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Research Article

Formulation and Optimization of Guggul Lipid Phytosomal Gel of Thymoquinone Using 2^2 Factorial Design

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ABSTRACT

Thymoquinone is a bioactive principal constituent of Nigella Sativa. Thymoquinone is hydrophobic, having low water solubility and less permeability. The study aimed to formulate and optimize Thymoquinone loaded phytosomes using guggul lipid by thin-film method to enhance permeation and stability. 2 factor 2 level design (2^2) using SigmaTech software was employed contour plots were used to predict the responses. The two independent variables used were: the amount of guggul lipid (X1), amount of cholesterol (X2); and the responses included entrapment efficiency (Y1), *in-vitro* drug release (Y2), and particle size (Y3). The optimized formulation of Thymoquinone phytosomes was incorporated into Carbopol 934 gel base and 0.4% thymoquinone phytosomal gel was prepared. The phytosomal gel was evaluated. The stability study was performed, and phytosomal gel formulation was found stable at 4°C for 45 days.

Introduction

The transdermal drug delivery system (TDDS) includes all topically administered drug formulations intended to deliver the active ingredient into circulation.[1] The vesicular approach is among the foremost illustrious drug delivery methods for topical delivery.[2]

The active constituents of plants are usually polar or water-soluble. However, water-soluble phytoconstituents like flavonoids, glycosidal aglycones, tannins, etc. are poorly absorbed due to their poor lipid solubility or larger molecular size, a barrier in passive diffusion, resulting in their poor bioavailability.[3] Phytosomal drug delivery system is a newly introduced patented technology developed to incorporate the water-soluble phytoconstituents or standardized plant extracts into lipids to produce lipid compatible molecular complexes.[4] A stoichiometric ratio of 1:1 is considered to be the most efficient ratio for preparing phospholipid complexes.[5] These complexes enhance the bioavailability of the active constituents and protect the valuable component of herbal extract from being destructed by the digestive secretion and gut bacteria. As a result, they showed better absorption and improved pharmacological and pharmacokinetic parameters than conventional herbal extract.[6]

Thymoquinone is the major bioactive principal constituent of Nigella Sativa. It showed multiple health beneficial activities like antihistaminic, antibacterial, anti-hypertensive, hypoglycemic, anti-inflammatory, and anti-arthritic actions.[7] The in-vivo attenuation of the expression of cyclooxygenase-2 (COX-2) enzyme and the in-vivo induction of cytoprotective enzymes are attributes

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to the anti-inflammatory effect of Thymoquinone. It can inhibit 5-lipooxygenase (LOX) enzyme and COX-2 enzyme-induced arachidonic acid metabolism in rat peritoneal leukocytes.[8]

Guggul is a natural gum resin obtained from the plant Commiphora Mukul or Commiphora Wightii belonging to the family Burceacea. The anti-inflammatory effect is produced from Myrrhanol A, a triterpene of guggul, by decreasing regulation of inflammatory mediators such as interleukins, collagenase, hyaluronidase enzymes, and transcription factor.[9,10] In the phytosomal gel, guggul is used as a lipid, which has anti-inflammatory properties resembling the property of Thymoquinone and causes the synergistic effect. This helps in reducing the drug dose of the formulation and minimizing adverse effects.[11]

The design of experiments (DOE) technique was used to optimize the process variables of phytosomal gel composition efficiently. DOE is a technical approach wherein the cause and effect relationship between numerous process variables and the output can be effectively and efficiently explored. A sequence of experiments was performed to provide information about the factors and their interactions in minimum experiments as possible.[12] A 2-factor 2-level factorial experimental design technique was employed to investigate the variables like particle size, percentage entrapment efficiency and percentage drug release using the Sigmatech software.

Materials and Methods

Materials

Thymoquinone and Guggul lipid were obtained as gift samples from Sami Labs Limited, Bangalore, Karnataka, India. Cholesterol, potassium dihydrogen orthophosphate, sodium hydroxide, dichloromethane, and carbopol were purchased from S D Fine-Chem Limited, India. Methanol and triethanolamine were purchased from Fischer Scientifics, Mumbai, India.

Methods

Design of Experiment

A two-level factorial design was implemented using SigmaTech software which required an experiment to be carried out at all possible combinations of the two levels of each of the factors considered.[13] The independent variables used were, amount of Guggul lipid (X1) and the amount of Cholesterol (X2). The independent variables were screened using a multilevel factorial design (2^2), and four different formulations, four midpoints, and four replicates of Thymoquinone pyrosomes were obtained. All the formulations were prepared using the thin film hydration method and then evaluated for entrapment efficiency (Y1), *in-vitro* drug release (Y2), and particle size (Y3) to determine the optimized formulation.

Preparation of Phytosomes using Thin Film Method

The phytosomes of Thymoquinone were prepared using the thin-film method. An accurate amount of guggul lipid, cholesterol, and Thymoquinone were dissolved in a mixture of dichloromethane: methanol (2:1 v/v) in a dry, round bottom flask. The organic solvent mixture was allowed to evaporate in the rotary evaporator adjusted to 60 rpm, at 40°C for 15 minutes under low pressure to prepare a thin lipid film on the wall of the round-bottom flask. The film was hydrated with phosphate buffer pH 6.8 by rotating 60 rpm for 1 hour at room temperature. The multilamellar lipid vesicles (MLVs) were then sonicated using the ultrasonic probe sonicator for 30 minutes to reduce the vesicle size and stored at 4°C for further investigation.[14]

Characterization of Phytosomes

Entrapment Efficiency

5 mL of Thymoquinone (TQ) phytosomal complex was added to phosphate buffer pH 6.8 and was centrifuged at 4000 rpm for 45 minutes at 4°C. The unentrapped drug was separated by removing the supernatant. The sediment was lysed with methanol and analyzed at 255 nm using UV-visible spectrophotometer. The percentage drug entrapment was calculated by using the formula.[15]

EE% = 100*[Amount of Entrapped Thymoquinone/Total Amount of thymoquinone]

Size Determination by SEM Analysis

Approximately 5 µL of the TQ phytosomal suspension was transformed to a cover slip, which in turn was mounted on a specimen tab. The samples were dried at room temperature. The particle size of the formulation

Table 1: Formulation design of phytosomes of Thymoquinone

Formulation code	*Pf1*	*Pf2*	*Pf3*	*Pf4*	*Pf5*	*Pf6*	*Pf7*	*Pf8*	*Pf9*	*Pf10*	*Pf11*	*Pf12*
Combinations	I	X_1	X_2	X_1X_2	MP 1	MP 2	MP 3	MP4	R 1	R 2	R 3	R 4
Thymoquinone (mg)	200	200	200	200	200	200	200	200	200	200	200	200
Guggul lipid (mg)	237.5	580	237.5	580	408.7	408.7	408.7	408.7	751.2	66.2	408.7	408.7
Cholesterol (mg)	77.5	77.5	207.5	207.5	142.5	142.5	142.5	142.5	142.5	142.5	12.5	272.5
Dichloromethane (mL)	15	15	15	15	15	15	15	15	15	15	15	15
Methanol (mL)	5	5	5	5	5	5	5	5	5	5	5	5
Phosphate buffer pH 6.8 (mL)	20	20	20	20	20	20	20	20	20	20	20	20

was viewed and photographed using Scanning Electron Microscope.[15]

Determination of Particle Size and Zeta Potential

Size distribution and zeta potential of phytosomes were measured by a dynamic light scattering (DLS) method with Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.) at a temperature of 25°C and a scattering angle of 173. The samples (50 mL) were diluted to 1.0 mL with phosphate buffer pH 6.8 and each sample was measured three times. Zeta potential of samples was calculated.[16]

In-vitro Diffusion Study

The dialysis membrane was soaked in distilled water for 24 hours. In the Franz diffusion cell, the receptor compartment was filled with pH 6.8 phosphate buffer The donor compartment contained 5 mL of phytosomes on the dialysis membrane with an exposure area of 2 cm^2 to the receptor medium. The whole assembly was kept on a magnetic stirrer at 600 rpm for 10 hours, and samples were withdrawn at an interval of 1 hour for 10 hours and replaced with an 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/cm^2) against a time (h) plot. Permeability coefficient (Kp) =Jss/Co, (Co= initial Thymoquinone concentration).[15]

Preparation of Phytosomal Gel

The phytosomes were formulated into gel for ease in application. Carbopol 934 wasdispersed in water to prepare 1% w/w dispersion. The dispersion was mechanically stirred and then neutralized with 0.5% v/v triethanolamine solution. The neutralized dispersion was kept overnight to remove any entrapped air. Finally, Phytosomes were then added to the dispersion.[17]

Characterization of Phytosomal Gel

Homogeneity

Homogeneity of the phytosomal gel was tested by visual inspection by pressing a small quantity of the gel between the thumb and the index finger. The gel was tested for its appearance and presence of any aggregates. The consistency was determined as homogeneous or not.[18]

Spreadability

Two glass slides of 20 cm × 20 cm were selected. The phytosomal gel was placed between the slides. A 100 g was placed on the upper slide to press the gel uniformly, to form a thin layer. Without even the slightest disturbance, the weight was removed and fixed to a stand so that the upper slide slid off freely due to the force of weight tied to it. The time taken for the separation was noted using a stop-clock.

The following equation was used for this purpose:[18]

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

Where,

S - Spreadability

m - Weight tied to the upper slide l - Length of the glass

t - Time taken in seconds.

Viscosity

The viscosity of phytosomal gel was measured using Brookfield viscometer using spindle number S64 rotated at a speed of 12 rpm for a 10-s run time at 37°C.[19]

Measurement of pH

One gram of gel was dispersed in 20 mL of distilled water, and a digital pH meter was used to determine the pH value. The measurement was performed three times and the mean ± SD was calculated.[19]

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.[19]

In-vitro Drug Release study

The dialysis membrane was soaked in distilled water for 24 hours. The receptor compartment was filled with pH 6.8 phosphate buffer, and donor compartment contained 1 g of Phytosomal gel (equivalent to 5 mg) on dialysis membrane with an exposure area of 2 cm^2 to receptor medium. The whole assembly was kept on a magnetic stirrer at 600 rpm for 10 hours and samples were withdrawn at a specified time interval of 1 hour and replaced with an 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/cm^2) against a time (h) plot. Permeability coefficient (Kp) =Jss/Co, (Co = initial Thymoquinone concentration).[19]

Stability Study

The stability study of TQ Phytosomal gel (phospholipid and guggul lipid) was conducted at refrigerated temperature (4°C) and room temperature (30°C) as per Guidelines of International Conference on Harmonization (ICH). Samples were analyzed for physical appearance drug content and *in-vitro* diffusion study after 15, 30, and 45 days.[20]

Release Kinetic Profile for TQ 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 a system where the release rate is concentration-dependent. Higuchi's model described the release of drugs from the insoluble matrix as a square root of a time-dependent process based on Fickian diffusion.

The results of *in-vitro* release profile obtained for all the formulations were plotted in models of the 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

The following equation would predict Zero-order release:

$$At=A_0 - K_{0t}$$

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, the data obeys zero-order kinetics and its slope equals zero order release constant K_0.

First order kinetics

The following equation could predict first order kinetics:

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

Where,

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

C_0= Initial amount of drug.

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

The data plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows 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:

$$Q= [D\varepsilon/\tau\ (2A-\varepsilon C_{S)}\ C_S t]\ ½$$

Where,

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

D= Diffusion coefficient of the drug in the matrix.

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

C_S = Solubility of the drug in matrix.

ε= Porosity 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).[20]

Results and Discussion

Characterisation of Thymoquinone Phytosomes

As shown in Table 2, it was found that the prepared Thymoquinone phytosomes exhibited a good EE%,

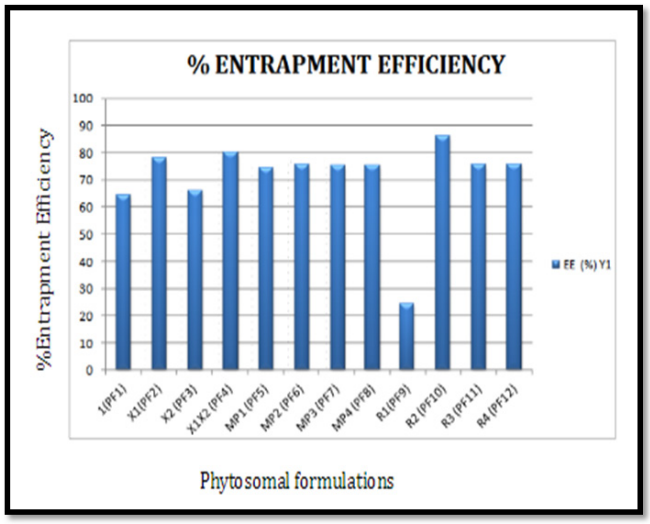

Fig. 1: Entrapment efficiency of Thymoquinone phytosomes

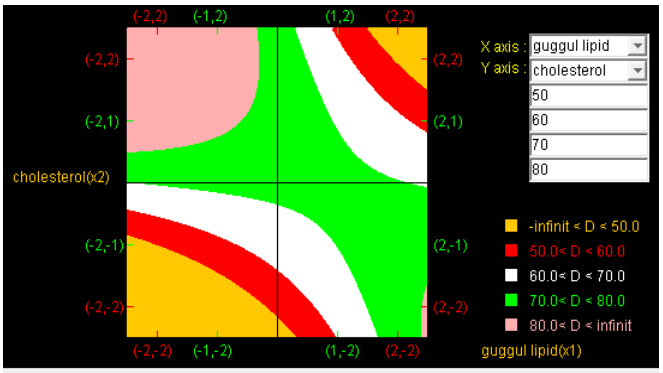

Fig. 2: Contour plot of Entrapment efficiency

Table 2: Characterization of Thymoquinone phytosomes

Combinations	*Entrapment Efficiency (%), Y1*	*In-vitro Drug Release (%), Y2*	*Particle Size (nm), Y3*
1(PF1)	64.33 ± 0.2	84.28 ± 0.9	228 ± 0.7
X1(PF2)	78.23 ± 0.5	76.16 ± 0.6	245 ± 0.4
X2(PF3)	65.87 ± 0.4	67.08 ± 0.5	202 ± 0.3
X1X2(PF4)	80.20 ± 0.6	82.28 ± 0.5	133 ± 0.9
MP 1 (PF5)	74.70 ± 0.7	65.86 ± 0.4	198 ± 0.6
MP 2 (PF6)	75.80 ± 0.7	66.51 ± 0.8	178 ± 0.4
MP 3 (PF7)	75.40 ± 0.3	69.12 ± 0.6	184 ± 0.7
MP (PF8)	75.30 ± 0.9	69.21 ± 0.7	173 ± 0.9
R1 (PF9)	44.40 ± 0.5	77.08 ± 0.3	200 ± 0.8
R2 (PF10)	86.27 ± 0.8	94.7 ± 0.9	112 ± 0.5
R3 (PF11)	75.80 ± 0.8	67.82 ± 0.2	202 ± 0.4
R4 (PF12)	75.7 ± 0.4	63.91 ± 0.4	188 ± 0.3

All values represent mean ± standard deviations (SD), n = 3

Table 3: *In-vitro* diffusion studies of phytosomal formulations

FC	1	X1	X2	X1X2	MP1	MP2	MP3	MP4	R1	R2	R3	R4
Time(min)	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	14.71 ± 0.18	17.68 ± 0.56	12.21 ± 0.35	18.27 ± 0.89	11.50 ± 0.43	11.62 ± 0.55	12.07 ± 0.24	11.77 ± 0.83	18.27 ± 0.34	18.45 ± 0.84	11.85 ± 0.47	11.16 ± 0.47
60	25.88 ± 0.3	31.84 ± 0.87	23.49 ± 0.4	32.84 ± 0.24	20.41 ± 0.37	20.61 ± 0.35	21.42 ± 0.37	24.80 ± 0.12	32.84 ± 0.12	33.16 ± 0.36	21.02 ± 0.24	19.8 ± 0.241
120	30.40 ± 0.83	38.68 ± 0.40	28.49 ± 0.24	39.81 ± 0.45	25.47 ± 0.21	25.72 ± 0.24	26.73 ± 0.11	31.21 ± 0.84	39.81 ± 0.83	40.21 ± 0.36	26.23 ± 0.48	24.72 ± 0.36
180	33.41 ± 0.23	42.95 ± 0.23	33.04 ± 0.24	46.88 ± 0.40	30.39 ± 0.19	30.69 ± 0.97	31.90 ± 0.98	34.99 ± 0.66	46.88 ± 0.34	57.64 ± 0.57	31.29 ± 0.82	29.49 ± 0.86
240	39.01 ± 0.52	48.29 ± 0.13	37.58 ± 0.83	49.80 ± 0.69	36.57 ± 0.13	36.93 ± 0.12	38.38 ± 0.10	39.15 ± 0.23	49.80 ± 0.24	61.62 ± 0.63	37.66 ± 0.74	35.49 ± 0.59
300	42.47 ± 0.76	54.86 ± 0.84	39.44 ± 0.73	57.08 ± 0.7	37.39 ± 0.47	37.76 ± 0.08	39.24 ± 0.37	42.26 ± 0.9	57.08 ± 0.12	68.05 ± 0.36	38.50 ± 0.37	36.28 ± 0.8
360	52.93 ± 0.13	59.09 ± 0.9	43.45 ± 0.78	61.01 ± 0.23	42.72 ± 0.24	43.15 ± 0.23	44.84 ± 0.55	50.31 ± 0.23	61.01 ± 0.24	77.85 ± 0.47	43.99 ± 0.24	41.45 ± 0.37
420	69.91 ± 0.89	61.93 ± 0.24	48.53 ± 0.46	72.17 ± 0.28	46.77 ± 0.13	47.24 ± 0.36	49.09 ± 0.23	55.03 ± 0.24	63.84 ± 0.25	83.96 ± 0.84	48.16 ± 0.57	45.38 ± 0.27
480	77.08 ± 0.24	65.57 ± 0.64	52.03 ± 0.24	77.08 ± 0.63	54.19 ± 0.51	54.72 ± 0.35	56.87 ± 0.98	58.19 ± 0.33	67.37 ± 0.73	88.15 ± 0.84	55.80 ± 0.45	52.58 ± 0.83
540	83.13 ± 0.35	69.76 ± 0.12	58.95 ± 0.32	83.13 ± 0.14	57.78 ± 0.25	58.35 ± 0.88	60.64 ± 0.22	66.88 ± 0.32	72.17 ± 0.55	90.8 ± 0.62	59.50 ± 0.84	56.07 ± 0.26
600	84.28 ± 0.2	76.16 ± 0.81	67.08 ± 0.38	82.28 ± 0.11	65.86 ± 0.19	66.51 ± 0.55	69.12 ± 0.84	69.21 ± 0.54	77.08 ± 0.84	94.7 ± 0.84	67.82 ± 0.28	63.91 ± 0.24

All values represent mean ± standard deviations (SD), n = 3

Table 4: Flux and permeability coefficient of the formulations

Formulations	*Flux (Jss) (μg/cm²/h)*	*Permeability Coefficient (kPa) (cm/hr)*
I (PF1)	2.811 ± 0.11	0.818 ± 0.62
X_1(PF2)	2.44 ± 0.55	0.467 ± 0.57
X_2(PF3)	2.18 ± 0.36	0.679 ± 0.46
X_1X_2(PF4)	2.402 ± 0.21	0.811 ± 0.76
MP 1 (PF5)	2.771 ± 0.32	0.456 ± 0.42
MP 2 (PF6)	2.310 ± 0.41	0.672 ± 0.39
MP 3 (PF7)	2.78 ± 0.93	0.609 ± 0.58
MP 4 (PF8)	2.82 ± 0.67	0.730 ± 0.65
R1 (PF9)	3.119 ± 0.88	0.822 ± 0.02
R2 (PF10)	3.876 ± 0.18	0.921 ± 0.78
R3 (PF11)	2.935 ± 0.57	0.758 ± 0.68
R4 (PF12)	3.119 ± 0.79	0.865 ± 0.82

All values represent mean ± standard deviations (SD), n = 3

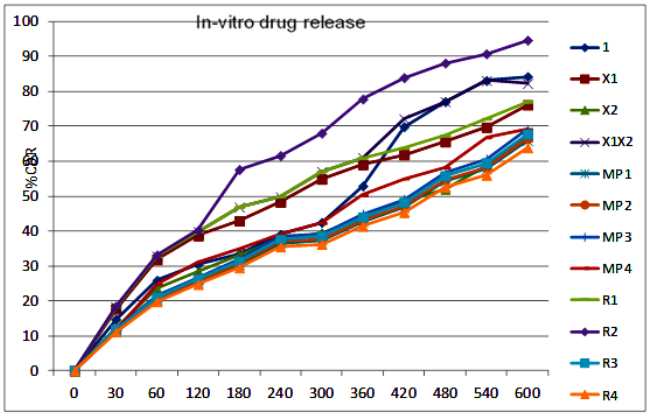

Fig. 3: *In-vitro* diffusion studies of phytosomal formulations

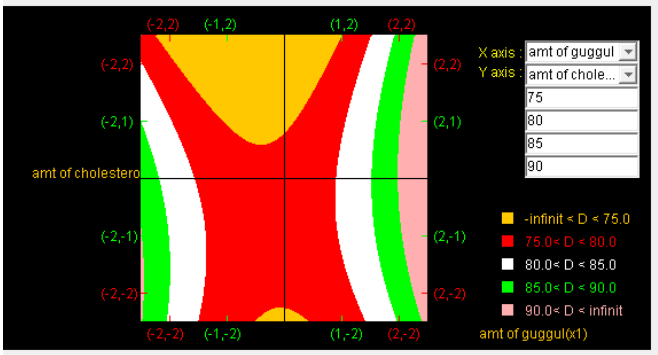

Fig. 4: Contour plot of *in-vitro* diffusion release

with values ranging from (44.4 ± 0.5) for R1 (PF9) to (86.27 ± 0.8%) for R2 (PF10).

In-Vitro Diffusion Studies:

Diffusion studies of all formulations were carried out using dialysis membrane for 600 minutes and samples were analyzed using double beam UV Visible Spectrophotometer.

Particle Size (Y3) and Zeta Potential of Thymoquinone Phytosomes

From the results it is found that all the Thymoquinone phytosomes have particle size less than 250 nm, and as such are effective for transdermal application.

Optimize Formulation

Table 5 showed the composition of the optimized formula R2 (PF10). After optimization of formulation variables it was found that the optimized formulation was suggested to contain 66.25, 142.5 mg of X1 and X2, respectively. Fig. 7 and 8 showed zeta potential and particle size distribution of the optimized formulation.

Characterization of the Optimized Formulation

Surface Morphology of Phytosomes

The Thymoquinone phytosomes were found to be spherical in shape and vesicle size was found to be in the range of 250 nm.

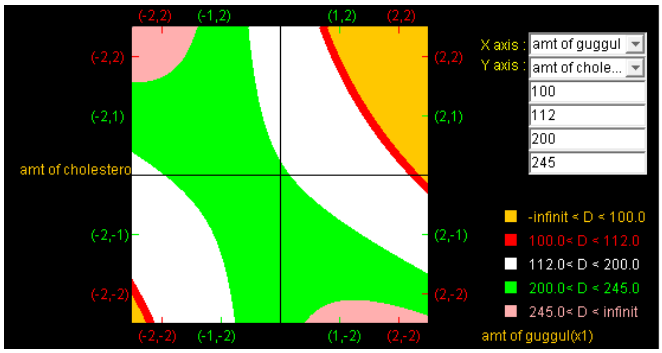

Fig. 5: Contour plot of particle size

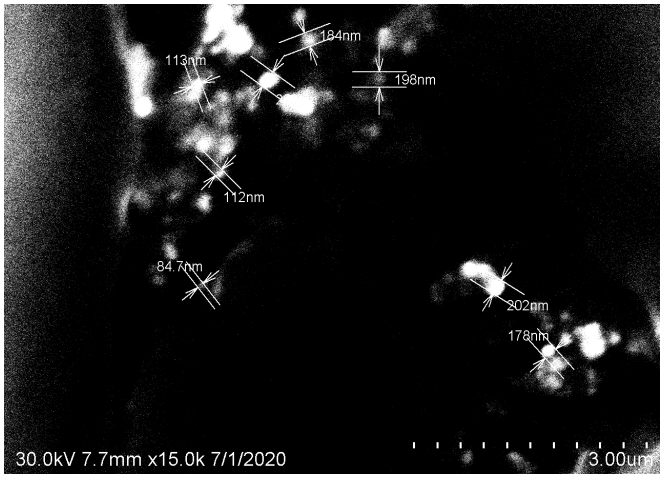

Fig. 6: SEM image of R2 (PF10)

Table 5: Composition of the factors and the responses of the optimized formulation

Optimize formulation		*Responses*	
X1	66.25 mg	Y1	86.27 ± 0.8 %
X2	142.5 mg	Y2	94.7 ± 0.84 %
		Y3	112 ± 0.5 nm

Particle size distribution and zeta potential determination

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

Formulation of Thymoquinone Phytosomal Gel

The thymoquinone phytosomal gel was prepared using guggul lipid with 1% Carbopol 934 as a gelling agent. The concentration of TQ in the prepared phytosomal gel was 0.4% w/w.

Evaluations of Thymoquinone Phytosomal Gel

0.4% Thymoquinone guggul lipid phytosomal gel

TQ phytosomal gel was smooth with a homogenous appearance. The spreadability value was 77.5 ± 0.26 cm, which indicates that the gel can be spared easily on

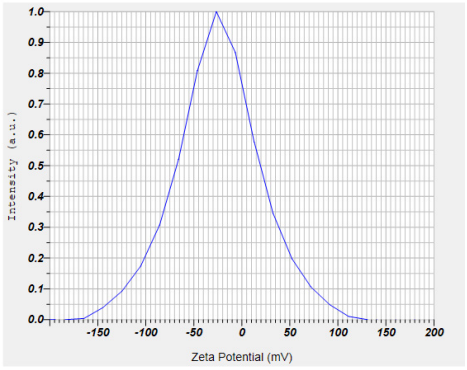

Fig. 7: Zeta potential of R2 (PF10) formulation

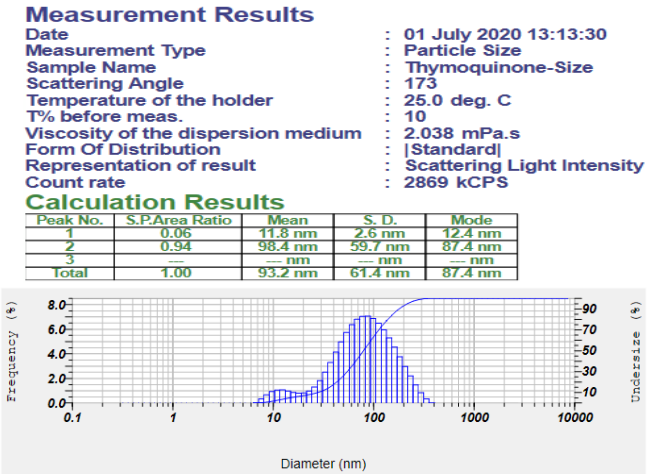

Fig. 8: Particle size distribution of R2 (PF10) formulation

Table 6: Evaluation of 0.4% Thymoquinone gel

Evaluation	*Results*
Homogeneity	Good
Spreadability (cm)	77.5 ± 0.26
Viscosity (pa/s)	18521 ± 0.75
pH measurements	6.5 ± 0.47
Drug content (%)	98.4 ± 0.66
In vitro drug release (%)	95.4 ± 0.36

the skin surface with little stress. The viscosity of 0.4% thymoquinone guggul lipid phytosomal gel was found to be 18521 ± 0.75 cps. The pH value was found to be 6.5 ± 0.47 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 98.4 ± 1.66 %, which represents good content uniformity. The in vitro drug release was found to be 95.4 ± 0.36 (Table 6).

In-vitro Drug Release

Pharmacokinetic Profiles for the Phytosomal Gel

The drug release kinetic studies were estimated to determine the type of release mechanism followed. Release kinetic study of guggul lipid phytosomal gel of Thymoquinone optimized was performed for different kinetic equations.

R^2 value for the optimized formulation of TQ phytosomal gel was found to be highest for the Higuchi model. This indicated that the drug release from all the formulations followed diffusion controlled 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.

Stability Studies

Stability studies were performed as per the conditions of ICH guidelines for climatic zone IV. Table 10 shows stability studies of the phytosomal gel. The studies showed that the phytosomal gel was found to be more stable at 4°C when compared to other temperatures. Phytosomal gel prepared using guggul lipid was more stable at 4°C in comparison to the stability of the phytosomal gel prepared

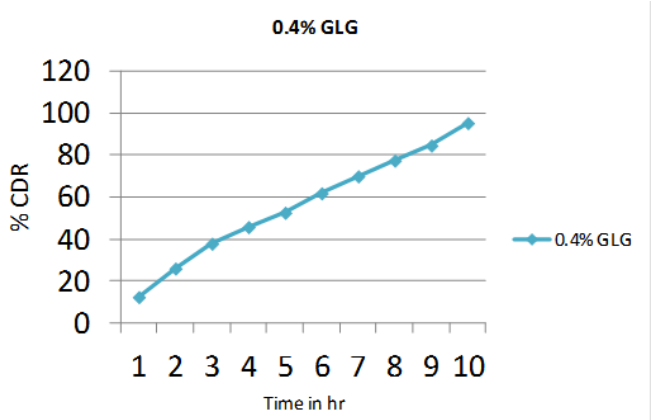

Fig. 9: *In vitro* drug release of 0.4% TQ phytosomal gel

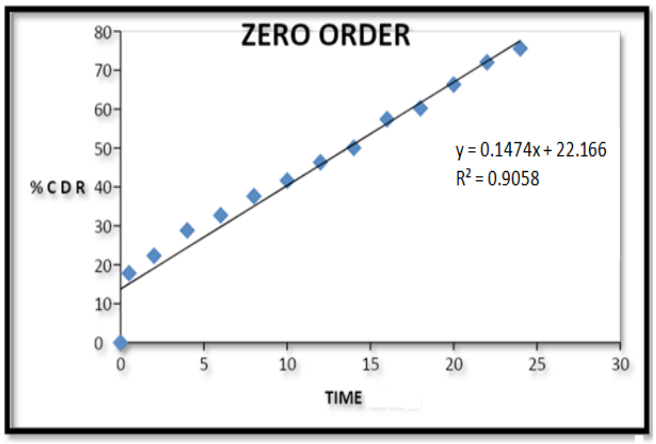

Fig. 10: Zero Order Kinetics for the Phytosomal gel

Table 7: Drug content and content uniformity

Formulation	*Drug content and content uniformity (%)*			*Mean ±SD*
Thymoquinone gel	98.2	98.8	98.6	98.4

Table 8: Cumulative drug release, flux and permeability coefficient of TQ gelOF 0.4% TQ phytosomal gel.

Formulation	*CDR (%)*	*Flux (Jss)(μg/cm²/h)*	*Permeability coefficient (kPa) (cm/h)*
0.4% thymoquinone gel	95.4 ± 0.36	4.75	1.174 ± 0.73

Table 9: Release kinetics for the phytosomal gel

PLOT	*ZERO*	*FIRST*	*HIGUCHI*	*PEPPAS*
x/y axis	*%CDR Vs T*	*Log % Remain Vs T*	*%CDR Vs √T*	*Log C Vs Log T*
Slope	2.657280722	-0.05333783	14.23132743	0.69450863
Intercept	13.78182621	2.097605075	0.71982	0.921521334
Correlation	0.979600184	-0.65822379	0.986315442	0.768192152
R^2	0.905816521	0.433258566	0.972818151	0.590119183

Table 10: Stability studies of 0.4% Thymoquinone phytosomal gel at refrigerated and room temperature

PARA-METERS/ TEMP (°C)	*0th day*		*15th day*		*30th day*		*45th day*	
	4°C ± 2°C	30°C ± 2°C	4°C ± 2°C	30°C ± 2°C	4°C ± 2°C	30°C ± 2°C	4°C ± 2°C	30°C ± 2°C
DC (%)	98.4 ± 0.66	98.4 ± 0.66	96.7 ± 0.32	92.4 ± 0.45	95.3 ± 0.88	87.8 ± 0.46	94.5 ± 0.56	83.9 ± 0.74
CDR (%)	95.4 ± 0.36	95.4 ± 0.36	95.7 ± 0.36	93.2 ± 0.85	96.3 ± 0.77	90.6 ± 0.38	96.9 ± 0.65	87.5 ± 0.36

All values represent mean ± standard deviations (SD), n = 3
DC- Drug Content, C.D.R- Cumulative Drug Release

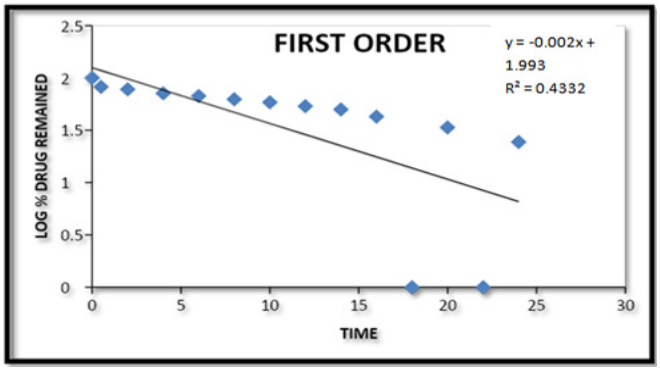

Fig. 11: First Order Kinetics for the Phytosomal gel

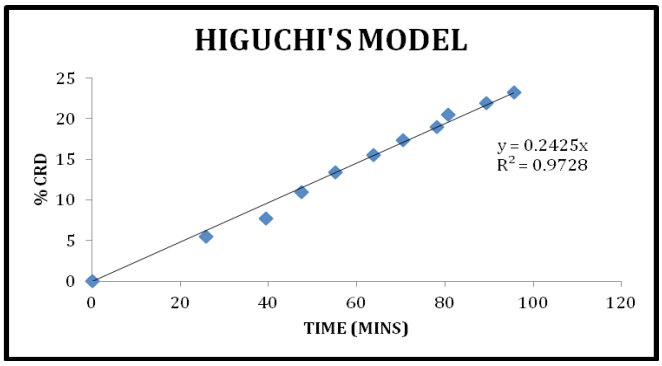

Fig. 12: Higuchi model for the Phytosomal gel

using phospholipid reported by Mannan, Safiya, et.al.[20] There was a change in color for the sample kept at room temperature. Thymoquinone phytosomal gel made up of Guggul lipid showed good physicochemical parameters along with good stability and permeation.

Conclusion

The aim of the study was to formulate and optimize Thymoquinone loaded phytosomes with guggul lipid using 2^2 factorial design. The optimized formulation contained 66.25 and 142.5 mg of X1 and X2, respectively, which gave high %EE (86.27), %CDR (88.15) and small particle size (112 nm). SEM of optimized TQ phytosomes appeared as spherical, well identified, unilamellar nanovesicles. The optimized formulation was incorporated into gel with concentration 0.4 % w/w of Thymoquinone. The spreadability value was 77.5 ± 0.26 cm, which indicates that it spreads easily on skin surface. The pH value was 6.5 ± 0.47 which was considered within the normal range of pH for topical preparations. The drug content of the gel was 98.4 ± 0.66%, which represents good content uniformity. The viscosity of was 18521 ± 0.75cps, the percentage drug release was 95.4 ± 0.36 which indicates that the phytosomal gel has high release and permeability. When release kinetics was applied, it followed Higuchi model and Quasi fickian mechanism. Finally, stability studies showed that phytosomal gel prepared using guggul lipid was more stable at 4°C when compared to room temperature. Thymoquinone phytosomal gel made up of Guggul lipid showed good release kinetics along with good stability.

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