# Synergistic Effect of Guggul-liposomes Lipid Nano-Vesicles for the Treatment of Inflammation

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## ABSTRACT

A gum resin i.e, guggul, obtained from *Commiphora Mukul,* was used to identify the anti-inflammatary effects. The present work of research is to identify the synergistic effects of Guggul-liposome lipid as a transporter and also as an anti- inflammatory drug. The preparation of guggul-liposomes was carried out by the usage of different concentration of cholesterol and guggul lipids with phenylbutazone by sonication method. A further characterization was performed and results of formulation F3 by sonication method were, average size (89.87± 1.294 %), entrapment efficiency (168.4± 1.41nm), sustained action (89.81±0.7) for 8hr and 85% protection .

*Keywords:* Guggul, guggul-liposomes, phenylbutazone, carbopol 934K, sonication, factorial design.

## 1.INTRODUCTION

Guggul-liposomes are new vesicular drug delivery system which owns several properties of liposomes, ethosomes and uses skin as the alternate route for delivery [1-2]. Inflammation can be described as 4 signs i.e., tumor or redness, swelling, heat and pain. Guggul produces biologically active compounds such as guggul lipid and Guggul-sterone (Z and E stereoisomer). From *Commiphora Mukul* a new Myrrhanol a triterpene is isolated, that reduces the directive of mediators of inflammation such as, collagenase, interleukins, hyaluronidase enzymes, transcription factor and produces potent anti-inflammatory effect [3]. In guggul-liposomes, phenylbutazone a drug was used, which consists of anti-inflammatory stuff and possess properties of guggul-lipid and ground effects i.e, synergistic, likewise benefits in plummeting dose of drug in formulations and minimize the side effect.

## 2. MATERIALS AND METHODS

Phenylbutazone (PBZ) was purchased from Sigma-Aldrich (New Delhi, India). It was obtained from Metro trading cooperation and Carbopol 934, ethanol (95%) was purchased from Merck and remaining other chemicals were gotten of analytical grade from Fisher Scientific with uppermost purity and quality [4-5].

### 2.1 Purification of Guggul

Hot water immersion technique was used to extract the Guggul from *Commiphora Mukul*. The 2 main objective of the Guggul purification was first to remove impurities and second to increase its medicinal value [6-7]. Purification was mainly done in 2 steps, first manually remove the external impurities and Second, Guggul was kept in muslin cloth bag and hanged in beaker for its immersion in double quantity of hot water for overnight. Next morning, muslin cloth content was thrown; water was heated (50 – 60ºC) with continuous stirring. Water reduction till half quantity, filtered and lenient mass was dried out in sun. Then guggul i.e, in pure form was obtained with minor quantity of ghee triturated. [8-9].

## 3. PREPARATIONS OF GUGGUL-LIPOSOMES

### 3.1 Sonication method

Accurately weigh Guggle lipid and dissolves in 10 milliliter of purified water on agitator on 700 rpm until complete dissolution, an another mixture of phenylbutazone and cholesterol in ethanol was prepared till the formation of thin layer [10]. Both the mixtures were diversified and attuned volume by 5% PVA solution upto 20 ml. The mix was sonicated (3 cycles for 5 min.) to form fine vesicles of guggul-liposomes [11].

### 3.2 Amalgamation of prepared Guggul-liposomes into carbopol gel

For an hour carbopol was sodden in lowest quantity of water with continuous stirring [12]. Prepared guggul-liposomes suspension (6ml) which contains phenylbutazone (150 mg), added to Carbopol solution, stirring at 30ºC on 700 rpm till even formation of guggul-liposomal gel. Tri-ethanolamine was additional to adjust pH 7.4 and Glycerin was added as humectant [13-15]. The Guggul-liposomal gel was leftward for 24 hr on (25 ± 1 ºC) [16]

## 4 EVALUATIONS OF GUGGUL-LIPOSOMES

### 4.1 Entrapment efficiency (%EE)

Entrapment was resolute by UV/Vis-spectrophotometry on 213nm and ultracentrifuge armed through rotor TLA-45 at 4℃ at 10,000 rpm intended for 1hr [17-18]. The quantity of drug tricked in the vesicles was considered by below given equation:-

$$\textbf{Entrapment Efficiency } \% = \frac{\textbf{amount of free drug}}{\textbf{total amount of drug}} * 100$$

### 4.2 Zeta potential and particle size analysis

This particular analysis of optimized guggul-liposomes suspension was strong-minded by dynamic light scattering (DLS) and the particle size dissemination was categorized using PDI, which govern width of size distribution [19]. Particle with highest positive zeta potential or lowest zeta potential are generally considered electrophoretically stable.

### 4.3 Viscosity

Brookfield DV III ultra V6.0 RV cone and plate rheometer was castoff to determine the viscosity of Guggul-liposomal gel at 25 ± 0.5ºC [20].

### 4.4 Scanning electronic microscopy (SEM)

This was done by SEM, using sample which was lyophilized was equestrian onto carbon-tape which were scheduled copper stubs secured by platinum coated, operated at 20 kV and examined on dissimilar exaggeration, 3300X.

### 4.5Atomic force microscopy (AFM)

AFM image was taken in AC mode and software was used to examine the images. Mica slip-ups were cast-off to make the slides for guggul-liposomal suspension. About 10 µl of the guggul-liposomal suspension was released on the carefully washed mica and permissible to dry on spin coater machine to make a thin film. Slide thickness was attuned physically by plummeting additional or fewer suspension accordingly. The arranged slide was reserved below the lens & examined at dissimilar intensifications and 3D structures were observed [21].

### 4.6 Attenuated total reflection- Fourier transform infrared spectroscopy (ATR-FTIR)

Guggul-liposome's IR spectra, laden through guggul (GL), cholesterol (CH), phenyl butazone and formulation with PVA were examined by means of spectra at room-temperature by Bruker spectrophotometer through MCT detector at a trifling determination of 2 $cm^{-1}$. A progressive ATR alteration was functional to entirely spectra, & the section from 400to 4000 cm-1 was peak fit using software(Opus).

### 4.7 Differential scanning calorimeter (DSC)

Contact among polymer and drug was resolute by DSC. The models (samples) were positioned in pan of aluminum & shut through lid trailed through heating system over $N_2$ (flow 30ml/min) on perusing proportion of 5ºC /min at 25ºC to 200ºC. The examination was documented and strategically display temperature on the X-axis &heat flow (w/g) on the Y-axis [22].

### 4.8 Thermo gravimetric analysis (TGA)

TGA study of cholesterol (CH), phenylbutazone (PBZ), poly-vinyl alcohol (PVA), guggul (GL), and lyophilized formulations was completed by the assistance of (TGA 400) PROTEUS thermal analysis. It was also castoff to govern vaporization, absorption, weight loss, sublimation, adsorption etc. The minor volume of testers was reserved in a crucible & afterward tarring the mass of crucible was set aside in assemblage and run the software. The quantity of mass loss graphs were found and reported [23].

### 4.9 *In- vitro* drug release

The study of release of phenyl butazone guggul-liposomal gel was considered on cellulose acetate membrane, was waterlogged for 24 hrs, previous to work. Clamp the diffusion tube and immersed in phosphate buffer in a beaker of pH 7.4. At 37ºC, it was kept and 1gm of phenyl butazone guggul-liposomal gel & phosphate-buffer 7.4 was supplementary in (donor section) diffusion tube and enclosed with para-film to evade evaporation of formulation. In the receiver compartment, the phosphate buffer 7.4 was reserved and continuously stirred at 500 rpm. As after receptor section 3ml solution was with-drawn at particular time interval and replaced by buffer solution in order to kept constant volume of receptor [9]. The concentrations of drug in the aliquot were unwavering at 213 nm in contradiction of suitable blank. The data of drug release was regularized by changing the drug concentrations in explanation to a %CDR and are shown graphically [20-23].

### 4.10 Anti-inflammatory activity by edema inhibition

This study was conducted on healthy albino rats by Carrageenan induced paw edema method. Rats which were healthy of weight 140-200 gm of any sex remained abstained for 12 hrs. Preceding tests, rats were alienated into 2 groups, first served as control and second for guggul-liposomes (100mg/Kg). 30 minutes later, all collections of animals were injected 0.1ml of 1% Carrageenan, a sub-plantar injection in left-hind paw. After 1hr, foot size was leisurely by plethysmometer on a time interval of hours [11]. The calculation of % edema inhibition was done by following formula:

$$\% \text{ Edema inhibition} = \frac{(Vt-V0)control-(Vt-V0)treated}{(Vt-V0)control} * 100$$

Where, Vt = mean paw volume of test

Vo = mean paw volume of control

### 4.11 Stability study

It was achieved, both for guggul-liposomes suspension and lyophilized formulations to examine several loss of drug from guggul-liposomes and stability effect during storage conditions [24]. Both formulation F1 and F3 guggul-liposomes suspensions and lyophilized samples were stowed in the vials of glass at 4ºC for 3 months. Subsequently 7, 15, 30, 60 and 90 days of storage, samples that are lyophilized were dispersed in HPLC water and guggul-liposomes suspension was sonicated directly for 10-15sec in bath-sonicator & considered aimed at zeta-potential, size, and PDI and competence of encapsulation. DSC thermal analysis study was performed to study the effect of time and temperature on the thermal properties of both the guggul-liposomes samples.

### 4.12 Response surface methodology

Prepared, six Guggul-liposome formulations & the independent variable's effect on reactions (dependent influences) were assessed by means of RSM & are accessible in Fig 3, [29-30]. 2 variables that are independent were selected: quantity of cholesterol (CH, $X_2$) and quantity of guggul lipid (GL, $X_1$) and every variable was verified at diverse concentrations, the amounts of which are articulated in their own units. The responses appraised were: Entrapment efficiency (%EE, $Y_1$), cumulative drug release (%CDR, $Y_3$) and particle size (PS, $Y_2$). Statistical software V.10_ (Stat Soft, Inc. USA) was castoff aimed at cohort and estimation of surface's response.

## 5. RESULTS AND DISCUSSIONS

### 5.1 Percentage entrapment efficiency (% EE)

With upsurge in concentration of cholesterol with persistent quantity of drug, the % EE were initiate to be 62.4 ± 0.141, 67.6 ± 1.414, 72.3 ± 1.414, 78 ± 0.989, 86.2 ± 1.060, 89.87 ± 1.294. F3 formulation with uppermost quantity of cholesterol (400mg), guggul-lipid (400mg) through continuous quantity of drug presented well & extreme efficiency ofen trapment. A suitable ratio of cholesterol concentration and guggul plays a significant part in the efficacy of entrapment; this owes to rise in complete lipid concentration. Hence, this reveals that the main optimization parameters for preparation of guggul-liposomes were the molar ratio of guggul lipid, cholesterol and drug present.

### 5.2 Particle size analysis

The PDI & particle size were resolute by using, dynamic light scattering technique and are shown in Table 1**.** F3 Formulation containing cholesterol (400mg) & guggul lipid (400mg) with the similar quantity of drug (150mg) has revealed (168.4± 1.41) mean particle size, which is an formulation which is optimum,consuming PDI (0.564±0.19). There was no significant change in guggul-liposomes particle size and PDI before and after lyophilization.

**Table 1: Composition and characteristics of guggul-liposomes by sonication method**

| **Composition** | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** |
|---|---|---|---|---|---|---|
| Guggul (mg) | 400 | 300 | 400 | 300 | 200 | 100 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Drug (mg) | 150 | 150 | 150 | 150 | 150 | 150 |
| Cholesterol (mg) | 300 | 200 | 400 | 300 | 100 | 200 |
| Span (%) | 3 | 3 | 3 | 3 | 3 | 3 |
| Ethanol (ml) | 5 | 5 | 5 | 5 | 5 | 5 |
| Water (ml) | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. |
| **Characterization** | | | | | | |
| Entrapment Efficiency (%EE) | 86.2±1.060 | 72.3± 1.414 | 89.87±1.294 | 78±0.989 | 67.6±1.414 | 62.4±0.141 |
| Particle size (nm) | 190.4± 1.55 | 192.2± 1.06 | 168.4± 1.41 | 189.7± 1.83 | 192.3±1.484 | 197.8±1.838 |
| Cumulative Drug Release (%CDR) | 64.2 ± 0.70 | 67.89±0.72 | 89.81±0.7 | 70.19±0.707 | 79.5 ±0.701 | 77.59±1.41 |
| Zeta-Potential (mV) | -17.8 | -19.2 | -58.5 | -20.2 | -17. 1 | -17.5 |
| PDI | 0.255±0.21 | 0.222±0.11 | 0.564±0.19 | 0.268±0.22 | 0.398±0.12 | 0.387±0.10 |
| Spread-Ability(cm) | 14.73±0.41 | 12.22±0.28 | 12.97±0.58 | 11.24±0.15 | 14.21±0.56 | 14.6±0.45 |
| Viscosity (Cps) | 5034±0.635 | 5846±0.587 | 6965±0.761 | 5097±0.770 | 5498±0.610 | 5285±0.796 |

### 5.3 Zeta potential

The variation showed by the measurements, revealed that the vesicle sizes might use an effect on the charges, the vesicles carries. The outcomes of zeta-potential for dissimilar formulations are show in Table 1. Formulations F5, F6 and F1 with low cholesterol concentration shows low zeta potential though with uppermost conc., of cholesterol in formulations F2, F3 and F4, shows an upsurge in zeta-potential worth. These conclusion discloses that an increase in the cholesterol concentration, charge of surface (-ive) on the guggul-liposome upsurges and therefore lead to be augmented in the constancy. This consequence obviously shows that the F3 (formulation) is the greatest unchanging formulation ready through sonication method [25].

### 5.4 (SEM) Scanning electron microscopy

Thespitting image of drug loaded F3 (formulation)exposed in Fig 1**,** indicating that the guggul-liposomes ready by sonication technique (F3) recollect their sphere-shaped vesicles by fewer size range of 200-550 nm.

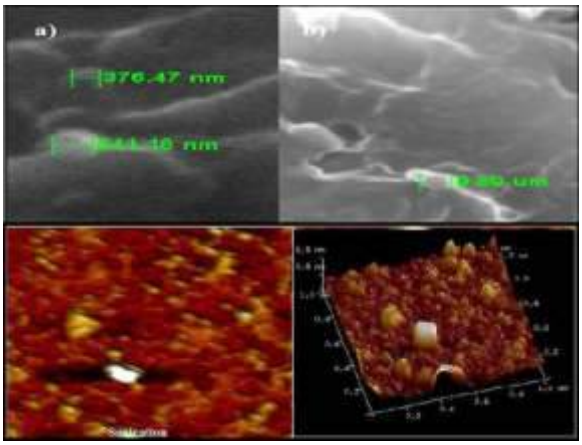


**Figure 1: Scanning electron microscopy (SEM) and atomic force microscopy (AFM) of guggul-liposomes.**

### 5.5 (AFM) Atomic force microscopy

The 3-D image of guggul-liposomes of formulation F3 was shown in Fig 1, which delivers info about the morphological features of guggul-liposomes suspension which was prepared. Additional, the superficial morphology establish the elevation of the guggul-liposomesready by sonication method (F3) obligate height 3.157(nm), zone 3593.191(nm²), diameter 63.287(nm), which clearly indicates that the formulation (F3) shows interaction with the substrate resulted in a flattened structure since the measured diameter of the average sized structure was ≃200 nm. With the assistance of AFM, it was too conceivable to detect that the exhibitedguggul-liposomes was having smooth surface and spherical in shape.

### 5.6 (ATR-FTIR) Attenuated total reflection-Fourier transform infrared spectroscopy

The respective outcomes of drug (pure), guggul-liposomes loaded withphenylbutazoneand polymer comprisingforemostpeaks with absorption (bands) are portrayed in Fig2 **(a)** guggul **(b)** F3 **(c)** phenylbutazone.

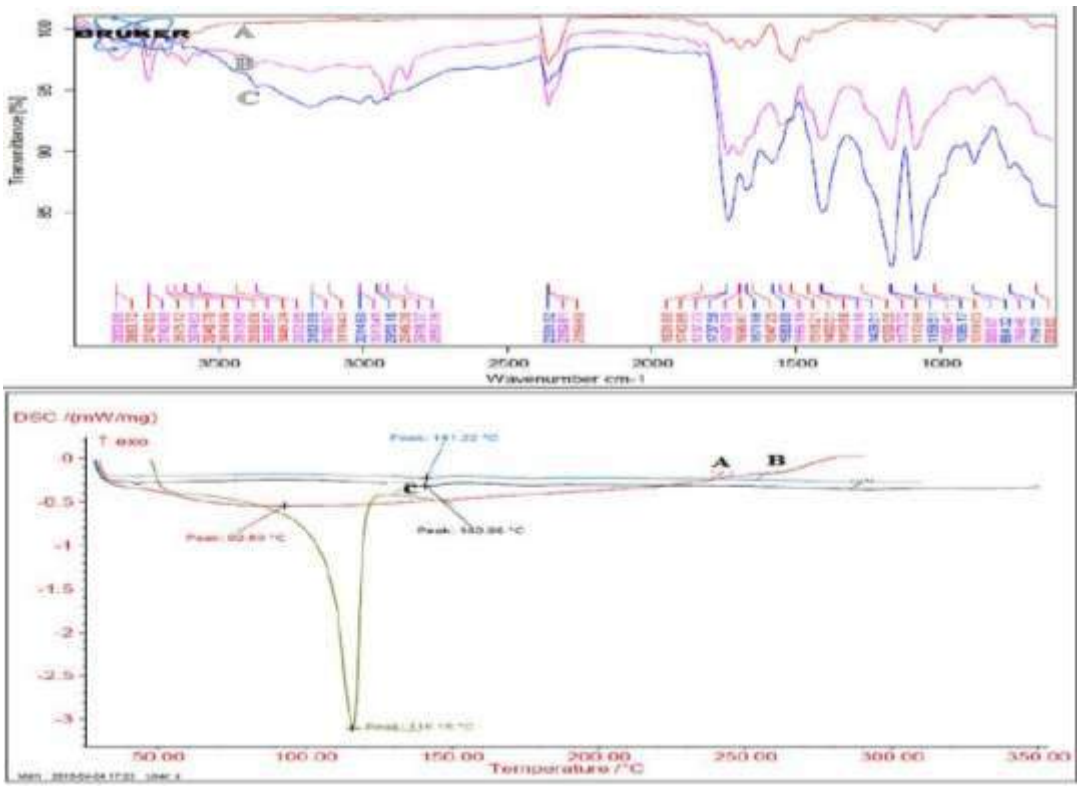


**Figure 2: ATR-FTIR spectra and (DSC) thermogram of (a) Guggul (b) F3 (c) Phenylbutazone.**

The drug spectra displays most important distinctive bands of absorption, peaks at 3014.60 cm-1 due to C-H stretching, 3183.97cm-1 due to N-H stretching,2955.18 cm-1 due to C-H vibration, due to -C=C stretching 1671.98 cm-1, 1737.58 cm-1 due to C=0 (ketone), 1409.31cm-1due to $CH_2$ bending, due to stretching of aromatic (C=C) group1583.80 cm-1, 1383.07, 1260.96 cm-1&1340.77 due to O-H bending, 1026.82 cm-1and 1095.11 due to C-F bending, 884.52 cm-1 due to distribution of aromatic protons, due to C-0 stretch 1169.51 cm-1 &759cm-1 due to $CH_2$ rocking correspondingly. Formed formulations were likewisego through scanning for the similararea and establish that the peaks lienearly in the precisenearby range. The formulation F3 peakswere obtained to be actualnearer to the peaks of drug i.e, pure,phenylbutazone i.e. 884.52 cm-1,1085.17, 1169.51, 1583.80, 1737.58, and3014.60, the outcomeexposed that there was no substantialmodification in the IR peaks of optimized formulation &phenylbutazone which representing the nonappearance of any interactionamong the polymer&drug.

### 5.7 (DSC) Differential scanning calorimetry analysis

The results of the lyophilized guggul-liposomes having same melting peaks and heat of enthalpy compared to pure drug are exposed in Fig 2, i.e, A) guggul-lipid B) F3 C) phenylbutazone. The thermogram of DSC of guggul-liposomes displayed a piercing endothermic-peak of drug at 116.15°C. The melting point of cholesterol, guggul-lipid, was gotten at 148.63$^{o}$C, 92.89$^{o}$C. The melting point peaks obtained was near about the drug, with low intensity and broad endothermic peaks compared to the pure drug. Thus it concludes that the results of DSC analysis for drug

loaded guggul-liposomes F3 indicate all the lipid and polymer components interact with each other to a great extent [26].

### 5.8 Thermo gravimetric analysis (TGA)

In this examination, the formulation F3 (A) which was optimized, guggul lipid (B), and drug phenylbutazone (C) were exposed to measure programming of temperature in a controlled atmosphere. TGA graph of pure drug presented that the mass endured continuous with cumulative temperature but as it move toward drug's melting point, the ongoing to tumble down. Alike marvel was also experimental by the formulated guggul-liposome F3, which exposed a sharp dropping of the bend at the 250ºC, that discloses that the amalgamation of guggul-lipid through the drug augments its constancy. All these conclusions exposed that moisture content or excipients had no adverse-effect on formulations.

### 5.9 *In-vitro* release study

F1 to F6 formulations was considered for the release study and the consequences of the similar were displayed in Fig 3. F3 formulation ready by sonication technique showed extreme % CDR profile owing to great drug loadingin guggul-liposomes in contrast to additional formulation. Release of 89.8±0.7% phenylbutazone from guggul-liposomes displayed release of drug for 24hour and spurt release in primarily 8hour and slowly release declines &develops continuous in 18 hours and the release of drug was constant due to adding of cholesterol. F1- F2 formulation exhibited least phenylbutazone release,due to upsurge in quantity of cholesterol which surges the inflexibility of guggul-liposomes vesicles. The outcomes portrayed that difference in lipid concentrations might modify the patterns of release of drug in all formulation which was also obtained by [27], that the formulations with higher drug content have higher release rates.

### 5.10 Anti-inflammatory activity by edema inhibition

The % defender of carrageenan persuaded paw edema in rats was treating with phenylbutazone guggul-liposomes. The optimized formulation F3 was given to rats of ready guggul-liposomes. The treated and controlled rats were observed for an interval of hrs on plethysmometer and anti-inflammatory effect was resolute by rat's paw size, therefore the interpretation gotten by % inhibition ofedema on certain intermission of time exposed that there was higher percent protection in phenylbutazone loaded guggul-liposomes. The same was obtained by that formulation with higher concentration of guggul showed maximum percent edema inhibition.

### 5.11 Stability studies

The guggul-liposomes were reserved in RT long-established reduction in % entrapment efficiency from 89.87± 1.294 to 77.82± 2.642 & noteworthy surge in particle size (from 168.4±1.41 nm subsequently 7 days 174.6± 1.07nm). This was associated with noteworthy increase in PDI from less than 0.564± 0.19 to 0.573± 0.47 and lessening zeta potential about -24.5 to -23.8 mV [27]. These are cyphers accumulation of particlesand seepage of drugencapsulated. Afterward 7 days the RT samples had too abundantaccumulation with cumulative in particle size 198.7± 1.26, 233.2± 0.14, 256.1± 2.73, 289.5± 1.63nm,with reduction

in % EE, 73.61± 1.201 , 68.64± 1.532 , 61.07± 1.223 , 59.10± 1.016 afterward 90 days correspondingly. This end result also reinforced through the declining value of zeta potential respectively & cumulative value of PDI. In contrast, the guggul-liposomes at 4ºC stowed were fairly unchanging as smallest development in PDI; size and reduction in zeta potentials & % EE later stowage of 90 days were detected. The optimized storage state for F3 formulation, was establish to be steady at 60 ± 5% /RH 4 ± 2 °C.

**5.12 Response surface analysis**

The response surface plots representing the relationship between the studied factors and measured responses are demonstrated in Fig 3.

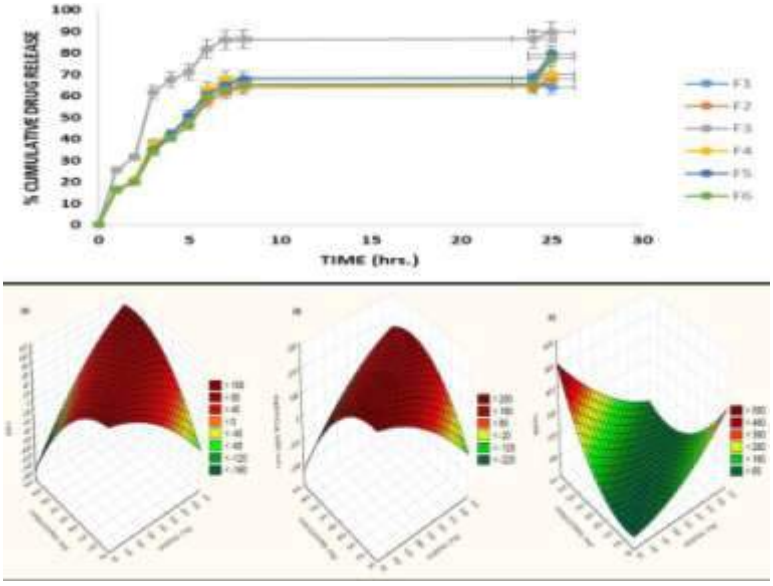


**Figure.3. Cumulative drug release and response surface methodology of F1-F6 guggul-liposomal formulations.**

A positive value in polynomial equations corresponds to an effect that favors the optimization, while a negative value corresponds to an inverse relationship between the factor and the response. In accordance with the above stated equation for %EE, an increase in the concentration of cholesterol and guggul lipid leads to upsurge in the efficiencyof entrapment. The conceivable motive behindhand was that morelipid contented averts the leakage of drug to external milieu through efficiently enfolding it. Whereas, it was investigated that,upon declining the conc. of the cholesterol, &guggul-lipid, the size of the particles of the formulation rises. Accordingly, the RSM forecast a precise significance outcome of formulation with the purpose of optimized one.

## 6. CONCLUSIONS

Guggul-liposomes were set by sonication technique by means of diverse lipids ratio in order to increase topical delivery. As of all overhead consequences exposed that prepared F3 formulation with ratio (guggul:400::phenylbutazone:150mg::cholesterol:400) was augmented as anticipated zeta potential,particle size,*in-vitro* drug release and entrapment efficiency was attained & also guggul-liposomes with phenylbutazone display wanted effects on paw edema's inflammation

treatment. Therefore results exposed that guggul-lipid is a brilliant carrier and with phenylbutazoneits possessions i.e,synergistic, provides sustainable achievement, well entrapment and improved defense as of inflammation. Consequently, this system of delivery can be castoff as design for improved continuous & synergistic activities aimed at inflammation.

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