173: 67–74. [Crossref], [PubMed],

[Web of Science®], [Google Scholar]

25. Csizmazia E, Berko S, Maroda M, et al. [Modelling of pecutaneous drug permeation and investigation of penetration enhancer effect]. Acta Pharm Hung, 2012; 82: 15–22. [PubMed], [Google Scholar]
26. Degano P, Schneider J, Hannan CM, et al. Gene gun intradermal DNA immunization followed by boosting with modified vaccinia virus Ankara: enhanced CD8+ T cell, 1999.