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Formulation, Development and Evaluation of Phytosomal Gel of Thymoquinone

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Abstract
Phytosomes is vesicular drug delivery system in which phytoconstituents of herb extract surround and bound by lipid (one phyto-constituent molecule linked with at least one phospholipid molecule) Thymoquinone is the main active constituent of nigella sativa oil. Using Design of experiment (sigmatech software) 12 different formulations were prepared. Two different independent variables were used which include: Amount of lipids (X1), Amount of cholesterol (X2). The independent variables were screened using a multilevel factorial design (2^2) and four different formulations, four mid points and four replicates of Thymoquinone. All the formulations was prepared using thin film hydration method. The Thymoquinone was more soluble in Methanol than in Ethanol, phosphate buffer pH 7.4 and water. The prepared thymoquinone was subject to the %EE, *In vitro* drug release and particle size. The highest entrapment efficiency was found to be 90.8 ± 0.6 % and *In vitro* drug release was found to be 94.28 ± 0.39 % which is selected as optimized formulation and SEM was evaluated. 0.2% and 0.4% % gel was prepared taking optimized formulation by using 1% carbopol 934 and evaluated for Homogenicity, spreadability, pH, Viscosity, Drug content and *In vitro* drug release.

Keywords
Phytosomes, Thymoquinone, Thin film hydration method, carbopol 934

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1. INTRODUCTION:
The term 'phyto' means plant while 'some' means cell-like. Phytosomes is vesicular drug delivery system in which phytoconstituents of herb extract surround and bound by lipid (one phyto-constituent molecule linked with at least one phospholipid molecule) fig no.1. The phospholipid molecular structure included a water-soluble head and two fat soluble tails, because of their dual solubility, phospholipid acts as an effective emulsifier.[1]

Black seed (also known as black cumin, Fennel Flower, Nutmeg Flower, Black seed, Black Caraway, Roman Coriander, Damascena, Devil in-the-bush, Wild Onion Seed.; *Nigella sativa*) is an annual flowering plant belonging to the family Ranunculaceae and is a native of Southern Europe, North Africa, and Southwest Asia. Black cumin is cultivated in the Middle Eastern Mediterranean region, Southern Europe, Northern India, Pakistan, Syria, Turkey, Iran, and Saudi Arabia. Nigella sativa contain many constituents like thymoquinone, Thymohydroquinone, dithymoquinone, Thymol, Protein, Fat, Carbohydrates, unsaturated fatty acids like linoleic acid, olic acid, saturated fatty acids like palmitic acid, steric acid etc. Thymoquinone (TQ) is the most abundant active constituent of the volatile oil of *Nigella sativa* seeds and most properties of *N sativa* are mainly attributed to TQ. A number of

pharmacological actions of TQ have been investigated including antioxidant, anti-inflammatory, immunomodulatory, anti-histaminic, anti-microbial, anti-tumor effect etc. It has also gastroprotective, hepatoprotective, nephroprotective and neuroprotective activities. In addition, positive effects of TQ in cardiovascular disorders, diabetes, reproductive disorders and respiratory ailments, as well as in the treatment of bone complications as well as fibrosis have been shown. Thymoquinone is abundantly present in seeds of Nigella sativa that is popularly known as black cumin or black seed. A large number of studies have revealed that thymoquinone is the major active constituent in N. sativa oil this constituent is responsible for the majority of the pharmacological properties. Thymoquinone has also been shown to alter numerous molecular and signaling pathways in many inflammatory and degenerative diseases including cancer. In addition, a large body of data shows that TQ has very low adverse effects and no serious toxicity.[2]

Hence the present study was used to formulate, optimized and evaluate the phytosomal gel of thymoquinone in different percentages (0.2% and 0.4% phytosomal gel of thymoquinone)

2. MATERIALS AND METHODS:

2.1 Materials:

Thymoquinone was obtained from Sigma Aldrich, soya lecithin was obtained from Himedia, Mumbai, India, cholesterol, Methanol, Dichloromethane, triethanolamine was obtained from Fischer Scientifics, Mumbai, India. Potassium dihydrogen phosphate and sodium hydroxide abd Carbopol 934 was procured from S.D fine chemicals.

2.2 Method:

2.2.1 Preparation of Thymoquinone loaded phytosomes:

The Thymoquinone loaded phytosomes are prepared by using Thin film method. Accurate amounts of Phospholipids (X_1), Cholesterol(X_2) (shown in table no.1) and drug were dissolved in a mixture of organic solvents consisting of Dichloromethane: Methanol (2:1, v/v) in a dry, round-bottom flask. The organic solvent was allowed to evaporate using a rotary evaporator (Aditiya scientific evaporator) adjusted to 60 rpm, at 40° C for 15 mins under low pressure to prepare a thin lipid film on the wall of the round-bottom flask. The dry thin lipid film was subjected to hydration with phosphate-buffered saline (pH 7.4) by rotation for 1 h at 60 rpm at room temperature. The multilamellar lipid vesicles (MLVs) were then sonicated using the ultrasonic probe Sonicator (Mangaldeep tech solutions) for 30 min to reduce the vesicle size and stored at 4°C for further investigation.[3]

2.2.2 Characterization of thymoquinone loaded phytosomes:

a. Determination of Entrapment Efficiency (EE %)

5 ml of Thymoquinone phytosomal complex was added to phosphate buffer pH 7.4 and were centrifuged at 4000 rpm for 45 min 4^0C to allow the separation the entrapped drug from the un-entrapped drug using a Remi ultra centrifuge (Remi-CM12plus). Results in formation of sediment and supernant. From this unentrapped drug is separate by removing the supernant then the sediment was lysed with methanol and then analyzed at 254nm using UV-Visible spectrophotometer (Elico, PG instruments). The percentage drug entrapment was calculated by using the formula.[4]

EE% = [Amount of Entrapped TQ/Total Amount of TQ] /100

b. Surface morphology of Phytosomes

Scanning electron microscopy is used to determine the shape and size of formulated Thymoquinone loaded phytosomes. After the samples have been cleaned, fixed, rinsed, dehydrated and dried using appropriate protocol then samples are mounted on holder that can be inserted into scanning electron microscope. [5]

c. Determination of Particle Size and Zeta Potential

Particle size and zeta potential were measured for all prepared TQ phytosomes using the dynamic light scattering (DLS) technique at 25°C using the Particle Size System. [6]

d. Determination of In-Vitro diffusion of TQ phytosomes

A diffusion study of formulations was carried out using Franz diffusion cell through dialysis membrane. Dialysis membrane was soaked in distilled water for 24 hours. Franz diffusion cell contain two compartments upper donor and lower receptor compartment. The receptor compartment was filled with 7.4 phosphate buffer and donor compartment contain 5 ml of phytosomes 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 600 minutes and samples were withdrawn at an interval of 1hour for 10 hours and replaced

with equal volume of buffer. Samples were appropriately diluted with buffer and analyzed using UV spectrophotometer at 254nm.[7]

Steady state Flux (**Jss**) was calculated from the slope of the linear part of the cumulative amount of drug permeated per unit area (µg/cm^2) against a time (h) plot. Permeability coefficient (**Kp) =Jss/Co**, (Co= initial Thymoquinone concentration)

2.3 Preparation of thymoquinone phytosomal gel

0.2% and 0.4% of thymoquinone phytosomal gel was prepared.The accurate weight of polymer was sprinkled into a beaker containing 60 mL boiling distilled water, and then soaking was allowed overnight. The Phytosomal dispersion containing 0.2% and 0.4% TQ was added with continuous stirring to allow homogeneous distribution of TQ Phytosomes within the gel base. Sodium benzoate is used as preservatives. The dispersion was neutralized by addition of Triethanolamine dropwise, with continuous mixing until a homogenous gel was obtained Prepared gels were stored in suitable containers at room temperature for further studies.[8]

2.3.1 Characterization of optimised thymoquinone phytosomal gel

a. Homogenicity:

Homogeneity of the developed gels were tested for by visual inspection by pressing small quantity of both gel (0.2% and 0.4% Thymoquinone phytosomal gels) between the thumb and the index finger. They were tested for their appearance and presence of any aggregates. The consistency was determined as homogeneous or not.[9]

b. Spreadability :

Two glass slides of 20 cm × 20 cm were selected. A small amount of gels was sandwiched between the two glass slides. A 500 g weight was placed on the upper slide so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and then 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. The following equation was used for this purpose: [9]

$$S = m \times L/T$$

c. Viscosity:

Viscosity of both the formulated gels 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. [9]

d. Measurement of pH:

One gram of both the gels 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 times and the mean ± SD was calculated. [9]

e. Drug content:

1gm of each gel was dissolved in 100 ml volumetric flask by using methanol. This was then stirred for 2 h. The resultant solution was filtered, and drug content was analyzed spectrophotometrically at 254nm. [9]

f. *In vitro* diffusion studies:

A diffusion study of 0.2% and 0.4% Thymoquinone loaded Phytosomal gel was carried out using Franz diffusion cell through dialysis membrane. Dialysis membrane was soaked in distilled water for 24 hours. The receptor compartment was filled with 7.4 pH and donor compartment contain 1g of Phytosomal gel (equivalent to 5mg) on dialysis membrane with exposure area of 2cm2 to receptor medium and whole assembly was kept on magnetic stirrer at 600rpm 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 254nm. Steady state Flux (Jss) was calculated from the slope of the linear part of the cumulative amount of drug permeated per unit area (µg/cm^2) against a time (h) plot. Permeability coefficient (Kp) =Jss / Co, (Co = initial Thymoquinone concentration).[9]

2.3.2 Stability studies:

The stability studies of TQ Phytosomal gel (0.2% and 0.4%) were conducted at refrigerated temperature (4°C) and room temperature as per Guidelines of International Conference on Harmonization (ICH). Samples were analyzed for physical appearance drug content, and *in vitro* diffusion studies after 15, 30 and 45 days.[10]

2.3.3 Release kinetic profile for thymoquinone phytosomal gel:

To analyze the *In-vitro* release data various kinetic models were used to describe the release kinetics. The zero-order rate describes the systems where the drug release rate is independent of its concentration. The first order describes the release from system

where release rate is concentration dependent. Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion.

The results of *in-vitro* release profile obtained for all the formulations were plotted in models of data treatment as follows:

- Zero-order kinetic model- Cumulative % drug released versus time.
- First-order kinetic model- Log cumulative percent drug remaining versus time.
- Higuchi's model- Cumulative percent drug released versus square root of time.

Zero order kinetics:

Zero order release would be predicted by the following equation:

$At=A_0 – K_{0t}$

Where,

At= Drug release at time 't'

A_0= Initial drug concentration

K_0= Zero-order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order kinetics and its slope is equals to zero order release constant K_0.

First order kinetics:

First order kinetics could be predicted by the following equation:

$Log\ C= log\ C_0-K_t / 2.303$

Where,

C= Amount of drug remained at time 't'.

C_0= Initial amount of drug.

K= First order rate constant (hr^{-1})

When the data plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follow first order kinetics. The constant 'K_t' can be obtained by multiplying 2.303 with the slope value.

Higuchi's model:

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$Q= [DЄ/τ(2A-ЄC_S)\ C_St]1/2$

Where,

Q= Amount of drug release at time 't'.

D= Diffusion coefficient of the drug in the matrix.

A= Total amount of drug in unit volume of matrix.

C_S = Solubility of drug in matrix.

Є= Posrosity of the matrix.

τ = Tortuosity.

t = Time (hrs at which q amount of drug is released).

Above equation can be simplified as if we assume that 'D', 'C_S', and 'A' are constant. Then equation becomes:

$Q= Kt_{1/2}$

When the data is spited according to the equation i.e, cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K' (Higuchi's 1963). (See table 9).

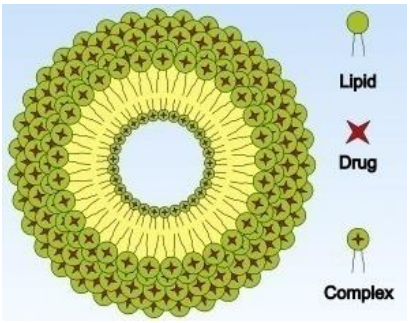

Figure 1: Structure of phytosomes

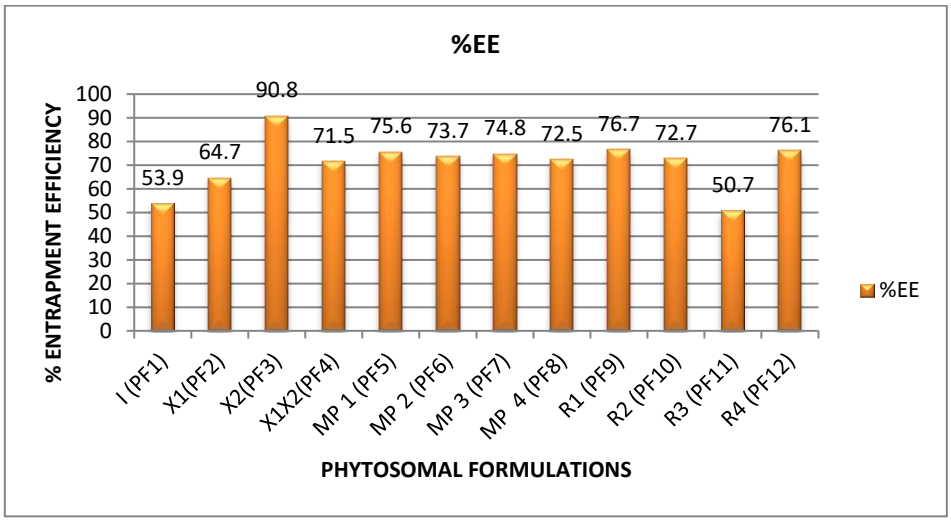

Figure no.2: Entrapment Efficiency of all prepared TQ phytosomal formulations

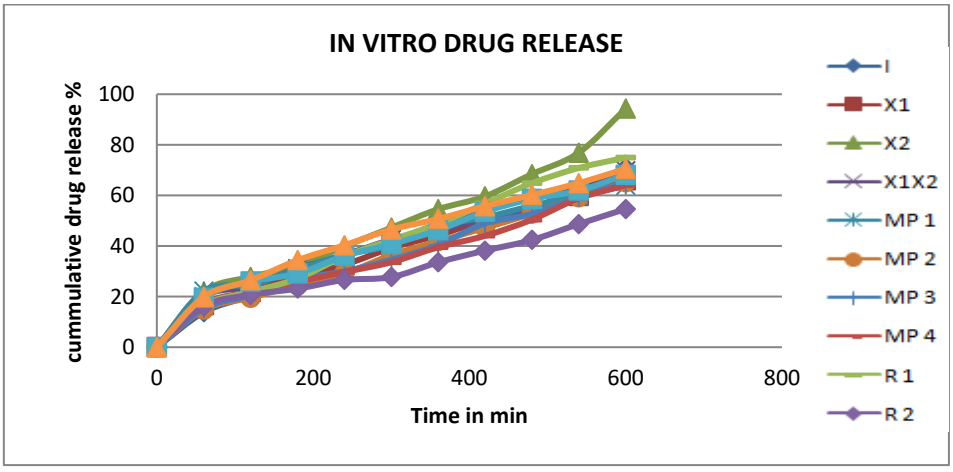

Fig no.3: *In vitro* diffusion studies of Phytosomal formulations

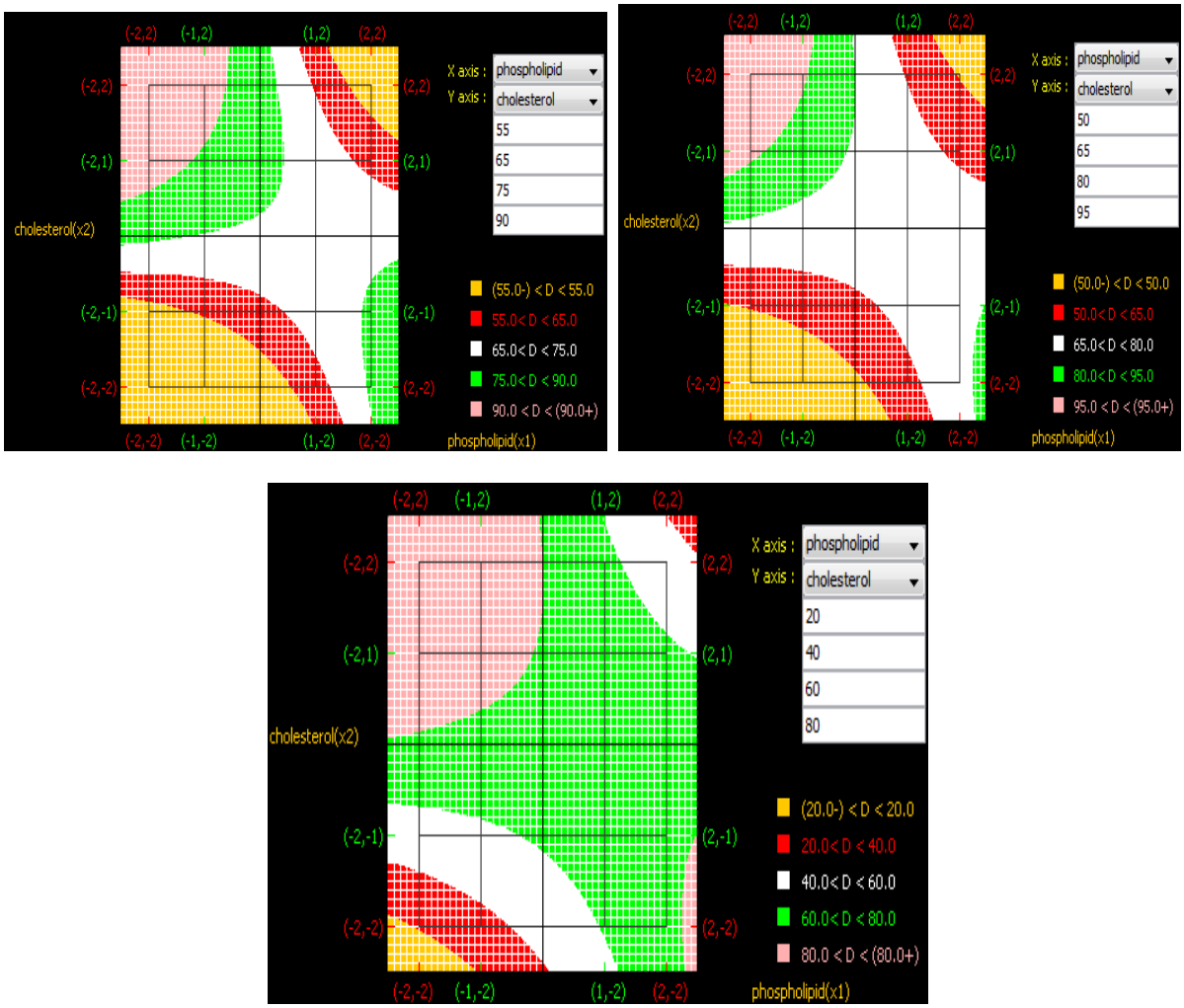

Figure 4: Contour plots(clockwise)%EE, *in vitro* diffusion and particle size

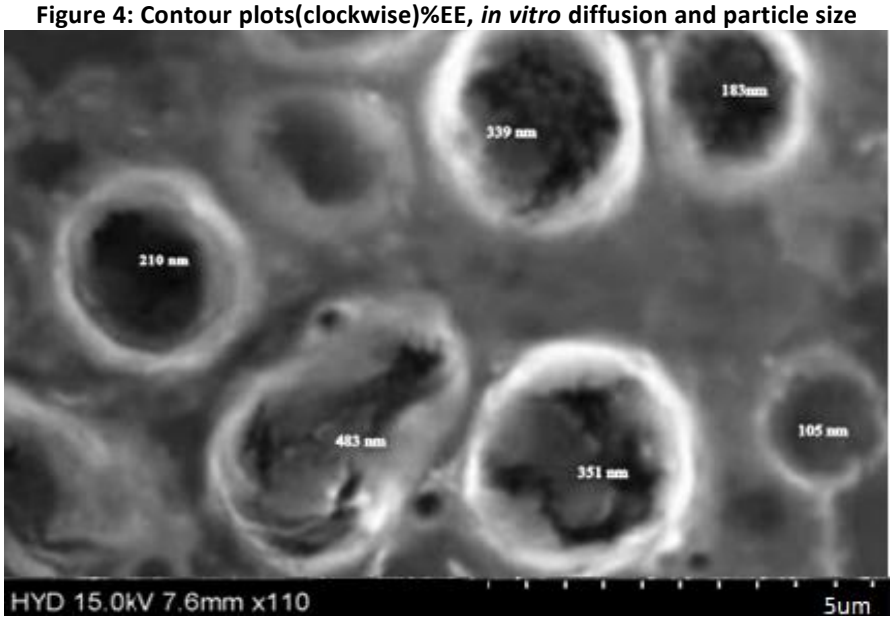

Figure 5: SEM image of X_2(PF3)

Zeta Potential (Mean)	: -35.4 mV
Electrophoretic Mobility mean	: -0.00027 cm^2/Vs

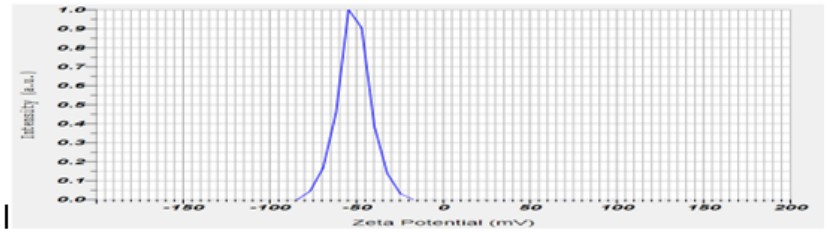

Figure 6: Zeta potential of X_2(PF3) formulation

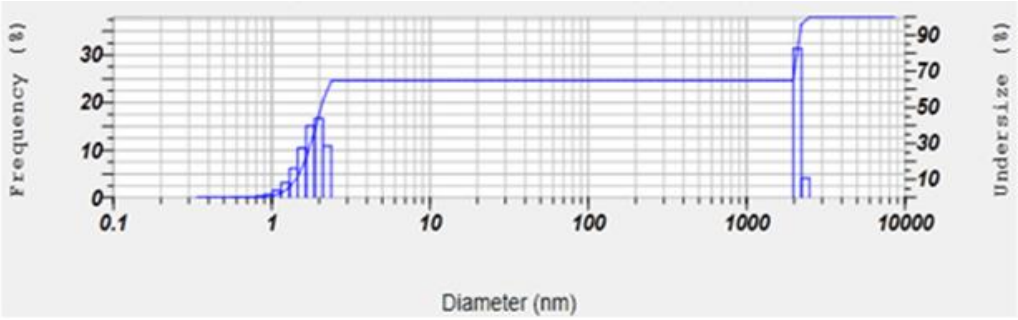

Figure 7: Particle size distribution of X_2(PF3) formulation

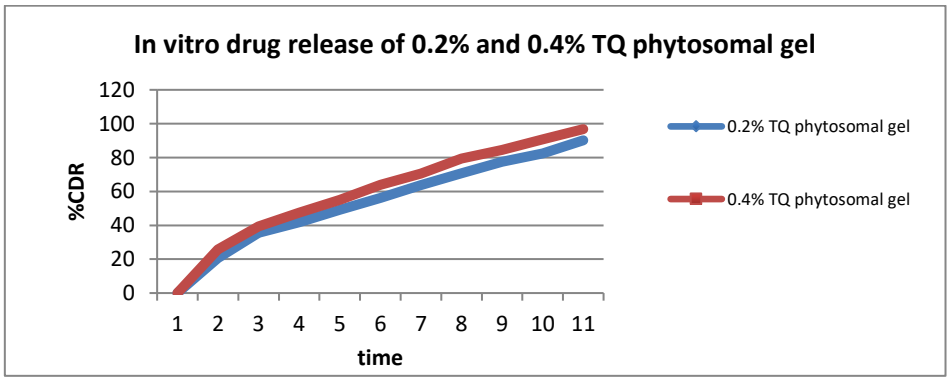

Figure 8: *In vitro* drug release of phytosomal gels

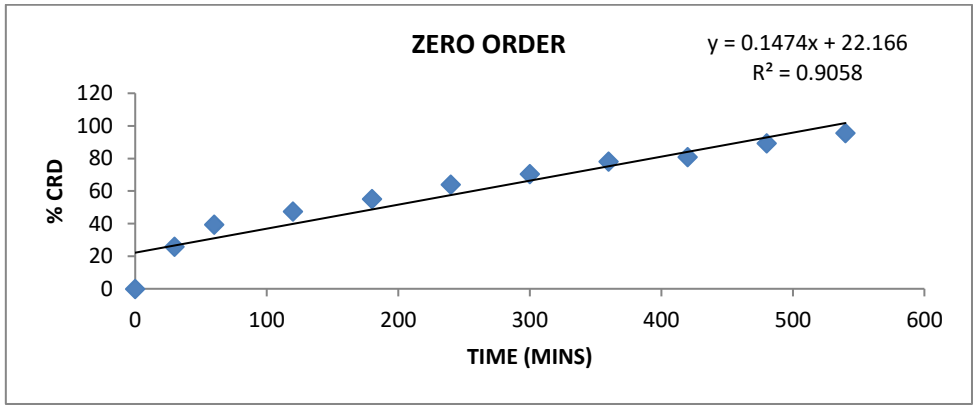

Figure 9: Zero order Kinetics for TQ Phytosomal gel

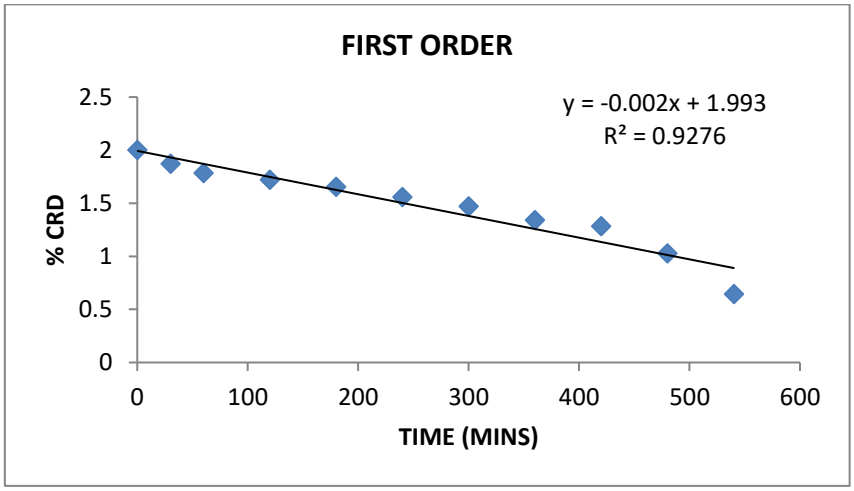

Figure 10: First order Kinetics for TQ Phytosomal gel

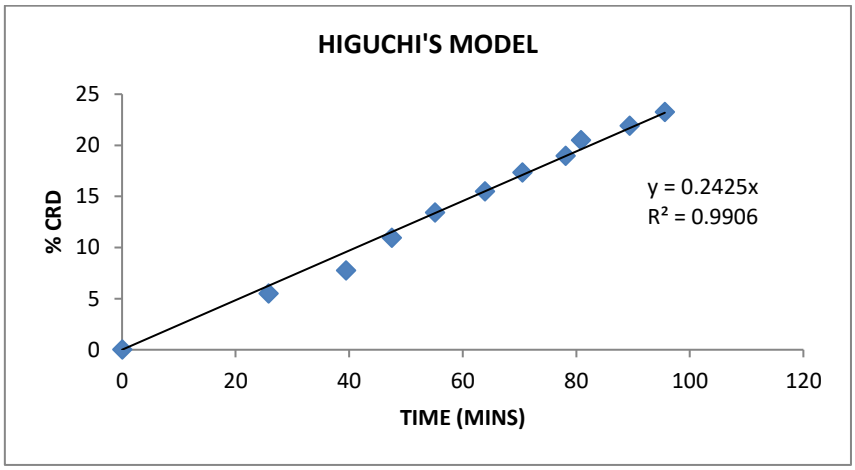

Figure 11: Higuchi's model for TQ Phytosomal gel

3. RESULTS AND DISCUSSION:

See the table 2 for Formulation design with responses by using design of experiment

3.1 Entrapment efficiency:

As shown in Table 2, it was found that the prepared Thymoquinone phytosomes exhibited a good EE%, with values ranging from (50.7±0.2) for R3 (PF11) to (90.8±0.6%) for X2 (PF3). (See figure 2)

3.2 *In-vitro* diffusion studies:

Diffusion studies of all formulations were carried out using dialysis membrane for 10 hours and samples wereanalysed using double beam UV spectrophotometer. (see table no.4)

Diagrammatic representation of *In vitro* drug release of all the formulations are shown in the figure 3

Jss and Kp of Phytosomal formulations are shown in table 3.

3.3 Particle size (Y3) and Zeta Potential of Prepared TQ phytosomes:

The prepared TQ phytosomes were tested for particle size and zeta potential. From the results of particle size, it was found that all prepared TQ phytosomes have a particle size less than 200 nm, and as such are effective for transdermal applications.

The contour plots of entrapment efficiency, *in vitro* drug release and particle size is given in the figure 4

4. Optimize formulation:

Table 4.7 shows the composition of the optimized formula (X2). After optimization of formulation variables, it was found that the optimized formulation was suggested to contain 550, 87.5 mg, of X1 and X2, respectively. As shown in Figure 4.7, SEM photographs of optimized Tq phytosomes appeared as spherical, well identified, unilamellar nanovesicles. Figure 4.8 and 4.9 showed the particle size distribution and zeta potential of the optimized formula.

4.1 Characterisation of optimised formulations:
See the table 5 for the Composition of factors and response for the optimized TQ Phytosomes.

a. Surface morphology of phytosomes:
The formulated Thymoquinone phytosomes were found to be almost spherical in shape and vesicle size was found to be in the range of 200 nm.(Shown in figure 5)

b. Particle size distribution and zeta potential determination
Zeta potential of Thymoquinone loaded phytosomes of formulation showed good stability.(See figure 6 & 7)

4.2 Evaluations of Thymoquinone phytosomal gel:

a. 0.2% thymoquinone phytosomal gel:
TQ phytosomal gel is smooth with a homogenous appearance. The Spreadability values is 67.6±0.31cm, respectively, which indicates that they can be spared easily on skin surface with little stress. The viscosity of TQ phytosomal gel is found to 15206 ± 0.83 cps. The pH value is found to be 6.4±0.34 which is considered within the normal range of pH for topical preparations. The actual drug content of the TQ phytosomal gel was found to be 90.5±1.67%, which represents good content uniformity. *In vitro* drug release was found to be 91.2 ± 0.51. (see table 6)

b. 0.4% thymoquinone phytosomal gel:
TQ phytosomal gel is smooth with a homogenous appearance. The Spreadability values is 72.5±0.52cm, respectively, which indicates that they can be spared easily on skin surface with little stress. The viscosity of 04% Thymoquinone phytosomal gel is found to 17291±0.25 cps. The pH value is found to be 6.5±0.58 which is considered within the normal range of pH for topical preparations. The actual drug content of the TQ phytosomal gel was found to be 95.5±1.29 %, which represents good content uniformity. The *in vitro* drug release was found to be 97.8 ± 0.85 (see table 6)

***In vitro* drug release**:
In vitro drug release of 0.2% thymoquinone phytosomal gel and 0.4% thymoquinone phytosomal gel is shown in the figure 8

5. Stability studies

5.1 Stability of 0.2% Thymoquinone phytosomal gel and 0.4% thymoquinone phytosomal gel at 4±2^{0}Cand R.T is shown in table 7 and 8.
DC- Drug Content, C.D.R- Cumulative Drug Release
Stability studies showed that Phytosomal gel is more stable at 4°C when compare to other temperatures.There was a change in color for the samples kept at room temperature, leakage of drug was minimum due to gel formulation because it is viscous in nature and also decreases the fusion of vesicles which otherwise will be responsible for drug leakage.

6. Release kinetic profile for tq phytosomal gel:
TQ phytosomal gel formulation shows (see Figure 9, 10 and 11) Higuchi kinetics that indicated the order of release of drug i.e, square root of time (Higuchi) model indicates that the release of drug is by diffusion.
R^2 values for the optimized formulation were found to be highest for the Higuchi model. This indicated that the drug release from all the formulations followed diffusioncontrolled release mechanism. 'n' value was estimated from linear regression of log (Mt/M) vs log t and it was found that drug release follows Quasi fickian mechanism (See table 10)

DISCUSSION:
The aim of the study was to Formulate, develop and evaluate phytosomal gel of thymoquinone. TQ phytosomes was prepared by Rotary evaporation technique (thin film method) and formulations was designed by using design expert software(table no.1) so according to the design of experiment 12 formulations was prepared (4 formulations, 4 mid points and 4 replicates) using 2^2 full factorial design by using sigma tech software. Independent variables used were phospholipid and cholesterol as X1 and X2 and dependent variables as entrapment efficiency, in vitro drug release and particle size. The concentration of phospholipid was responsible for the drug release and the concentration of cholesterol is responsible for the entrapment efficiency. Entrapment efficiency (figure 2), *in vitro* diffusion studies (Figure 3) and particle size was performed for all the formulations (Table no.2) and contour plots was obtained (figure 4). The Optimised formulation was selected (table 5) based on the dependent variables and the optimised sample was subjected to SEM (figure 5). 0.2% and 0.4% thymoquinone Phytosomal gels was prepared of optimized formulation and subjected for different evaluations like homogenicity, spreadability, pH, viscosity, drug content and *in vitro* drug release (table 6). Stability studies were also performed for the 0.2% and 0.4% thymoquinone phytosoma gel. Stability studies showed that Phytosomal gel is more stable at 4°C when compare to room temperatures. 0.4% of the gel shows higher stability compare to 0.2% thymoquinone phytosomal gel(table 7 & 8). There was a change in color for the samples kept at room temperature, leakage of drug was minimum due to gel formulation because it is viscous in nature and also decreases the fusion of vesicles which otherwise will be responsible for drug leakage. Release kinetics

was determined for the gel and kinetics includes zero order, first order and higuchi's model. (table no.9).

Table-1 Formulation factors for the multilevel factorial design.

Independent Factors	Low level	High level
X_1 – Amount of lipid(mg)	550	850
X_2 – Amount of cholesyerol (mg)	62.5	87.5
Dependent variables	**Goals**	
Y1 – entrapment efficiency	Maximize	
Y2 – drug release	Maximize	
Y3- particles size	Minimize	

Table 2: Formulation design with responses

SNO.	Combinations	Phospholipid X1	Cholesterol X2	Y1 (%EE)	Y2 (% drug release)	Y3 (particle size)nm
1	I (PF1)	550	62.5	53.9 ± 0.8	70.84 ± 0.25	0.8 ± 0.06
2	X_1(PF2)	850	62.5	64.7 ± 0.2	65.91 ± 0.98	0.2 ± 0.08
3	X_2(PF3)	550	87.5	90.8 ± 0.6	94.28 ± 0.39	0.4 ± 0.02
4	X_1X_2(PF4)	850	87.5	71.5±0.45	69.72 ± 0.89	0.9 ± 0.05
5	MP 1 (PF5)	700	75.0	75.6 ± 0.7	63.88 ± 0.22	0.12 ± 0.06
6	MP 2 (PF6)	700	75.0	73.7 ± 0.9	65.25 ± 0.5	0.6 ± 0.04
7	MP 3 (PF7)	700	75.0	74.8 ± 0.8	64.91 ± 0.3	0.7 ± 0.07
8	MP 4 (PF8)	700	75.0	72.5± 0.43	64.02 ± 0.49	0.5 ± 0.05
9	R1 (PF9)	400	75.0	76.4± 0.95	74.98 ± 0.62	0.9 ± 0.01
10	R2 (PF10)	1000	75.0	72.9 ± 0.3	54.63 ± 0.86	0.10 ± 0.08
11	R3 (PF11)	700	50.0	50.3± 0.25	68.11 ± 0.75	0.7 ± 0.05
12	R4 (PF12)	700	100.0	77.5± 0.45	70.57 ± 0.5	0.3 ± 0.03

The values are expressed as mean, ±SD (n=3)

Table 3: Jss and Kp of Phytosomal formulations

FORMULATIONS	FLUX (J) (µg/cm²)	PERMEBILITY COEFFICIENT (KP)
I (PF1)	2.923 ± 0.21	0.769 ±0.23
X_1(PF2)	2.73 ± 0.35	0.718 ±0.27
X_2(PF3)	3.893 ± 0.12	0.919 ±0.35
X_1X_2(PF4)	2.860 ± 0.85	0.754 ±0.75
MP 1 (PF5)	2.805 ± 0.42	0.738 ±0.42
MP 2 (PF6)	2.612 ± 0.82	0.687 ±0.86
MP 3 (PF7)	2.619 ± 0.53	0.689 ±0.35
MP 4 (PF8)	2.548 ± 0.15	0.670 ±0.84
R1 (PF9)	3.086 ± 0.27	0.812 ±0.38
R2 (PF10)	2.175 ± 0.36	0.572 ±0.94
R3 (PF11)	2.881 ± 0.23	0.758 ±0.24
R4 (PF12)	3.057 ± 0.25	0.873 ±0.35

The values are expressed as mean, ±SD (n=3)

Table 4: *In vitro* diffusion studies of Phytosomal formulations

	I	X1	X2	X1X2	MP 1	MP 2	MP 3	MP 4	R 1	R 2	R 3	R 4
Time (mins)	CDR %	CDR%	CDR%	CDR%	CDR%	CDR%	CDR%	CDR%	CDR%	CDR%	CDR%	CDR%
0	0	0	0	0	0	0	0	0	0	0	0	0
30	13.84 ±0.2	16.58± 0.71	21.94± 0.3	19.92± 0.6	22.12± 0.34	14.82± 0.51	15.28± 0.35	17.05± 0.6	16.52± 0.37	16.15± 0.61	19.52± 0.19	19.92± 0.67
60	20.56 ±0.8	21.78± 0.3	27.79± 0.4	24.05± 0.23	24.7± 0.27	19.58± 0.36	20.49± 0.72	21.86± 0.26	21.82± 0.46	20.72± 0.19	25.94± 0.37	26.42± 0.31
120	29.74 ±0.54	27.82± 0.73	32.13± 0.28	31.08± 0.49	30.75± 0.49	24.51± 0.43	26.78± 0.75	25.86± 0.31	27.14± 0.41	23.14± 0.68	29.13± 0.67	34.4± 0.46
180	36.72 ±0.7	32.59± 0.12	39.85± 0.58	36.37± 0.51	36.52± 0.36	29.09± 0.21	30.01± 0.25	29.72± 0.37	35.93± 0.21	26.59± 0.37	36.08± 0.56	40.28± 0.37
240	41.81 ±1.2	39.21± 0.3	47.23± 0.22	42.52± 0.42	40.29± 0.49	36.42± 0.20	34.57± 0.21	33.59± 0.27	42.42± 0.64	27.71± 0.16	40.79± 0.37	46.52± 0.64
300	48.75 ±0.84	44.28± 0.80	54.55± 0.8	46.52± 0.34	47.85± 0.34	41.87± 0.19	40.42± 0.58	39.51± 0.39	48.67± 0.38	33.55± 0.28	46.23± 0.81	50.72± 0.46
360	54.86 ±0.91	50.21± 0.23	59.62± 0.8	50.72± 0.21	51.29± 0.68	47.21± 0.29	48.94± 0.69	44.03± 0.82	56.75± 0.81	38.18± 0.37	53.55± 0.89	55.74± 0.59
420	59.09 ±0.5	54.85± 1.89	68.42± 1.8	55.28± 0.61	55.86± 0.26	52.78± 0.37	52.71± 0.67	50.42± 0.76	64.84± 0.69	42.39± 0.64	58.62± 0.37	60.21± 0.28
480	62.75 ±0.52	59.77± 0.2	76.89± 0.21	61.88± 0.37	59.79± 0.43	59.21± 0.61	59.28± 0.34	58.84± 0.21	70.84± 0.37	48.69± 0.52	61.86± 0.37	64.85± 0.37
540	70.84 ±0.62	65.91± 0.7	94.28± 0.5	69.72± 0.63	63.88± 0.59	65.25± 0.49	64.91± 0.27	64.02± 0.34	74.98± 0.92	54.63± 0.81	68.11± 0.67	70.57± 0.64

The values are expressed as mean, ±SD (n=3)

Table 5: Composition of factors and response for the optimized TQ Phytosomes.

Independent variables		Responses	
X1	550	Y1	81.8 ± 0.6
X2	87.5	Y2	85.28 ± 0.7
		Y3	148.4 ± 0.82

Table 6: Evaluation of 0.2% and 0.4% TQ phytosomal gel

Evaluation	0.2% Phytosomal gel	0.4% Phytosomal gel
Homogenicity	Good	Good
Spreadability (cm)	67.6±0.31	72.5 ±0.52
Viscosity (cps)	15206±0.83	17291±0.25
pH measurements	6.4±0.34	6.5±0.58
Drug content %	90.5±1.67	95.5±1.29
In vitro drug release	91.2 ± 0.51	97.8 ± 0.85

Table 7: Stability of 0.2% Thymoquinone phytosomal gel at 4±2^{0}Cand R.T

DURATION	4±2^0 C		R.T	
	DC	%CDR	DC	%CDR
INITIAL	92.5±1.25	90.6. ±0.42	92.5±1.25	90.6. ±0.42
15 days	93.89±1.01	94.94±1.5	90.5±0.54	89.72±0.19
30 days	93.52±0.21	93.4±0.21	88.1±0.84	86.57±0.64
45 days	90.03±1.22	91.1±1.41	85.9±0.16	80.21±1.51

The values are expressed as mean, ±SD (n=3)

Table 8: stability of 0.4% Thymoquinone phytosomal gel at 4±2^{0}Cand R.T

DURATION	4±2^0 C		R.T	
	DC	%CDR	DC	%CDR
INITIAL	95.5±1.25	96.6.±0.42	96.5±1.41	95.4±0.42
15 days	96.89±1.01	97.4±1.5	94.5±0.54	92.72±0.19
30 days	96.02±0.21	97.1±0.21	91.1±0.84	90.57±0.64
45 days	94.53±1.22	98.1±1.41	89.9±0.16	88.21±1.51

The values are expressed as mean, ±SD (n=3)

Table 9 - Diffusion Exponent and Solute Release Mechanism

S no.	Diffusion	Exponent (n) Overall solute diffusion mechanism
1	0.45	Fickian diffusion
2	0.45 < n < 0.89	Anomalous (non-Fickian) diffusion
3	0.89	Case-II transport
4	n > 0.89	Super case-II transport

% Thymoquinone phytosomal gel

	ZERO	FIRST	HIGUCHI
	% CDR Vs T	Log % Remain Vs T	%CDR Vs √T
Slope	0.147353	-0.00204	0.254319
Intercept	22.16607	1.99297	-0.84499
Correlation	0.95176	-0.96312	0.996605
R 2	0.905848	0.927597	0.993221

CONCLUSION:

The aim of the study to enhance the permeability by formulating the Thymoquinone loaded Phytosomes. Thymoquinone has use to treat diabetes, cancer, wound healing, bacterial infection even in diabetic wound. Preformulation studies shows high solubility in methanol and FTIR shows no interaction between drug and polymer, it was concluded that the factorial design (2^2) had the ability to obtain an optimized formula of Thymoquinone phytosomes, with high EE% (90.8 ± 0.6), small particle size (156± 0.02), *In vitro* drug release (94.28 ± 0.39) and high transdermal flux (3.893 ± 0.12). After optimization of formulation variables, it was found that the optimized formulation was suggested to contain 550 and 87.5 mg of X1 and X2, respectively. SEM of optimized Thymoquinone phytosomes appeared as spherical, well identified, unilamellar nanovesicles. Incorporation of optimize Phytosomes formulation into gel, The concentration of Thymoquinone in the prepared Phytosomal gel was 0.2% and 0.4% w/w. 0.4% was considered to be optimized gel compare to 0.2% based on evaluations. 0.4% gek shows The Spreadability values 72±0.52 cm, which indicates that they can be spared easily on skin surface with little stress. The pH value is found to be 6.5±0.58 which is considered within the normal range of pH for topical preparations. The actual drug content of the Thymoquinone phytosomal gel was found to be 95.5±1.29%, which represents good content uniformity. The viscosity of Thymoquinone phytosomal gel is found to 17291±0.25cps. The percentage drug release and Flux for Thymoquinone phytosomal gel is 97.8± 0.85 and 4.92 respectively which indicate Thymoquinone phytosomal gel is having high release and permeability. When release kinetics is applied it follows Higuchi model, it was found that drug release follows Quasi fickian mechanism.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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