Guggulosomes: A herbal approach for enhanced topical delivery of Phenylbutazone

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Abstract

Background: Guggul, the gum resin obtained from Commiphora mukul, is one of the components of various formulations of traditional Ayurvedic medicine to treat inflammation, atherosclerosis, and weight loss. This research work describes Guggul lipid as a carrier and synergistic effect for anti-inflammatory drugs.

Method: Guggulosomes prepared by trituration method was further incorporated into carbopol gel, developed for the enhanced topical drug delivery system for the phenylbutazone. This preparation was characterized by using Attenuated total reflection- Fourier transform infrared spectroscopy (ATR-FTIR), Scanning electron microscopy (SEM), Atomic force microscopy (AFM), Differential scanning calorimeter (DSC), Thermo gravimetric analysis (TGA), Particle size & Zeta potential and further guggulosomes was tested for Anti-inflammatory activity and % cumulative drug release. All these study was carried out by using standard protocol.

Results: The guggulosomes shows all the characteristic peaks in ATR-FTIR. The optimized formulation was spherical in shape having diameter >200nm. The % entrapment efficiency was 77.2±0.212 with good % Cumulative drug release 60.80±0.707%. The anti-inflammatory activity of the optimized formulation was found satisfactory (89%).

Conclusion: The guggulosomes prepared by trituration method was found to give a synergistic effect along with the phenylbutazone when incorporated into the carbopol gel. Thus, this present research shows that it can be good perspective for enhanced topical drug delivery system.

Key words: Guggulosomes, Trituration, Full Factorial design, Anti-inflammatory activity

1. Introduction
The topical drug delivery system offers major obstacle of low diffusion rate of drug across the skin. Several strategies were proposed to increase permeation of drugs across the membrane. One of the most promising strategies is vesicle based permeation [16]. Vesicles in topical drug delivery can be used as permeation enhancer, deliver entrapped drug through skin and act as rate limiting membrane barrier and modulate systemic absorption.

Guggul is a natural gum resin which is obtained from plant Commiphora Mukul or Commiphora Wightii belonging to family Burceaceae. This oleo gum resin is generally used in Ayurvedic formulations for treatment of obesity, inflammation, rheumatism, acne, arthrosclerosis etc. The purpose of using guggul is to produce synergistic effect and to provide medicinal value to Guggulosomes. Guggul produces bioactive compounds such as guggulsterone (E and Z stereoisomer) and guggul lipid. A new triterpene Myrrhanol A is isolated from Commiphora Mukul which produces potent anti-inflammatory effect by reducing regulation of inflammatory mediators such as interleukins, transcription factor, and collagenase and hyaluronidase enzymes [5]. Guggulsterones also supports in platelet functioning and fibrinolytic activity and also in maintain cardiovascular support [6-7]. Phenylbutazone is an acidic, lipophilic, NSAID, categorized under anti-inflammatory category has been recommended for arthritis, pain, inflammation property. It bounds highly to plasma protein. Phenylbutazone exerts its anti-inflammatory action by inhibiting the Cyclooxygenase enzyme and by inhibiting inflammatory mediators such as PGs. Using guggulosomes topically can eliminate side effects, increase patient compliance and avoid first pass metabolism. In guggulosomes, phenylbutazone was used as drug which has anti-inflammatory property which resembles with property of guggul lipid and causes synergistic effect also helps in reducing drug dose in formulations and helps in minimizing side effect.

2. Material and methods

Guggul was obtained from Metro trading cooperation, Phenylbutazone (PBZ) was purchased from Sigma-Aldrich (New Delhi, India) and ethanol (95%), carbopol 934 was purchased from Merck specialties Pvt. Ltd., Mumbai, India and all other chemicals were obtained from Fisher Scientific of analytical grade with highest quality and purity.
2.1. Purification of guggul

Guggul was extracted from Commiphora Mukul by hot water immersion technique. Guggul purification provides with two objectives first to increase its medicinal value and next to remove impurities. Purification is done in 2 steps. Firstly external impurities are removed manually such as dry leaves and other foreign material. Secondly by keeping guggul in muslin cloth bag was hanged in beaker so that it is immersed in hot water for overnight. Quantity of water should be more than guggul. Next morning, content of muslin cloth is thrown; water is heated gently at low temperature to prevent loss of volatile oils and other constituents (50 – 60°C). Mixture is stirred continuously. When water is heated till half quantity it is filtered (generally when hot as it solidifies on cooling) and soft mass is dried in sun. After pure guggul is obtained it is triturated with small amount of ghee or butter [8].

2.2. Preparation of Guggulosomes by trituration method

Guggulosomes were prepared by using different ratios of purified guggul lipid and cholesterol with ethanol and water. Guggul lipid was weighed accurately and dissolved in 10 milliliters of distilled water with continuous stirring on magnetic stirrer at 700 rpm till guggul was completely dissolved in distilled water, phenylbutazone and cholesterol was dissolved in ethanol till a thin layer is formed. Both mixtures were mixed and adjusted volume up to 20 milliliters by 5% PVA solution. Mixture was triturated continuously to result in fine vesicles.

2.3. Incorporation of prepared Guggulosomes into Carbopol gel

Carbopol 934 K 1% w/v was soaked in minimum amount of water for an hour. The swelled mass of Carbopol was stirred till carbopol completely dissolved in distilled water. Prepared guggulosomes suspension (6ml) containing phenylbutazone (150 mg) was added to Carbopol solution on continuous stirring at 700rpm at 30°C until uniform guggulosomal gel was formed. The pH was then adjusted to 7.4 by tri-ethanol amine. Glycerin was added to the formed guggulosomal gel which serves as a humectant which enhance the skin hydration, thus increases the drug penetration through skin. The guggulosomal gel was left equilibrating for 24 h at room temperature (25 ± 1°C) [8].
2.4. Characterization of guggulosomes

2.4.1. Attenuated total reflection-Fourier transform infrared spectroscopy (FTIR)

Infrared spectra of guggulosomes loaded with phenylbutazone, guggul (GL), cholesterol (CH), and polyvinyl alcohol formulation were analyzed by using a ATR-FTIR spectra at room temperature by Bruker EQUINOX 55 FTIR spectrophotometer equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector at a nominal resolution of 2 cm\(^{-1}\). The internal reflection element (IRE) was a diamond, with an incidence angle of 45°, scans 32, 21 resolutions leading to one internal reflection. An advanced ATR correction was applied to all spectra, and the region from 4000 to 400 cm\(^{-1}\) was peak fit using Opus software.

2.4.2. Scanning electronic microscopy (SEM)

Surface morphology was conducted by scanning electronic microscopy SEM (CSIR-CEERI, Kota, Rajasthan) by using lyophilized sample was mounted onto double sided carbon tape secured on copper stubs and coated with platinum operated at 20 kV and then analyzed at different magnification of 3300X.

2.4.3. Atomic force microscopy (AFM)

In this study, Atomic Force Microscope (AFM) was carried out on AIST-NT (model no. Smart SPM 1000). The images of the optimized guggulosomes were captured in AC mode and imaging tip is used for the guggulosomes is AIST FP Tip no. 01. The software used for the capturing of the images is AIST-NT SPM Control software and Mica slips were used to prepare the AFM slides for guggulosomal suspension. Approx. 10 µl guggulosomal suspension was dropped onto the mica slip and then a thin coating was formed using spin coater dryer machine. Thickness of slide adjusted manually by dropping more or less suspension consequently. The prepared slide was kept under the lens and analyzed at different magnifications and three dimensional structures were observed.

2.4.4. Differential scanning calorimeter (DSC)
Interaction between drug and polymer was determined by differential scanning calorimeter NETEZCH DSC 204 F1 phoenix comprised of calorimeter (DSC 60), flow controller (FCL 60), Thermal analyzer (TA 60) and operating software TA 60. The phenylbutazone (PBZ), guggul (GL), cholesterol (CH) ,polyvinyl alcohol (PVA)and lyophilized formulations was performed by using DSC-60. The samples were kept in aluminum pan and closed by lid followed by heating through nitrogen (flow 30ml/min) at scanning rate of 5°C /min from temperature range 25°C to 200°C. In same quantity indium was used as reference in aluminum pan. Heat flow as a function of temperature was measured of drug, excipients and mixtures. The scan was recorded and plotted showing heat flow (w/g) on the Y-axis and temperature on the X-axis [20].

2.4.5. Thermo gravimetric analysis (TGA)

TGA studies of phenylbutazone(PBZ), guggul (GL), cholesterol (CH) ,polyvinyl alcohol (PVA)and lyophilized formulations was done in order to study physical and chemical properties with the help of PROTEUS Thermal analysis (TGA 400). TGA is also used to determine weight loss, vaporization, sublimation, absorption, adsorption, etc. TGA is generally used to conclude selected characteristics of samples that show either weight loss or gain due to decomposition. The small amount of samples was taken in a crucible and after tarring the weight of crucible was kept in assembly and software was made to run. The amount of weight loss graphs were obtained and reported [21].

2.4.6. Entrapment efficiency

Amount of drug entrapped is calculated by deducting amount of un-entrapped drug by amount of total drug added initially. Entrapment capacity of phenylbutazone loaded guggulosomal suspension was determined by ultracentrifuge (Remi) equipped with TLA-45 rotor at 10,000rpm at 4°C for 1hr. After separation of phenylbutazone entrapped guggulosomes vesicles, amount of un-entrapped determined by using UV/Vis-spectrophotometry at 213nm. Each sample was analyzed in triplicate [22-23]. The amount of drug entrapped in vesicles was calculated by equation given below:

\[
\text{Entrapment Efficiency} \% = \frac{\text{amount of free drug}}{\text{total amount of drug}} \times 100
\]
2.4.7. Particle size analysis and Zeta potential

The guggulosomal vesicle size and zeta potential of optimized guggulosomes suspension was determined by dynamic light scattering (DLS) using a Malvern Zeta master UK. Guggulosomes were dispersed in Millipore water and system was set at an angle of 90° at 25°C, a medium with viscosity of 0.8872 and refractive index of 1.330. The particle size distribution was characterized using PDI, which determine the width of size distribution. Zeta potential was determined by using Malvern Zetasizer Nano ZS (Malvern Instruments, UK) performed by using a combination of laser Doppler velocimetry and phase analysis light scattering (PALS) [13,18]. All measurements were taken as triplicate [13-14].

2.4.8. Viscosity

In this study, Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) was used to determine the viscosity of Guggulosomal gel using spindle number CPE40 at 25 ± 0.5 °C [34]. All measurements were taken as triplicate for an accurate result.

2.4.9. In vitro drug release

The in vitro drug release study of phenylbutazone guggulosomal gel was studied on cellulose acetate membrane which was soaked for 24hr prior work so that it can easily tie to diffusion tube. Diffusion tube was clamped and dipped in phosphate buffer 7.4 in beaker. It was kept at 37°C of phosphate buffer 7.4 and 1gm of phenylbutazone guggulosomal gel was added in donor compartment of the diffusion tube followed by parafilm covering to avoid evaporation of the solvent system. The phosphate buffer 7.4 was kept in receiver compartment and stirred continuously at 500 rpm. From receptor compartment 3ml solution was withdrawn at 0,1,2……8,…,24, & 25 hours respectively at particular time interval and replaced by buffer solution so that volume of receptor solution kept constant during drug release[2,8]. The drug concentrations in the withdrawn samples were determined at 213 nm against appropriate blank. The in-vitro drug release were carried out in triplicate for each preparation and expressed as the Mean ± SD., and further the cumulative drug release (%CDR) was calculated and a plot of time
versus %CDR was constructed and shown graphically. In vitro skin permeation studies were conducted for all guggulosomes formulation and reported [23-24].

2.4.10. Anti-inflammatory activity

In this study, the Carrageenan induced paw edema method was used to perform the Anti-inflammatory activity of guggulosomes. In this study, six healthy rats were taken of weight 140-150 gm of either sex and they were further divided into two groups one for control and another for treatment (guggulosomes, 100mg/Kg). These two groups of rats were fasted overnight prior to the experiment period accordingly to the experimental protocol. Healthy Rats of Weight 140-200 gm of either sex were fasted for After 30 minutes, all groups of animals were given a subplantar injection 0.1ml of 1% Carrageenan in left hind paw. After 1 hour, paw volume was measured by plethysmometer at 0.5, 1, 2, 3 & 4 hours [11]. The percentage edema inhibition was calculated by following formula:

\[
\% \text{ edema inhibition} = \left(\frac{(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated}}}{(V_t - V_0)_{\text{control}}} \right) \times 100
\]

Where, \( V_t \) = mean paw volume edema inhibition by treatment (guggulosomes)

\( V_0 \) = mean paw volume edema inhibition by control (standard drug)

2.4.11. Stability studies

Stability studies were performed for guggulosomes suspension to investigate any loss of drug from guggulosomes and effect of stability during storage condition. From the study, optimized formulation F3 guggulosomes formulation was subjected to accelerated stability studies as per ICH guidelines [33]. Than the guggulosomes suspensions was stored in the glass vials at room temperature (25 °C) and 4°C to 3 months. Further the stability study was performed with the function of time (in days) as 7, 15, 30, 60, and 90 days according to the ICH guidelines. Then the guggulosomal suspension was withdrawn according to the given protocol and stability was justified by particle size, zeta potential, PDI, entrapment efficiency and thermal analysis. PDI and encapsulation efficiency [24].
2.4.12. Response surface methodology

In this present study, Response surface methodology (RSM) is used to describe the influence of guggul lipid and cholesterol on the formulations. RSM is a statistical technique that is basically used for the optimization of best experimental result with respect to their variables [28]. RSM explores the relationships between several independent variables (factors) and one or more dependent variables (responses) [29]. This experiment design is flexible and do not require any experts, it generates result itself and rejects if the data not fits to the selected model. If the experimental data fits to the proposed model then the software construct a polynomial equation that describes characteristics of the designed experiment.

In this experiment, Six Guggulosome formulations were prepared and the effect of independent variables on responses (dependent factors) were evaluated using RSM [30-31] and are presented in (Fig. 8). Following two independent variables were taken: amount of guggul lipid (GL, $X_1$) and amount of cholesterol (CH, $X_2$) and each variable was tested at different concentrations, the quantities of which are expressed in their respective units [32]. The experiment was evaluated against the following responses: Entrapment efficiency (%EE, $Y_1$), particle size (PS, $Y_2$), and cumulative drug release (%CDR, $Y_3$). Experiments were performed according to a randomized procedure and the scheme showing the values of process variables corresponding to the observed responses is reported in Table 3. Statistica V.10 software (StatSoft, Inc. USA) was used for generation and evaluation of response surfaces.

3. Result and discussion

The ATR-FTIR results of pure drug, guggul lipid, cholesterol and phenylbutazone loaded guggulosomes containing major absorption band peaks are shown in (Fig.1) A) Guggul B) Phenylbutazone C) Cholesterol and D) F3 (formulation). ATR-FTIR spectra of the drug shows major characteristic absorption bands peaks at 3183.97 cm$^{-1}$ due to N-H stretching, 3014.60 cm$^{-1}$ due to C-H stretching, 2955.18 cm$^{-1}$ due to C-H vibration, 1737.58 cm$^{-1}$ due to C=O (ketone), 1671.98 cm$^{-1}$ due to -C=C stretching, 1583.80 cm$^{-1}$ due to stretching of aromatic (C=C) group, 1409.31 cm$^{-1}$ due to CH$_2$ bending, 1383.07, 1340.77 and 1260.96 cm$^{-1}$ due to O-H bending, 1095.11 and 1026.82 cm$^{-1}$ due to C-F bending, 1169.51 cm$^{-1}$ due to C=O stretch, 884.52 cm$^{-1}$ due to distribution of aromatic protons and 759 cm$^{-1}$ due to CH$_2$ rocking respectively. Prepared
formulations were also scanned for the same region and found that the peaks lies almost in the very close range. The peaks of formulation F3 was found to be very closer to the peaks of pure drug phenylbutazone i.e. 3014.60, 1737.58, 1583.80, 1169.51, 1085.17, and 884.52 cm⁻¹ the result revealed that there was no considerable change in the IR peaks of phenylbutazone and optimized formulation.

Surface morphology and 3-dimensional nature of guggulosomes was confirmed by Scanning electron microscopy (SEM) which was performed by using lyophilized sample coated with gold and operated at 20 kV at a magnification of 3300X. SEM image of drug loaded formulation F3 shown in (Fig. 2) indicate that the guggulosomes prepared by trituration method (F3) retain their spherical shaped vesicles with less size range of 200-550 nm.

The 3-Dimensional image of guggulosomes of optimized formulation F3 was captured by AFM surface topographic imaging has shown in (Fig.2), which provides information about the morphological characteristics of prepared guggulosomes suspension. Further, the surface morphology demonstrate the height of the guggulosomes prepared by trituration method (F3) have height 7.277nm, area 6726.828(nm²) and diameter 268.196(nm). By the help of AFM it was also possible to observe that the displayed guggulosomes was spherical in shape and having smooth surface.

In this study, DSC experiments involved the study of thermal behavior for phenylbutazone, guggul lipid, and cholesterol and lyophilized (F3) guggulosomes samples. The results of DSC study was shown in (Fig.3) A) Guggul Lipid B) F3 C) Cholesterol and D) Phenylbutazone. The DSC thermogram of Phenylbutazone exhibited a sharp endothermic peak at 116.15°C of drug. Melting point of guggul lipid, cholesterol was seen at 92.89°C, 148.63 °C. The result shows the disappearance of the pure drug peak in the formulation due to an increase in heating rate cycle (above 5K/min.). Thus from this study we can conclude that the optimized formulation has no interaction with the other polymers.

In this analysis, the optimized formulation A) F3, B) Guggul lipid, C) Phenylbutazone and D) Cholesterol were subjected to controlled temperature program in a controlled atmosphere. The combined TGA graph shown in (Fig.4) , that the TGA graph of pure drug showed that the mass
remained constant with increasing temperature but as it approached melting point of drug, the started to fallen down. Similar phenomenon was also observed with the formulated guggulosome F3 which shown a sharp falling of the curve at the 250 °C, that reveals that the combination of guggul lipid with the drug enhances its stability. An another TGA study was done for the guggul alone and its shown a sharp falling curve at 140°C. all these findings revealed that excipients or moisture content have no adverse effect on formulations.

The entrapment efficiency of drug in guggulosomes is dependent on numerous factors like the ratio of lipid i.e. ratio of guggul lipid and cholesterol and the ratio of dug to total lipid. The entrapment efficiency of the phenylbutazone in the guggulosomes was determined by the centrifugation technique. We observed that with increase in cholesterol concentration and constant amount of drug and different amount of guggul lipid as 100, 200, 300, 400 mg, in the formulations F5-F6, F2- F1 & F3- F4 respectively, the percentage encapsulation efficiency were found to be 58.1± 0.84, 68.9 ± 0.21, 68.3± 0.56, 73.8± 0.282, 77.2 ± 0.212, and 55± 0.162. Formulation F3 with highest amount of guggul lipid (400mg), cholesterol (400mg) with constant amount of drug showed better & maximum entrapment efficiency. From the result obtained shown in (Table 2). we can say that the an appropriate ratio of guggul and cholesterol conc. play an important role in the entrapment efficiency, this is due to increase in overall lipid conc. that favors the nature of drug to be encapsulate in the core as a whole.

The particle size and polydispersity index (PDI) were determined by using dynamic light scattering technique and are show in Table 2. All the formulations, F1- F6 showed mean particle diameter sizes ranging from 230.2±0.14 to 342.3±3.04 nm with PDI ranging between 0.225±0.32 to 0.614±0.27. Formulation F3 containing guggul lipid (400mg) and cholesterol (400mg) with the same amount of drug (150mg) has shown the mean particle size (287.3± 1.13), which is an optimum formulation having PDI (0.289±0.23). By the help of above observations we can conclude that particle size of F3 shown in (Fig. 5) was suitable for topical use.

The guggulosomal formulations F1 to F6 showed zeta potential value ranging from -20.1to -35.7mV are shown in (Table 2.). Formulations F5, F6 and F1 with cholesterol concentration 100, 200, and 300 shown zeta potential –20.1, -21.2 and -25.9 mV respectively while with the highest concentration of cholesterol 200, 300, and 400 in formulations F2, F4, and F3 showed an
increase in zeta potential of value -26.5, -29.8 and -35.7 respectively. These finding reveals that upon increasing the cholesterol concentration, the surface charge (negative) on the guggulosomes increases and thus lead to be increased in the stability. This result clearly shown that the formulation F3 is the most stable formulation prepared by trituration method and has shown in (Fig. 5).

The viscosity of the prepared guggulosomes was measured by using Brookfield viscometer, and the formulation viscosity range lies between 4387±0.659 to 6934±0.865 cps and the viscosity of the optimized formulation was obtained as 6853±0.625 cps. The spreadibility of the formulation was measured by using glass slide and physical weight pulling method. The spreadibility of the all formulation lies between 14.38±0.42 to 16.1±0.61 cm, and the spreadibility of the optimized formulation was obtained as 15.7±0.41 cm. On the behalf of these finding it can be said that the optimized formulation of guggulosomes possess good viscosity and spreadibility.

The in-vitro release of all formulations loaded with phenylbutazone was studied. The drug release from guggulosomes was formulated by use of various concentrations of guggul lipid, cholesterol and phenylbutazone. The in vitro release studies of F1 to F6 formulations were studied and the results of the same were showed in (Fig. 6) The formulations F1 to F6 have shown the following drug release 37.50 ± 0.1.40, 55.48± 0.1.41, 60.80±0.707, 44.03±0.1.40, 44.21 ±1.41, and 46.53±0.707, after 24 hrs respectively. Formulation F3 prepared by trituration method show maximum percent cumulative drug release profile due to high loading of drug in guggulosomes in comparison to other formulation. 60.80±0.707% phenylbutazone release from guggulosomes showed drug release for 24hr and burst release in initially 8hr and gradually release decreases and becomes constant in 18 hrs. The drug release was sustained due to addition of cholesterol in formulation to provide rigidity to the lipid vesicles of phenylbutazone. Thus cholesterol and guggul lipid acts as rate-limiting membrane for release of the phenylbutazone loaded guggulosomes. Formulation F1 showed minimum release of phenylbutazone due to increase in amount of cholesterol which increases the rigidity of guggulosomes vesicles. The results depicted that variation in guggul lipid and cholesterol concentration may affect the drug release pattern which is also mention by (Jithan AV et all; 2010) [25].
The % protection of Carrageenan induced paw edema in rat was treated with phenylbutazone guggulosomes. The rats were treated with optimized formulation F3 of prepared guggulosomes. The controlled and treated rats were observed for 0.5, 1, 2, 3 & 4 hours on plethysmometer and anti-inflammatory effect was determined by paw volume of rat, thus the reading obtained by percent edema inhibition at certain interval of time was 62± 0.0057, 79± 0.015, 82± 0.0057, 88± 0.0208 & 92± 0.208 % of control and 58± 0.0057, 64±0.0305, 71± 0.0264 and 85± 0.0115 & 87± 0.532% for treated. The results revealed that there were higher percent protection in phenylbutazone loaded guggulosomes and provides desired anti-inflammatory effect and percent edema inhibition was shown in (Fig.7)

The optimizations of physical and chemical stability are required in guggulosomes. The stability of vesicles is one of the major problems in the formulation of guggulosomes because of leaching and drug accumulation from the polymer core and lipid layers. The results of physical stability of optimized formulation F3 in various intervals of time are shown in (Table 4). guggulosomal suspension was divided into two batches and kept in sealed vials (10ml) at refrigerator temperature 4 ± 2 ºC/ 60 ± 5% RH and room temperature 25 ± 2 ºC/ 60 ± 5% RH. The study was held as the function of time as 7, 15, 30, 60 and 90 days of storage. Then the guggulosomal suspension was directly sonicated for 15 second in bath sonicator and characterized for size, zeta potential and encapsulation efficiency. The triplicate analysis was done for a concordance reading and the results are reported as mean ± SD. The guggulosomes were kept in RT confirmed significant increase in particle size (from 287.2±1.41 nm after 7 days 289± 1.07nm) and decrease in percent entrapment efficiency from 60.80± 0.707 to 59.95± 2.642. This was allied with significant increase in PDI from less than 0.226± 0.19 to 0.573± 0.47 and diminishing zeta potential from about -35.7to -34.8mV. These are signs of particle accumulation and leakage of the encapsulated drug. After 7 days the RT samples had too much aggregation with increasing in particle size 302± 1.26, 355.46± 0.14, 364.46± 2.73, 384.52± 1.63nm with decrease in % encapsulation efficiency 58.86±1.201 , 58.80± 1.532 , 55.46±1.223 , 54.21± 1.016 after 15, 30, 60 and 90 days respectively. This result also supported by the increasing value of PDI 0.591± 0.05, 0.625± 0.72, 0.640± 1.20, 0.664± 0.11 with decreasing value of zeta potential -32.5, -30.4, -29.7, and -27.4 respectively. In comparison, the guggulosomes stored at 4 ºC were quite stable as there was minimum increase in size; PDI and decrease in zeta potentials and encapsulation
efficiency after storage of 90 days were observed. The optimized storage condition for guggulosomes formulation F3 was found to be stable at 4 ± 2 °C/ 60 ± 5% RH.

The response surface models fit between the factors and measured responses are shown in the following polynomial equations:

%EE = -39.8 + 0.425*GL - 0.0385*CH + 0.0041*GL^2 - 0.0102*GL*CH + 0.006*CH^2

PARTICLE SIZE (nm) = -597.3 + 2.5195*GL + 3.374*CH + 0.0283*GL^2 - 0.077*GL*CH + 0.0394*CH^2

%CDR = -181.78 + 1.3248*GL - 0.1333*CH + 0.0053*GL^2 - 0.017*GL*CH + 0.0102*CH^2

Where, GL= Guggul Lipid; CH= Cholesterol

The response surface plots representing the relationship between the studied factors and measured responses is demonstrated in (Fig. 8)

Only statistically significant (p < 0.05) coefficients are included in the equations. In this analysis the positive value in the polynomial equation suggest that the effects are favoring the optimization, and the negative value give an inversion relation between the variables and their corresponding effect. In accordance with the above stated equation for %EE, an increase in the concentration of Guggul lipid and Cholesterol lead to decrease in the entrapment efficiency but in the case of F3 formulation an equal ratio of Guggul and cholesterol give the high entrapment efficiency than the others. The possible reason behind this could be that higher lipid content prevents the escape of drug to outer milieu by effectively enclosing it. On the other hand it was observed that upon decreasing the concentration of the Guggul lipid and Cholesterol, the Particle size (PS) of the formulation increases. For %CDR, an increase in the amount of total lipid causes a non-significant increase in % drug release, shown only in the optimized formulation, however the other formulation shown better drug release upon decrease in total concentration of drug lipid. Thus the Response Surface Methodology predicts an accurate relevance result about formulation so that we can optimized the better one.

4. Conclusion

Guggulosomes were prepared by trituration method by using different ratios of lipids to enhance topical delivery. From all above results revealed that formulation F3 of ratio (guggul:400::cholesterol:400::phenylbutazone:150mg) was optimized as desired particle size,
zeta potential, entrapment efficiency and in-vitro drug release was achieved and also phenylbutazone guggulosomes exhibit desired effects in treatment of inflammation on paw edema. Thus all these findings discovered that guggul lipid is a promising carrier and due to its synergistic effects with phenylbutazone, provides better entrapment; sustain action and better protection from inflammation. Thus this delivery system could be used as strategy for better sustained & synergistic actions for inflammation.

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Table 1. Formulation table of guggulosomes by trituration method

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<tr>
<th>Ingredients</th>
<th>F1</th>
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Table 2. Physicochemical evaluation of developed guggulosomes formulations by trituration method

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<tr>
<th>Formulation</th>
<th>Entrapment Efficiency (%EE)</th>
<th>Particle size (nm)</th>
<th>Cumulative Drug Release (%CDR)</th>
<th>Zeta Potential (mV)</th>
<th>PDI</th>
<th>Spreadability (cm)</th>
<th>Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>73.8±0.282</td>
<td>268.2±3.04</td>
<td>37.50±1.40</td>
<td>-25.9</td>
<td>0.226±0.13</td>
<td>15.28±0.67</td>
<td>4387±0.659</td>
</tr>
<tr>
<td>F2</td>
<td>68.30±0.56</td>
<td>342.01±1.84</td>
<td>55.48±1.41</td>
<td>-26.5</td>
<td>0.301±0.34</td>
<td>14.38±0.42</td>
<td>4869±0.714</td>
</tr>
<tr>
<td>F3</td>
<td>77.2±0.212</td>
<td>287.3±1.13</td>
<td>60.80±0.707</td>
<td>-35.7</td>
<td>0.289±0.23</td>
<td>15.7±0.41</td>
<td>6853±0.625</td>
</tr>
<tr>
<td>F4</td>
<td>55±0.162</td>
<td>342.3±3.04</td>
<td>44.03±1.40</td>
<td>-29.8</td>
<td>0.321±0.16</td>
<td>14.62±0.68</td>
<td>6934±0.865</td>
</tr>
<tr>
<td>F5</td>
<td>58.1±0.84</td>
<td>232.1±6.08</td>
<td>44.21±1.41</td>
<td>-20.1</td>
<td>0.225±0.32</td>
<td>14.97±0.54</td>
<td>6693±0.774</td>
</tr>
<tr>
<td>F6</td>
<td>68.9±0.21</td>
<td>650.7±4.59</td>
<td>46.5±0.707</td>
<td>-21.2</td>
<td>0.614±0.27</td>
<td>16.1±0.61</td>
<td>5901±0.669</td>
</tr>
</tbody>
</table>

All data expressed as mean ± S.D.; n = 3; P ≤ 0.05.
Table 3. The experimental design matrix for the optimization of developed guggulosomes by trituration method

<table>
<thead>
<tr>
<th>Exp. run</th>
<th>Actual value</th>
<th>Dependent variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Guggul (mg)</td>
<td>Cholesterol (mg)</td>
</tr>
<tr>
<td>F1</td>
<td>400</td>
<td>300</td>
</tr>
<tr>
<td>F2</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>F3</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>F4</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>F5</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>F6</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>
Table 4. Stability study of the optimized formulation (F3) of developed Guggulosomes by trituration method

<table>
<thead>
<tr>
<th>Time(Days)</th>
<th>Particle size(nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>% Entrapment efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At 4 °C/60 ± 5% RH (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>287.2± 1.41</td>
<td>0.226± 0.19</td>
<td>-35.7</td>
<td>60.80±0.707</td>
</tr>
<tr>
<td>7</td>
<td>289.1± 1.63</td>
<td>0.254± 0.1</td>
<td>-35.0</td>
<td>60.77±0.201</td>
</tr>
<tr>
<td>15</td>
<td>284.6± 1.47</td>
<td>0.286± 0.12</td>
<td>-33.5</td>
<td>59.52±0.896</td>
</tr>
<tr>
<td>30</td>
<td>295± 2.68</td>
<td>0.298± 0.29</td>
<td>-31.1</td>
<td>56.24±0.84</td>
</tr>
<tr>
<td>60</td>
<td>296.33± 1.52</td>
<td>0.301± 0.13</td>
<td>-31.7</td>
<td>56.55±0.22</td>
</tr>
<tr>
<td>90</td>
<td>295.4± 1.35</td>
<td>0.345± 0.09</td>
<td>-30.5</td>
<td>56.45±0.876</td>
</tr>
<tr>
<td><strong>At 25 °C/60 ± 5% RH (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>287.2± 1.41</td>
<td>0.226± 0.19</td>
<td>-35.7</td>
<td>60.80±0.707</td>
</tr>
<tr>
<td>7</td>
<td>289± 1.07</td>
<td>0.573± 0.47</td>
<td>-34.8</td>
<td>59.95± 2.642</td>
</tr>
<tr>
<td>15</td>
<td>302± 1.26</td>
<td>0.591± 0.05</td>
<td>-32.5</td>
<td>58.86± 1.201</td>
</tr>
<tr>
<td>30</td>
<td>355.46± 0.14</td>
<td>0.625± 0.72</td>
<td>-30.4</td>
<td>58.88± 1.532</td>
</tr>
<tr>
<td>60</td>
<td>364.46± 2.73</td>
<td>0.640± 1.20</td>
<td>-29.7</td>
<td>55.46± 1.223</td>
</tr>
<tr>
<td>90</td>
<td>384.52± 1.63</td>
<td>0.664± 0.11</td>
<td>-27.4</td>
<td>54.21± 1.016</td>
</tr>
</tbody>
</table>