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FORMULATION DEVELOPMENT AND EVALUATION OF THYMOQUINONE LOADED TRANSFERSOMAL GEL

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Abstract: Transfersomes are particularly optimized, ultra deformable (ultra-flexible) lipid supramolecular aggregates, which can penetrate the mammalian skin intact. Transferosome is a type of carrier system capable of transdermal delivery of low and high-molecular-weight drugs. Transfersomes penetrate through the pores of the stratum corneum which are smaller than its size and get into the underlying viable skin in intact form. Currently, there are many marketed formulations available, but herbal formulations containing transfersomes will have their own advantages in terms of solubility, enhanced bioavailability, increased pharmacological action, stability of the active drug, and fewer side effects. Herbal drugs have a wide range of pharmacological activities, but the use of this herbal drugs is limited due to its poor stability and poor penetration through the skin. This NDDS approach with herbal phytoconstituent will be beneficial in enhancing therapeutical activity in topical and transdermal preparations. The aim of the present study was to formulate and evaluate the vesicular formulations (Transfersomes) containing Thymoquinone as an active phytoconstituent.

Keywords: transfersomes, thymoquinone (TMQ), transdermal drug delivery system, gel.

INTRODUCTION

Drug delivery is the method or process of administering a pharmaceutically active compound to achieve a therapeutic effect in humans. As developed solutions for the targeted delivery and/or controlled release of therapeutic substances into the body, drug delivery systems fall under this definition. Because of its excellent effectiveness, transdermal medication delivery systems have attracted a lot of attention recently. Transdermal administration has been suggested as a superior alternative to attain stable plasma levels for prolonged periods of time. This method has the potential to be more favorable because it requires less frequent dosing regimens.[1]
Transfersomes is an ultra-deformable vesicle possessing an aqueous core surrounded by complex lipid bilayer, because of their self-optimized and ultra-flexible membrane properties, they are able to deliver the drug reproducibly either into or through the skin depending on the choice of administration or application, with high efficiency. They consist of a bilayer structure that makes it easier to encapsulate drugs that are hydrophilic, lipophilic, and amphiphilic with higher penetration efficiencies than traditional liposomes. [6]
Transfersomes escape the obstacle of skin penetration by squeezing along the intracellular sealing lipid of stratum corneum. Transfersomes bypass the skin barrier by creating extracellular channels within the organ's cells and then bending to fit through them. Plant-derived bioactive chemicals form herbal medications. Chemically, thymoquinone, a monoterpene molecule, is 2-methyl-5-isopropyl-1, 4-benzoquinone. It is found extensively in the seeds of Nigella sativa L., a member of the Ranunculaceae family and commonly referred to as black cumin or black seed.[7]
The pharmacological effects of thymoquinone (TMQ) are numerous and include antioxidant, anti-hyperglycaemic, anti-inflammatory, anti-histaminic, immunomodulatory, and anticancer activities. Additionally, it has antioxidant, antiviral, antibacterial, and hepatoprotective properties. Although thymoquinone (TMQ) offers an acceptable and broad spectrum of pharmacological activity, its hydrophobicity, poor aqueous solubility, sensitivity to light, and pH make it difficult to deliver to the target site. Transfersomes have been researched to help with these TMQ delivery issues. A feasible way of administration for a range of therapeutic indications is transdermal delivery of drugs through the skin to the systemic circulation. One of the simplest and

easiest ways to administer drugs to the body is through topical distribution. These pathways are thought to be advantageous since they avoid the first pass metabolism.[7]

MATERIALS AND METHOD

Materials: Thymoquinone phytoconstituent was purchased from Yucca Enterprises, Mumbai, Maharashtra, India. LECIVA S80 was provided as a gift sample from VAV Lipids Private limited, Nariman point, Mumbai, Maharashtra, India. Sodium cholate was purchased by and Carbopol 974- were purchased. Chloroform and ethanol were purchased by MolyChem Mumbai, Maharashtra, India. Distilled water was used to prepare a buffer of pH 7.4.

Pre-formulation studies: Pre-formulation studies are an important tool for determination of physical and chemical properties of the drug before incorporating it in formulation development programmed.

- **Physical appearance**

The drug (thymoquinone) powder was examined for its organoleptic properties like color, and odour.

- **Melting point determination**

It is one of the parameters for the purity of drugs. Melting point was determined by using basic capillary method. The sample is placed in capillary tube with Thiele Tube containing liquid paraffin Oil and heated slowly. Melting point was recorded using thermometer.

- **Solubility studies:**

Saturation solubility study profile of drug was determined in various organic solvents like ethanol, methanol, DMSO, chloroform and isopropyl alcohol.

- **Determination of Wavelength Maxima:**

In a 10 ml volumetric flask, 10 mg of precisely weighed drug was dissolved in 10 ml of phosphate buffer, pH 7.4, then 1 ml of this stock solution was pipetted into a 10 ml volumetric flask and volume made up to the mark with phosphate buffer pH 7.4. Once again, 1 ml of the previous solution was pipette out, and volume was adjusted to the required level with phosphate buffer pH 7.4 to create 10 ppm solution. With the use of a UV/Vis double beam spectrophotometer, the final solution was scanned.

Preparation of calibration curve of Thymoquinone

(a) Calibration curve in Methanol:

Accurately weighing 10 mg of thymoquinone, it was dissolved in 10 ml of methanol in a 10 ml volumetric flask, and volume was made up to 10ml with methanol. To get 100 g/ml, 1 ml of this solution was diluted with methanol and added to a 10 ml volumetric flask. Aliquot of 1ml of this solution was withdrawn and transferred to 10 ml volumetric flask and diluted to 10ml with methanol to obtain a stock solution of 10µg/ml. From this stock solution, aliquots of 2ml, 4ml, 6ml...10ml were transferred to 10ml volumetric flasks and volume was made up to 10ml with methanol and was scanned using UV/Vis double beam spectrophotometer at obtained maximum wavelength. The calibration curve was obtained by plotting the absorbance of Thymoquinone versus the concentration of Thymoquinone. The straight line of best fit was obtained by using linear regression analysis program.

(b) Calibration curve in phosphate buffer pH 7.4:

Accurately weighing 10 mg of thymoquinone, it was dissolved in 10 ml of phosphate buffer pH 7.4 in a 10 ml volumetric flask, and volume was made up to 10ml with methanol. To get 100 g/ml, 1 ml of this solution was diluted with phosphate buffer pH 7.4 and added to a 10 ml volumetric flask. Aliquot of 1ml of this solution was withdrawn and transferred to 10 ml volumetric flask and diluted to 10ml with phosphate buffer pH 7.4 to obtain a stock solution of 10µg/ml. From this stock solution, aliquots of 2ml, 4ml, 6ml...10ml were transferred to 10ml volumetric flasks and volume was made up to 10ml with phosphate buffer pH 7.4 and was scanned using UV/Vis double beam spectrophotometer at obtained maximum wavelength. The calibration curve was obtained by plotting the absorbance of Thymoquinone versus the concentration of Thymoquinone. The straight line of best fit was obtained by using linear regression analysis program.

- **FTIR spectrum of Thymoquinone:**

The Fourier Transform Infrared Spectrophotometer was used to record the IR spectra of the drug using the KBr technique. Using dried potassium bromide pellets, a baseline correction was made. The 3-5 mg of drug and 100-150 mg of potassium bromide were crushed together in a KBr pellet press machine to create the potassium bromide-drug pellet, which had a diameter of around l mm. Infrared radiation was used to scan the sample pellet at wavelengths ranging from 4000 cm-1 to 400 cm-1.

• **DSC of drug**

Drug-excipient compatibility was determined using DSC of the drug. The thermal analysis was carried out between 30 ºC and 300ºC at a heating rate of 100 ºC /min in a nitrogen environment.

Selection of method of preparations of transfersomes: There are various reported method that are used for preparation of transfersomes. For selecting appropriate method of preparation for TMQ Transfersomes, preliminary trial batches were taken.

Trail batches:

Table no.1. Trail batches of different method.

Method	Drug	Lipid: surfactant	Organic solvent
Vortex sonication method	10mg	400:25	IPA
Modified hand shaking method	10mg	200:13	IPA
Ethanol injection method	10mg	200:25	Ethanol
Thin film hydration method	10mg	200:100	Chloroform: ethanol

FORMULATION AND DEVELOPMENT OF TMQ TRANSFERSOMES

Preparation of transfersomes: Conventional thin layer evaporation followed by sonication technique was used to prepare TMQ Transfersomes. 10 mg of thymoquinone, 25-100 mg of leciva S80 and 5-15 mg of 4 sodium deoxy cholate were dissolved in 10 mL of chloroform: ethanol mixture (1:1, v/v) in a round bottom flask. The thin lipid film was obtained after drying the solvent at room temperature under reduced pressure on a rotary evaporator. The deposited lipid film was kept in desiccator for 24 h to remove residues of organic solvent. The deposited lipid film hydrated with 10 mL of phosphate buffer of pH 7.4 by rotation at 150 rpm for 40 min at 37 ± 1 °C. The resulting vesicles were swollen for 2 h at room temperature to form a suspension. The suspension was sonicated for 10 min with a titanium probe ultrasonicator to produce transfersomes.

Selection of factors:

Table no.2. Factors for optimization.

Sign	Factors	Low (-)	High (+)
A	**Total lipid content**	**100mg**	**200mg**
B	**Concentration of Surfactant**	**50mg**	**100mg**
C	**RPM**	**120**	**130**
D	**Temperature**	**36^0C**	**38^0C**
E	**Hydration time**	**25 min**	**35 min**
F	**Sonication time**	**5min**	**10 min**
G	**Dummy**	**-**	**-**

Experimental design:

Plackett Burman Design:

Table no.3. Plackett-Burman Design for trial experiments

Run	A	B	C	D	E	F	G
1	+	-	-	+	-	+	+
2	+	+	-	-	+	-	+
3	+	+	+	-	-	+	-
4	-	+	+	+	-	-	+
5	+	-	+	+	+	-	-
6	-	+	-	+	+	+	-
7	-	-	+	-	+	+	+
8	-	-	-	-	-	-	-

Optimization:
Results based on plackett burman batches optimized batch was selected. To check repeatability of selected batch, selected batch was repeated thrice and evaluated

EVALUATION OF TRANSFERSOMES

- **Determination of Particle size, Polydispersity Index and zeta potential of transfersomal dispersions.**

The polydispersity index (PDI) and the mean diameter of the optimized vesicles were measured in triplicate using the dynamic light scattering technique and a Horiba Nano Partica instrument (SZ-100, Japan). Prior to PDI assessment, a little amount of the optimized transfersomal sample was combined with 10 ml of double distilled water and sonicated for 5 minutes. The same device was used to measure the zeta potential in triplicate using the laser Doppler electrophoresis method at 25 0C.

- **Determination of drug entrapment efficiency of thymoquinone transfersomes.**

The amount of free, unentrapped drug present in the aqueous medium was measured to evaluate entrapment efficiency. The centrifuge tubes were filled with the drug-loaded transfersomes dispersion, and they were centrifuged at 12,000 rpm for 30 minutes. The transfersomes along with unentrapped drug were separated at the bottom of the tubes as sediment. The supernatant of Transfersomal dispersion was separated from sediment and the UV absorbance of the supernatant was determined at 457 nm

- **Investigation of the morphology of the transfersomal dispersions.**

Transmission electron microscopy (TEM) was used to visualize the structure and shape of transferosomal vesicles. The vesicles were absorbed with filter paper after being dried on a copper grid. The sample was examined under a microscope at a 100k magnification and 100kV accelerating voltage after drying..

- **Microscopic image of transfersomes under microscope.**

Optimised transfersomes (2 mg) were diluted in a watch glass with 0.5 ml of water. A small amount of hydrated transfersomes were spread out on a glass slide, and observed at 40x magnification power using a compound microscope, and their vesicle structures were studied.

FORMULATION OF THYMOQ UINONE (TMQ) TRANSFERSOMAL GEL.

a. Preparation of carbopol gel base: 0.5 g Carbopol 934 was weighed and dispersed in distilled water with mild stirring and allowed to swell for 24 hours to obtain 0.5% gel. Later 2 ml of glycerine was added to for gel consistency. Similarly 1 and 2% carbopol gels were prepared.

Table no.4. Composition of different gel base Formulation.

Dummy Formulation	**Carbopol (%)**
Trial F1	0.5
Trial F2	1.0
Trial F3	2.0

b. Preparation of TMQ transfersomal gels: The suspension of TMQ transfersomes was centrifuged at 12000 rpm for 20 min and then sediment was separated from supernatant. Based on required TMQ dose, amount of sediment is added into the 1.0 % carbopol gel base. The incorporation of the TMQ transfersomes into gel base was achieved by slow mixing using stirrer for 10 minutes. Two to three drops of Triethanolamine was added to adjust the pH and consistency of gel.

FORMULATION OF THYMOQUINONE (TMQ) GEL.

Preparation of TMQ gel:
10mg of thymoquinone drug was weighed and dissolved in 10ml of ethanol. After complete dissolution of drug, the solution was added into the Carbopol gel base. The incorporation of the TMQ Solution into gel base was achieved by mixing using stirrer for 10 minutes. Two to three drops of Triethanolamine were added to adjust the pH and consistency of gel base.

EVALUATION OF (TMQ) TRANSFERSOMAL GEL AND TMQ GEL.

- **Determination of Spreadability:**

A modified apparatus suggested was used for determining spreadability. The spreadability was determined by using the below formula.

$$S = \frac{m \times l}{t}$$

where S, is spread ability, m is weight applied on upper slide and t is the time taken to travel a specific distance and l is the distance travelled. For the practical purpose the mass, time was kept constant.

- **Determination of Viscosity:**

The Brookfield viscometer was used to measure the viscosity of cream. The Brookfield viscometer with a 6 number spindle was used to measure the viscosity. The developed formulation was then transferred into the adaptor of the viscometer and determined the viscosity of the test sample as per standard operating procedure of viscometer. The spindle was rotated at speeds of 100 rpm. The reading near to 100% torque was noted.

- **Determination of pH:**

Weighed 50gm of gel formulation were transferred in 10 ml of beaker and measured it by using the calibrated digital pH meter by dipping the glass electrode completely into the gel so as to cover the electrodes. Study was done in triplicate and average of those triplicates was calculated as final pH of prepared formulation. pH of the topical gel formulation should be between 3–9 to treat the skin infections.

- **Drug content:**

0.5gm of prepared gel was mixed with 5ml methanol and then filtered. Aliquots of different concentrations were prepared by suitable dilutions using phosphate buffer after filtering the stock solution and the absorbance was measured at 257 nm. Drug content was calculated by linear regression analysis of the calibration curve.

- **Invitro release study:**

An in-vitro drug release study was performed using modified dialysis membrane method using with phosphate buffer, pH 7.4 as diffusion medium. Dialysis membrane was soaked in phosphate buffer, pH 7.4 for 24 hrs. The Transfersomal sediment was placed in the dialysis membrane bag and kept in diffusion medium.

The temperature of diffusion medium was maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 2 ml of aliquots were withdrawn from receptor compartment and immediately replaced with equal volume of fresh receptor solution and analyzed for drug content by UV Visible spectrophotometer. Similarly, invitro release study of TMQ transfersomal gel was also performed.

RESULTS AND DISSCUSSION

- **Results of preformulation studies:**

Table no.5. Preformulation studies.

Sr.no.	Physical parameter	Results
1.	Color	Pale yellow scaly crystals
2.	odor	Odorless
3.	Melting point	42^0C -45^0C

- **Solubility studies:**

Table no.6. Results of solubility studies

Sr. no.	Solvent used	Observation	Result
1.	Isopropyl alcohol (IPA)	Clear solution	Freely soluble
2.	Ethanol	Slight yellow solution	Soluble
3.	Chloroform	Clear solution	Freely soluble
4.	Methanol	Light yellowish solution	Soluble
5.	DMSO	Yellowish solution	Soluble
6.	Water	Yellow particles of drug	Insoluble

- **Results of FTIR studies:**

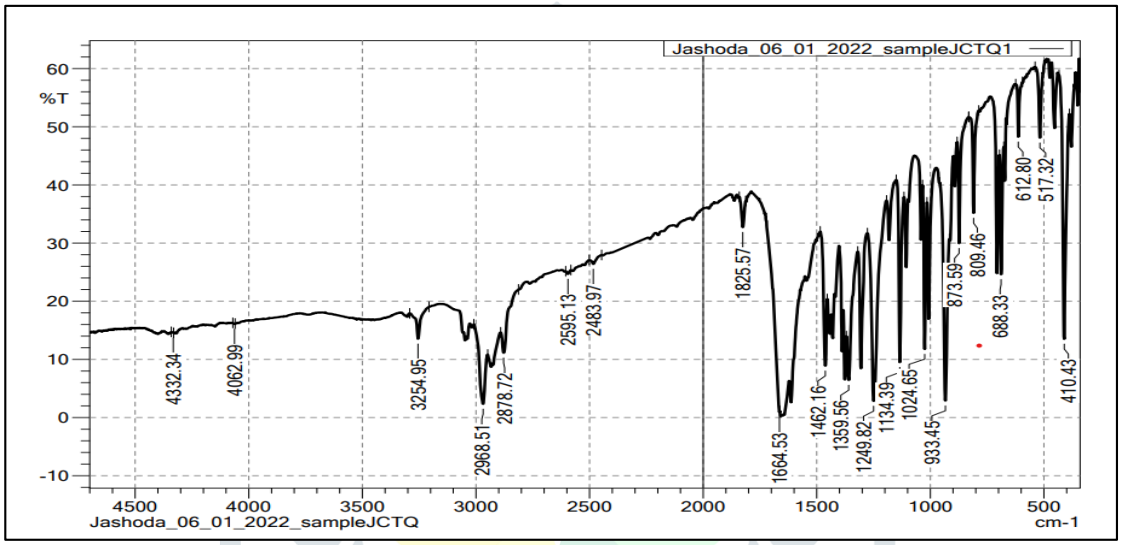

Fig no.1. FTIR of thymoquinone drug

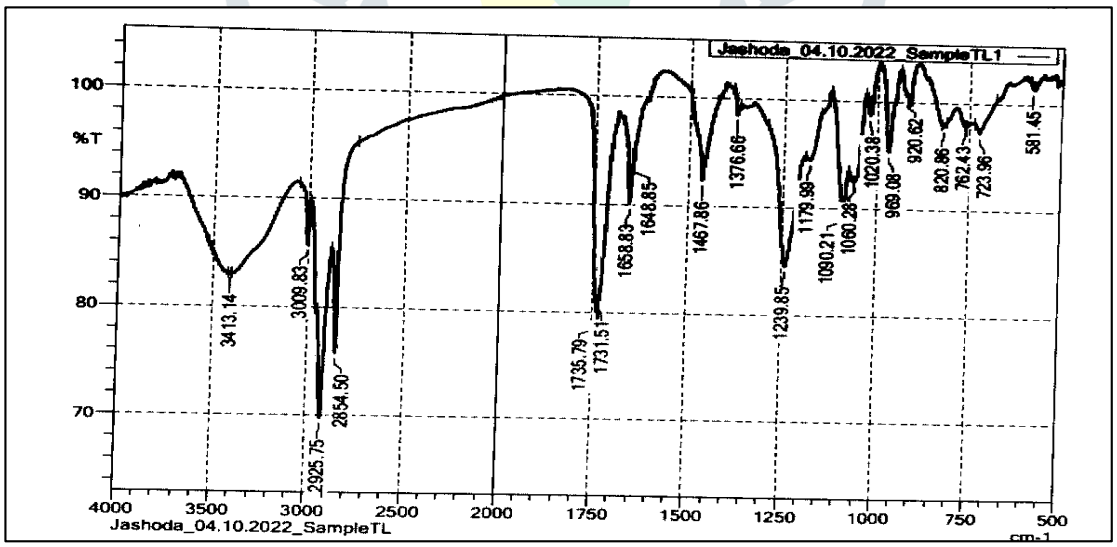

Fig no.2. FTIR of physical mixture.

- **Results DSC studies:**

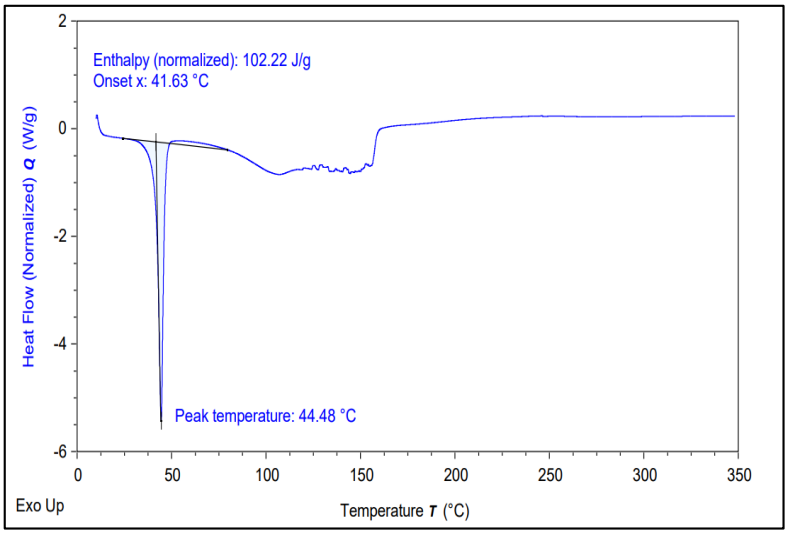

Fig no.3. DSC of Thymoquinone drug.

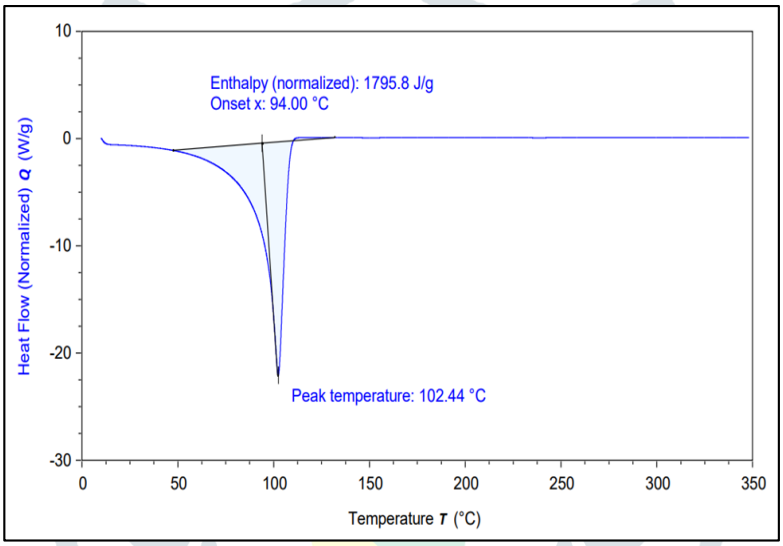

Fig no.4. DSC of Thymoquinone loaded Transfersomal suspension

- **UV spectra of thymoquinone:**

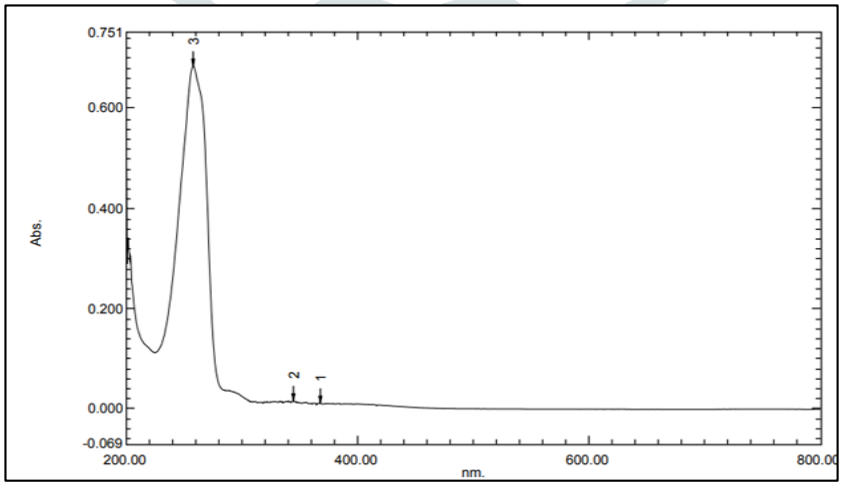

Fig no.5. UV spectra of thymoquinone in phosphate buffer.

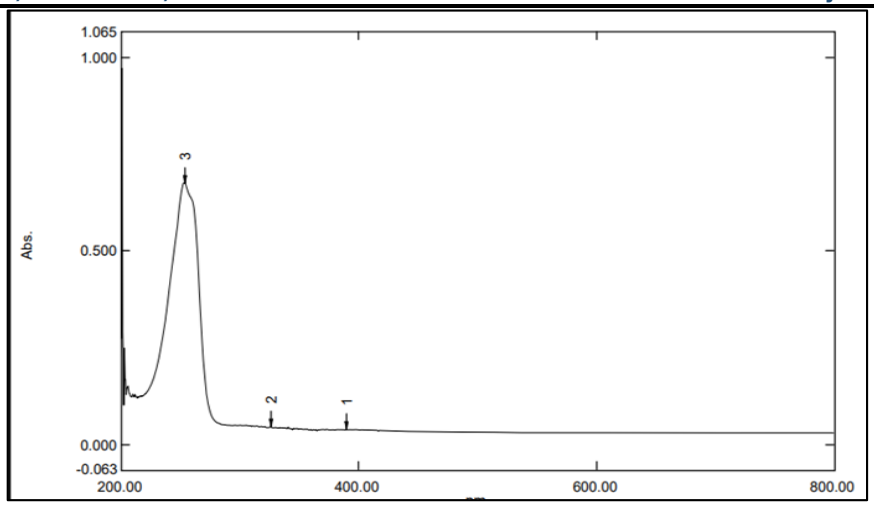

Fig no.6. UV spectra of thymoquinone in methanol.

- **Calibration curve:**

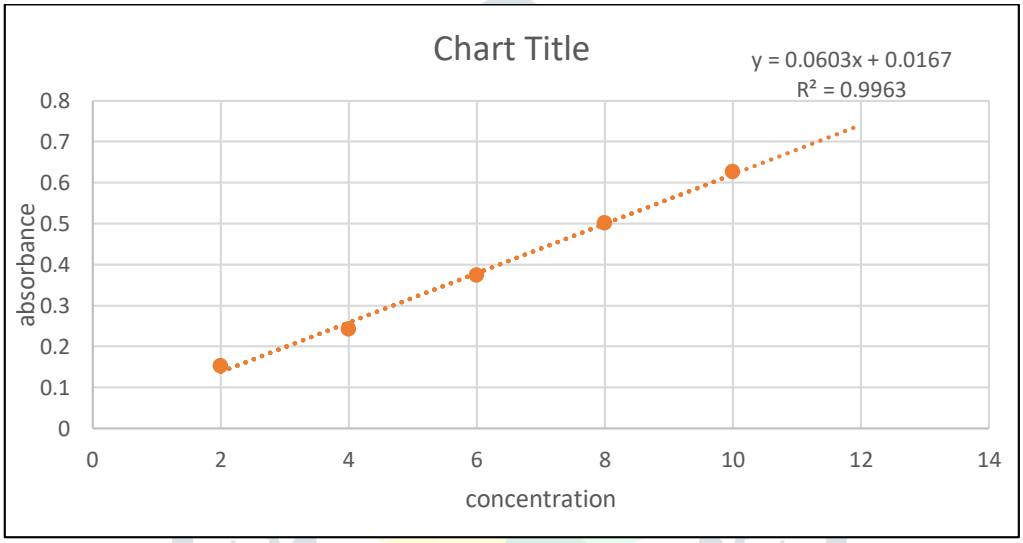

Fig no.7. Calibration curve in phosphate buffer pH 7.4

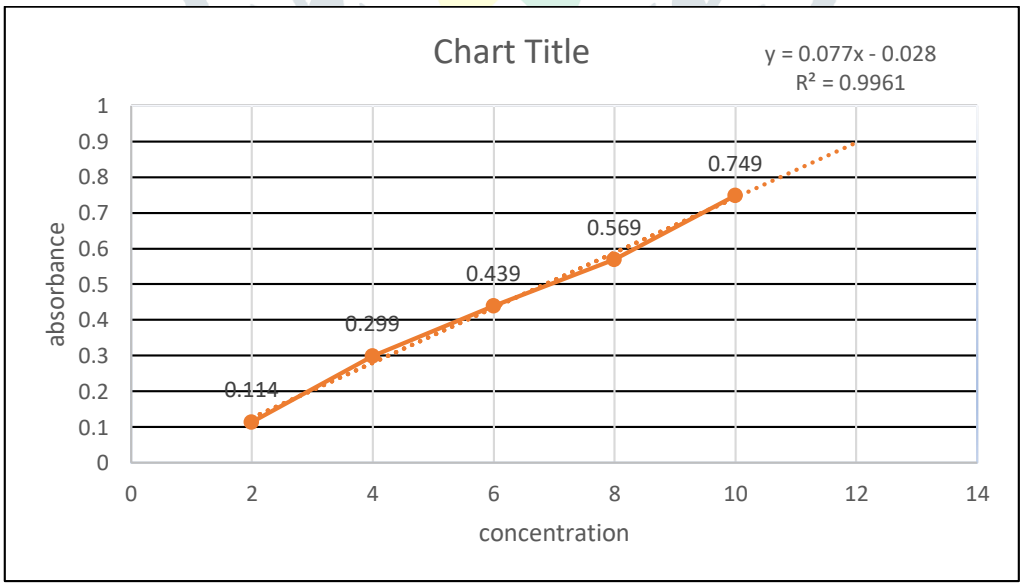

Fig no.8. Calibration curve in methanol

- **Method of preparation:**

Table no.7. Method of preparation.

Sr.no.	Method of preparation	Particle size	P. I
1.	Vortex sonication method	3173.9nm	1.616
2.	Thin film hydration method	143.2nm	0.236
3.	Modified hand shaking method	1437.4nm	1.312
4.	Ethanol injection method	210.4nm	1.144

Table no.8. Formulation and evaluation of trial batches for thin film hydration method.

Formula	F1	F2	F3	F4	F5
Thymoquinone	10mg	10mg	10mg	10mg	10mg
Phospholipid	200mg	200mg	200mg	400mg	100mg
Sodium cholate	100mg	100mg	100mg	200mg	50mg
Ethanol: chloroform	1:1	1:1	1:1	1:1	1:1
% Drug entrapment	76.8%	80.4%	63.8%	30.6%	86.8%
Particle size	37.5nm	38.4nm	602.4nm	1818.4nm	57.1nm

Evaluation of optimized batches of transfersomes:

Table no.9. The experimental variables and levels of PB Design

Run	A	B	C	D	E	F	G	Particle size (nm)	P. I	Drug entrap-ment efficiency (%)
1	+	-	-	+	-	+	+	**77.3nm**	**0.364**	**70.60**
2	+	+	-	-	+	-	+	**89.8nm**	**0.348**	**84.60**
3	+	+	+	-	-	+	-	**51.4nm**	**0.266**	**87.94**
4	-	+	+	+	-	-	+	**28.7nm**	**0.558**	**41.50**
5	+	-	+	+	+	-	-	**92.6nm**	**0.311**	**71.60**
6	-	+	-	+	+	+	-	**37.7nm**	**0.681**	**52.50**
7	-	-	+	-	+	+	+	**45.2nm**	**0.277**	**87.24**
8	-	-	-	-	-	-	-	**66.5nm**	**0.455**	**56.60**
Coefficient value for particle size.	**76.9**	**-19.5**	**23.9**	**-80.1**	**39.3**	**43.9**	**15.7**			
Coefficient value for entrapment efficiency.	**133**	**-74**	**-53.4**	**-16.6**	**41.4**	**-6.6**	**-7.2**			

- **Repeatability of optimized batch:**

Based on the results of trial batches and Placket Burmann Design, optimized batch was selected. Repeatability of same was checked thrice and following are the result.

Table no.10. Results of repeatability of optimized batch.

Sr. no.	Batch	Particle size	P.I	%DEE
1.	PBD 3a	51.4nm	0.266	87.9%
2.	PBD 3b	49.8nm	0.241	70.1%
3.	PBD 3c	52.1nm	0.261	80.1%

• **Particle size and zeta potential**

Zeta potential of optimized batch

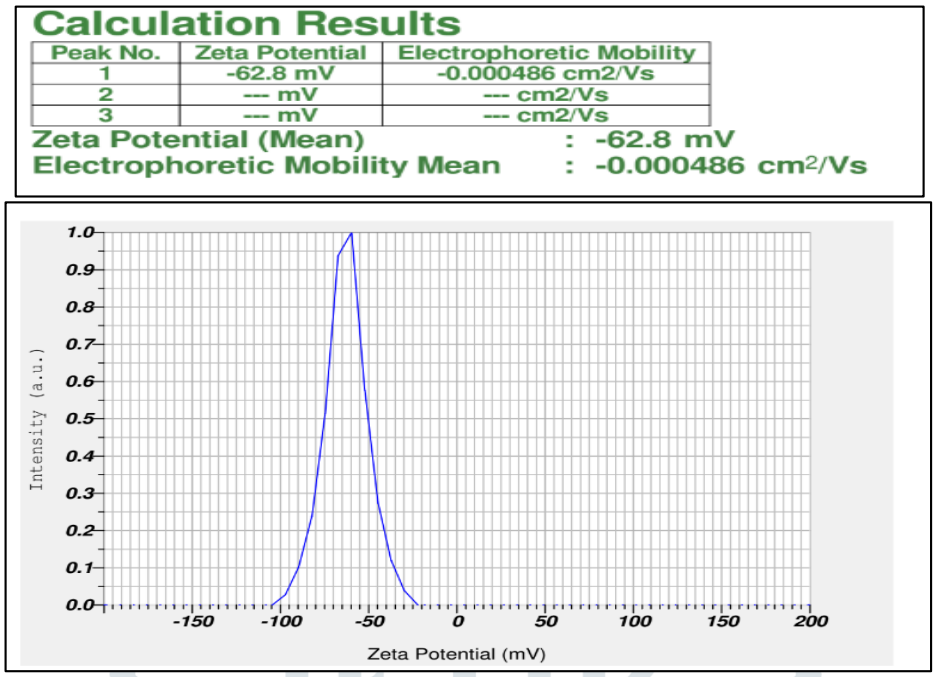

Fig no.9. Zeta potential of optimized batch.

Particle size of optimized batch

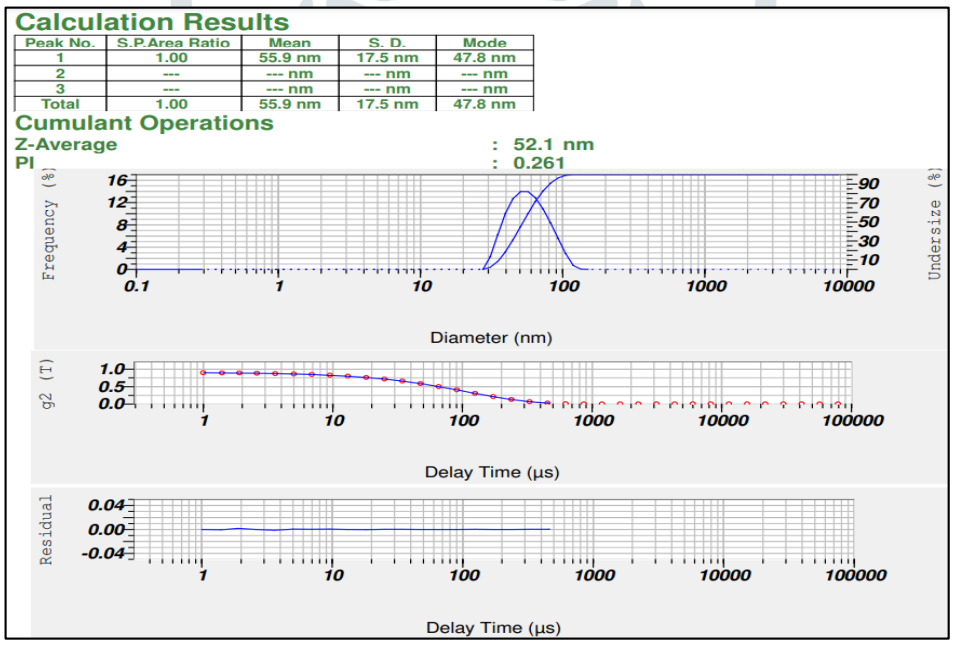

Fig no.10. Particle size of optimized batch.

• **Investigation of the morphology of the transfersomal dispersions**

Morphology of formulated transfersomes was studied by transmission electron microscopy (TEM).

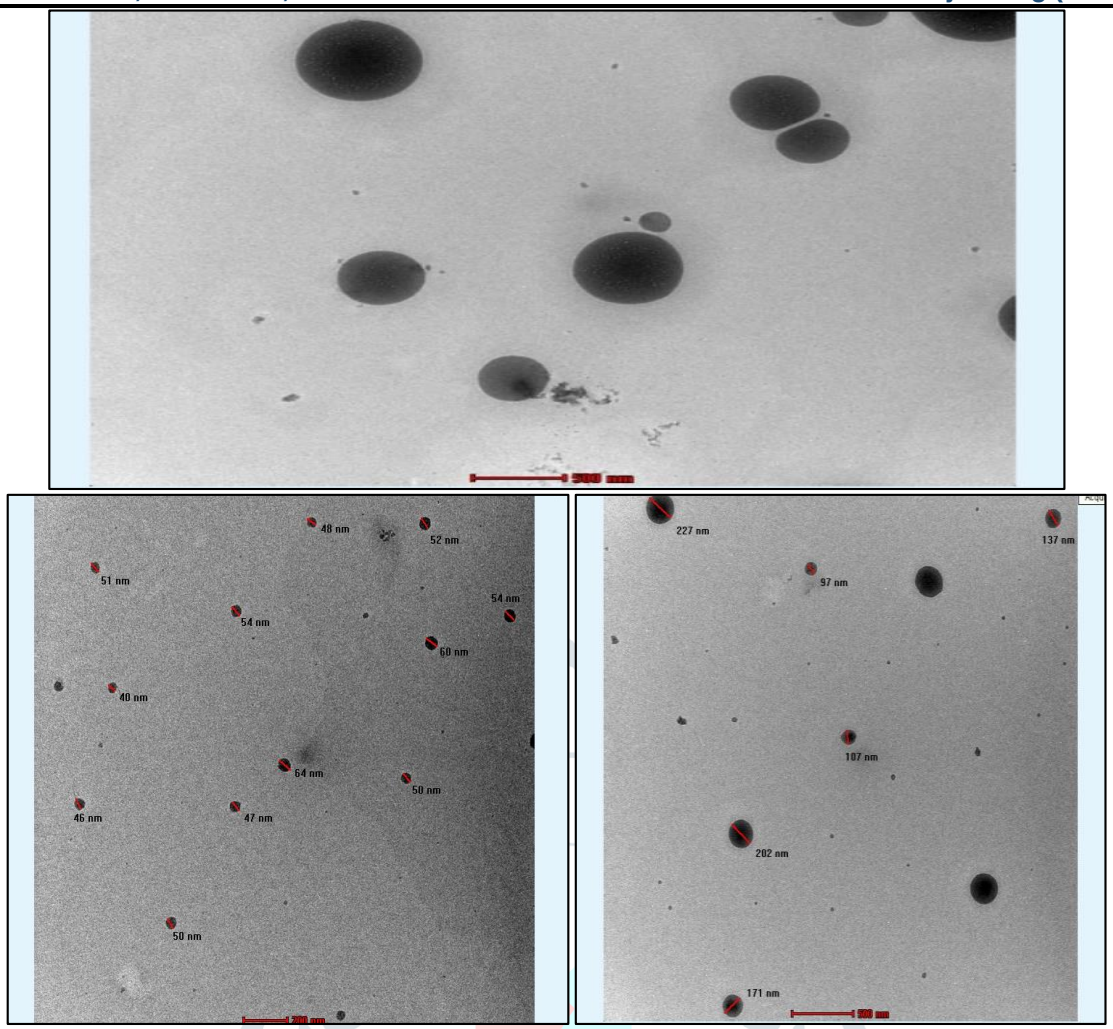

Fig no.11. Images of transmission electron microscopy (TEM) studies.

- **Microscopic image of thymoquinone transfersomes.**

An optical microscope was used to observe the shape of the prepared transfersomes formulation.

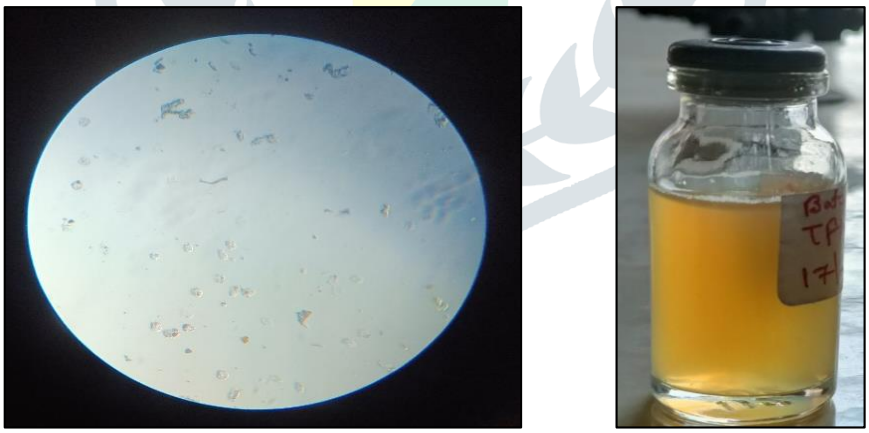

Fig no.12. Microscopic image and formulation of TMQ loaded Transfersomal suspension

Evaluation of thymoquinone Transfersomal gel:

Table no.11. Evaluation of thymoquinone Transfersomal gel

Sr.no.	Evaluation parameter	TMQ transferosomal Gel
1.	pH	6.58
2.	Appearance	Opaque with smooth texture and no particulate matter.
3.	Spreadability	2.05 gm cm sec^{-1}
4.	Viscosity	27500 centipoises

- **Invitro release study:**

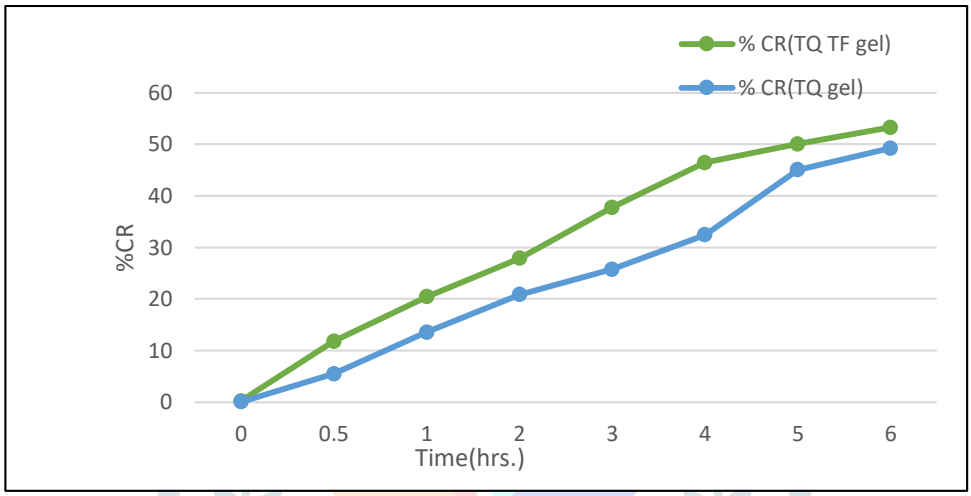

Fig no.13. Invitro release study

- **Kinetics:**

The Thymoquinone gel followed zero order kinetic model and thymoquinone Transfersomal gel following Higuchi model.

Table no.12. kinetic model of TMQ gel and TMQ tranfersomal Gel.

Sr. no	Model	R^2 Values for TMQ gel	R^2 Values for TMQ transfersomal gel
1.	Zero order kinetic	**0.9787**	0.9549
2.	First order kinetic	0.9722	0.9835
3.	Higuchi model	0.9363	**0.9872**
4.	Korsemeyer Peppas	0.8410	0.8166

- **Drug content:**

Drug content of TMQ transfersomal gel was found to be 97% and after one month it was found to be 88%

- **Stability studies:**

Table no.13. Stability studies.

Drug Content	Months	At freezing temperature	At room temperature	Accelerated temperature
105.5%	1 Month	105.1%	101.2%	98.2%
	2 Month	104.5%	99.4%	88.4%
	3 Month	99.8%	97.9%	85.7%

CONCLUSION:

According to the literature survey, thymoquinone has wide range of pharmacological activities but there is no marketed formulation containing thymoquinone for topical use. Thymoquinone (TMQ) is reported with good and wide range of pharmaceutical activity however, the hydrophobicity, poor aqueous solubility, light and pH sensitive nature of TMQ hinder its delivery to target site. To address these delivery challenges of TMQ, transfersomes were explored. This approach will improve patient acceptance towards the herbal drug formulation.

In present study transfersomes were prepared successfully by Thin Film Hydration method using Leciva S 80 as lipid and sodium cholate as surfactant. Prepared thymoquinone loaded transfersome was incorporated into Carbopol gel. All the evaluation parameters such as Entrapment efficiency, size distribution study, zeta potential study, Transmission Electron Microscopy, drug content, pH, viscosity, spreadability, in-vitro drug released study, in-vitro anti-fungal study were determined. In preformulation study, authentication of drug was carried out by UV. Melting point range 42^0C -45^0C found to be as reported in literature. Excipient were selected on the basis of solubility of drug in lipids. FTIR and DSC showed that thymoquinone and all other excipients used in formulation were compatible. Various process parameters were optimized to obtain significant factors using Plackett-Burman design.

Since the results of trial batches and results of placket burman design helped to conclude the optimized formula, hence no other factorial design was carried out further. Optimized batch of thymoquinone transfersome was formulated into gel and evaluated for various parameters for gel formulation. The stability study data showed promising results with respect to drug content and other physicochemical properties of the formulation. This approach will improve patient acceptance towards the herbal drug formulation**.**

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