Guggulusome - A Novel Vesicular Carriers for Enhanced Transdermal Delivery

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Abstract

The present work describes guggul as a novel carrier for some anti-inflammatory drugs. Guggulusomes containing different concentration of guggul with aceclofenac were prepared by sonication method and characterized for vesicle shape, size, size-distribution, pH, viscosity, spread ability, homogeneity, and accelerated stability *in-vitro* drug permeation through mouse skin. The vesicles exhibited an entrapment efficiency of 93.2 ± 12%, vesicle size of 0.769 ± 3µm and a zeta potential of - 6.21mV. In vitro drug release was analyzed using Franz's diffusion cells. The cumulative release of the guggulusomes gel (G2) was 75.8% in 18 hrs, which is greater than that all the gel formulation. The stability profile of prepared system assessed for 45 days. The vesicular suspension was kept in sealed vials (10ml) at 4 ± 2ºC and at room temperature for 45 days no change was showned in entrapment efficiency. The optimized guggulusomes formulation showed transdermal flux 216.1µg/cm²/hr. The result advocates the potential of guggulusomes formulation to treat diseases where facilitated penetration of the drug into muscle and synovial fluid is desirable. In the end of the tests guggulusomes gel G2 with carbopol 934K was the most stable. The paw edema and percentage inhibition of carrageenan-induced paw edema in rats treated with aceclofenac guggulusomes gels and commercial formulation of aceclofenac gel marketed gel. The formulation G2 were devoid of any irritation potential and no edema formation was observed in any case. Irritation score for aceclofenac guggulusomes gels was zero, which indicated its acceptability for topical administration.

Keywords: Aceclofenac, Gel, Carbopol, Guggulu carrier.

Introduction

New drug delivery system that offers numerous advantages compared to conventional dosage forms. Such systems often use macromolecules as carriers for the drugs [(1)]. Guggul is used as an anti-inflammatory, anti-hyperlipidemic, anti-obesity, anti-cancer and is also used in cosmetics as anti-wrinkle, anti-acne in dermatitis etc. If aceclofenac drug is incorporated in guggul, it may give the synergistic effect [(2)]. By this concept of guggulusomes we can reduce the dose amount of these drugs due its synergistic effect and minimize the dose dependent side effects of these drugs. Guggul is an ayurvedic extract sourced from the resin of the *Commiphora mukul* tree of India. Guggul contains guggulsterones, compounds that primarily promote healthy lipid metabolism. Guggul also shows initial promise for supporting joint function and comfort. Guggul moderated lipid peroxide levels, revealing important antioxidant action for lipid support. Additional proposed mechanisms for the lipid modulating potential of guggul includes supporting liver enzyme and cellular membrane function, promoting receptor function, enhancing bile acid excretion, and promoting thyroid function. In addition, guggulsterones have been shown to support platelet function and fibrinolytic activity, helping to maintain blood vessel health and cardiovascular support [(3,4)]. Guggul shows anti-inflammatory action as well as promises to deliver the drugs. The carrier that results is yet to be described named as 'guggulusomes' it has mixed properties of liposome, solid lipid particles and multiple emulsion etc. The purpose of this research was to find that guggul which has been proven medicinally could also function as a drug carrier. Aceclofenac, an NSAID, has been recommended orally for the treatment of rheumatoid arthritis and osteoarthritis. It also has anti-inflammatory, antipyretic, and analgesic activities. The short biological half-life (about 4 h) and a higher dosing frequency make aceclofenac an ideal candidate for sustained release. The oral administration of aceclofenac causes gastrointestinal ulcers and gastrointestinal bleeding with chronic use. Because of gastrointestinal bleeding, it also causes anemia. Using the transdermal route eliminates these side effects, increases patient compliance, avoids first-pass metabolism, and maintains the plasma drug level for a longer period of time.

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Received: 21/2/2014
Accepted: 24/4/2014

Guggul is one of the very ancient ayurvedic drugs, having been first described in atharva veda (2000 B.C). According to sushrut samhita, guggul is, when taken orally, curative of obesity, liver dysfunction, internal tumors, malignant sores and ulcers, urinary complaints, intestinal worms, leucoderma, sinus, edema and sudden paralytic seizures. It is also considered a cardiac tonic [(5)]. When drug materials, dyes or marker etc. are triturated with guggul in presence of water, the globules, particles or the vesicles formed in the dispersion, take them up. This uptake of is not limited to only lipophilic materials, the hydrophilic materials are taken-up as well. This behavior of guggul is analogous to the liposomes forming behavior of phospholipids. The entrapped moieties show much slower dialysis diffusion as compared to their true solution counterparts. The skin acts as a major target as well as a principle barrier for topical/transdermal (TT) drug delivery. The stratum corneum plays a crucial role in barrier function for TT drug delivery. Despite major research and development efforts in TT systems and the advantages of these routes, low stratum corneum permeability limits the usefulness of topical drug delivery. To overcome this, methods have been assessed to increase permeation. One controversial method is the use of vesicular systems, such as liposomes, ethosomes and niosomes, whose effectiveness depends on their physicochemical properties [(6)]. Hence the aim of present study was to develop sustained topical guggulusomes gel of aceclofenac and to evaluate with respect to various in vitro evaluation tests. Presently no scientific reports are available on the formulation of topical drug delivery system of guggulusomes gel of aceclofenac.

Materials and Methods

Purification of guggul

Guggul, which is a oleo-gum-resins, obtained from plant sources i.e. *Commiphora wightii* or *Commiphara mukul (*Mukul myrrh tree) is one of such material, with main source species of family *Burceacea*. Visible impurities like sand, glass, stone impurities were removed from guggul manually. Guggul was broken in to small pieces, put it in to muslin cloth bag and heat in a beaker with distilled water. The muslin cloth bag is hanged in a beaker so that the material remains immersed in liquid. The heating was continued until all guggul is converted in to liquid and coming out of muslin cloth bag. The content of muslin cloth is thrown; liquid is filtered and heated until soft mass is formed. [(7)] The soft mass is thus obtained was then dried in sunlight and triturated with mortar with addition of small amount of Ghee.

Aceclofenac was received as a gift sample from Lupin Research Park, Pune, guggul (resin) was extracted from *Commiphora mukul* tree which was authenticated by taxonomist, Ethanol, propylene glycol, chloroform, from Qualigens, Mumbai, Carbopol 934K and HPMC 15cps from SD fine-chemical limited, Mumbai, All chemicals used were of analytical grade.

Preparation of guggulusomes

Aceclofenac guggulusomes were prepared using different concentrations of guggul lipid, ethanol, propylene glycol and aceclofenac as given in the (Table 1). Guggulusomes and drug were dissolved in ethanol and propylene glycol. The mixture was heated to 30ºC in water bath. In this solution distilled water was added slowly in a fine stream with a constant mixing (Mechanical stirrer, Remi equipment, Mumbai) at 700rpm in a closed vessel. The temperature was maintained at 30ºC during the experiment. The mixing was continued for 5 minutes. The preparation was stored at 4ºC. guggulusomes prepared by the above procedure were subjected to sonication at 4ºC using probe sonicator in 3 cycles of 5 minutes with 5 minutes rest between the cycles. [(8)]

Incorporation into gel

Carbopol 934K 1%w/v and HPMC 15cps 1 % was soaked in minimum amount of water for an hour. Guggulusomes suspensions 20 ml containing aceclofenac (200mg) was added to the swollen polymer under stirring. Petroleum jelly was melted and liquid paraffin was mixed and the mixture was added to above prepared solution and mixed well. Stirring was contained were then incorporated in gel with continuous stirring at 700 rpm in a closed vessel and maintained at temperature 30ºC until homogeneous guggulusomes gels were achieved. The pH was then adjusted to neutral using triethanol amine (TEM) and stirred slowly till a gel was obtained. pH measurement of the formulations using pH meter by dipping the glass electrode completely into the semisolid formulation so as to cover the electrode [(8-10)].

Fourier transform infrared spectroscopy

Infrared spectroscopy was conducted using a Shimadzu FTIR 8300 Spectrophotometer and the spectrum was recorded in the region of 4000 to 400cm-1. The procedure consisted of dispersing a sample (drug and drug-excipient mixture, 1:1 ratio) in KBr (200-400mg) and compressing into discs by applying a pressure of 5 tons for 5min in a hydraulic press. The pellet was placed in the light path and the spectrum was

obtained. All spectra were collected as an average of three scans at a resolution of $2cm^{-1}$.

Differential scanning calorimetry

Differential scanning calorimetry was performed by using DSC-60. The instrument comprised of calorimeter (DSC 60), flow controller (FCL 60), Thermal analyzer (TA 60) and operating software TA 60 from (Shimadzu Corporation, Japan.) The samples were placed in aluminium pans and were crimped, followed by heating under nitrogen flow (30ml/min) at a scanning rate of 5^oC/min from 25^oC to 200^oC. Aluminium pan containing same quantity of indium was used as reference. The heat flow as a function of temperature was measured for both the drug and drug-excipient mixture. Duplicate determinations were carried out for each sample.

Optical microscope observation and vesicular shape and surface morphology

The guggulusomes dispersion was spread on the glass slide using a glass rod. Formation of multilamellar vesicles was confirmed by examining the guggulusomes suspension under an optical microscope with the magnification power of 40x and 100x. (Olympus CX41, Philippines) Photographs of vesicles were taken using Olympus camera. Scanning electronic microscopy (SEM) was also conducted to characterize the surface morphology of the guggulusomes vesicles were analyzed by scanning electron microscopy (SEM). Prior to analysis, the guggulusomes were mounted onto double-sided tape that has previously been secured on copper stubs and coated with platinum, then analyzed at different magnifications.

Vesicle size and size distribution

The vesicle size, size distribution and zeta potential of optimized guggulusomes formulation were determine by the dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster (ZEM 500962, Malvern, UK). The electric potential of guggulusomes, including its stern layer (ZETA POTENTIAL) was determine by injecting the diluted system into a zeta potential measurement cell.

Determination of viscosity

A Brookfield viscometer was used to measure the viscosities of all the formulations of gel prepared. A spindle (LV1) was rotated at 50rpm. Samples of gel formulations were left to settle over 30 minutes at the temperature 32 ± 1°C before measurements were taken. Viscosity was determined by using the following formula [11].

Viscosity = Dial reading × Factor

Determination of spreadability

One gram of gel is placed between two plates of 20×20cm. the mass of the upper plate was 125gm. After one minute the spreading diameter was measured. The spreadability was calculated by using the following formula [12,13].

$$S = mt/l$$

Where S = spreadability, m = weight of upper slide, l = length of the glass slide, t = time.

Determination of pH

pH is an important parameter to be considered in gels as irritation on skin, compatibility and drug release may be altered. The pH for skin should ranges in between 6.4 to 6.8 [14].

Determination of homogeneity

It is visually tested by vision test and also by elegance effect. The +ve sign indicates the confirmation of good clarity and good elegance effect while –Ve sign indicates the non homogeneity and non elegance effect [14-16].

Determination of drug content

Ten gram of the gel was taken and dissolve in 100ml buffer (pH 6.8) and shaken continuously until dissolved. The solution was ultrasonicated for 15 minutes. After filtration, the drug was suitably diluted and analyzed at UV Spectrophotometer at 276nm [15].

Entrapment efficiency

The entrapment capacity of aceclofenac guggulusomes was measured by the ultracentrifuge method Vesicular preparations containing 1% aceclofenac were kept overnight at 4˚C and centrifuged in a ultracentrifuge (Remi) equipped with TLA-45 rotor at 4˚C, at 30,000rpm for 2h. aceclofenac was assayed both in the sediment and in the supernatant. The entrapment capacity of aceclofenac was calculated from the relationship [(T-C)/T] 100, where T is the total amount of aceclofenac that is detected both in the supernatant and sediment, and C is the amount of aceclofenac detected only in the supernatant [16,17].

In-vitro permeation studies

The *in-vitro* skin permeation of aceclofenac from guggulusomes formulation was studied using Franz's diffusion cell. The *in-vitro* diffusion of the drug through mouse skin was performed. The mouse skin soaked in a buffer for 6-8 hours. The two cell compartments will be held together with a clamp. The receptor compartment has a volume of 11 ml and will be filled with pH 6.4 buffer. It will be kept at 37°C by circulating water through an external water jacket. After 30min of equilibration of the membrane with the receptor solution, 200µl of the drug

solution will be applied in the donor compartment by means of a pipet. The donor compartment will be then covered with parafilm to prevent evaporation of the solvent. The receptor solution will be continuously stirred by means of a spinning magnetic bar, at 500rpm. Receptor solution samples, 2.0ml aliquots, was withdrawn through the sampling port of the receptor compartment at various time intervals. The cells will be refilled with receptor solution to keep the volume of receptor solution constant during the experiment. The sample was withdrawn and replaced by 2.0ml with pH 6.4 phosphate buffer saline. The drug concentrations in the aliquot were determined at 276 nm against appropriate blank. This experiment was done in triplicate and average value was reported.[5,6]

Permeation enhancers

These are compound, which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant.

The flux, J, of drug across the skin can be written as:

$$J = D\, dc/ dx$$

Where D is the diffusion coefficient and is a function of the size, shape and flexibility of the diffusing molecule as well as the membrane resistance, C is the concentration of the diffusing species, X is the spatial coordinate. The in vitro skin permeation of acelecfenac from guggulusomes formulation was studied using diffusion cell specially designed in our laboratory according to the literates. The effective permeation area of the diffusion cell is 2.013cm². The temperature was maintained at 37 ± 0.5ºC. [5,6]

In-vivo efficacy study

The anti-inflammatory activity of Aceclofenac guggulusomes gels formulations was evaluated by the carrageenan-induced rat hind paw edema method [18,19]. The experimental protocol was designed and approval of Institutional Animal Ethics Committee (IAEC) (Reg. No. 0436) was obtained. Healthy Albino Wistar rats of either sex weighing between 150-200 g were obtained from the disease free small animal house of CCSHAU, Hisar. The animals were housed in institutional animal house under standard conditions with free access to food and water. Anti-inflammatory activity of the Aceclofenac guggulusomes gels was compared to the marketed gel of aceclofenac (AC Rub).

Six albino Wistar rats were divided into three groups of five animals each as follows:

Group 1 (Control group): animals were treated with plain guggulusomes gels.

Group 2 (Standard group): animals were treated with aceclofenac (AC Rub).

Group 3 (Carbopol 934K): animals were treated with guggul lipid loaded guggulusomes gels formulation.

Group 3 (HPMC): animals were treated with guggul lipid loaded guggulusomes gels formulation.

Inflammation was induced by sub-plantar carrageenan injection and after 1 hour, formulations were applied topically on the inflamed paw of rats by gently rubbing with index finger and the volume of the paw was measured. The thickness of paw was measured at 1h time intervals till 5h after carrageenan injection. A digital vernier caliper (Aerospace, China) was used for measuring paw thickness of rats [20,21]. The percentage inhibition of inflammation was calculated by the following formula:

$$\text{Percentage inhibition} = [(C-T)/C] \times 100$$

where C = control paw edema, T = test paw edema.

Draize skin irritation test

Lastly, each formulation was assessed for irritancy by conducting modified Draize skin irritation tests on male White New Zealand rabbits (3-4 kg) obtained from. Approval for the use of animals in the study was obtained from the Banasthali University. Animal Ethics Committee (Banasthali University. Rajasthan, India, Ref. No. BU/BT/184/11-12). For this purpose, a dorsal area on each restrained animal was shaved and then tape stripped three times to detach several upper layers of the stratum corneum. A 0.5 mL aliquot of each test guggulusomes gels was used in these areas which were then covered with a surface. After 4h, the gels were removed and the rabbits were observed over 14 days for signs of erythema, edema and ulceration. On days 1, 3, 7 and 14, visually-apparent cutaneous changes were assigned scores ranging between 0 and 4 with higher numbers signifying greater skin damage. The mean erythema scores were recorded depending on the degree of erythema: no erythema = 0, slight erythema (barely perceptible-light pink) = 1, moderate erythema (dark pink) = 2, moderate to severe erythema (light red) = 3, and severe erythema (extreme redness) = 4.

Stability study

All regulatory bodies accept only real time data for any drug or pharmaceutical for all purpose of assessing the shelf life and only accelerated stability studies my serve as a tool for formulation screening and stability issues related to shipping or storage at room temperature. The accelerated stability studies were carried out in accordance with the ICH guidelines. The ability of vesicles to retain the drug was assessed by keeping the

guggulusomes suspension at different temperature. Optimized guggulusomes formulation was selected for stability studies of vesicles. The vesicular suspension was kept in sealed vials (10ml) at 4 ± 2ºC and at room temperature for 45 days. Percent entrapment was determined at different time intervals. It was observed that the guggulusomes system was more stable at 4 ± 2°C.

Results and Discussion

Fourier transform infrared spectroscopy

The possible interaction between the drug and the excipients were studied by IR spectroscopy. IR spectra of pure aceclofenac, its physical mixture with guggul lipids and the prepared formulation are shown in Figure 1. Pure aceclofenac showed major peaks at 3317.3, 2970.2, 2935.5, 1716.5, 1589.2, 1506.3, 1479.3, 1344.3, 1280.6, 1255.6, and 665.4 cm^{-1} The result revealed no considerable change in the IR peaks of aceclofenac in the physical mixture or in the prepared crystals when compared to pure drug there by indicating the absence of any interaction.

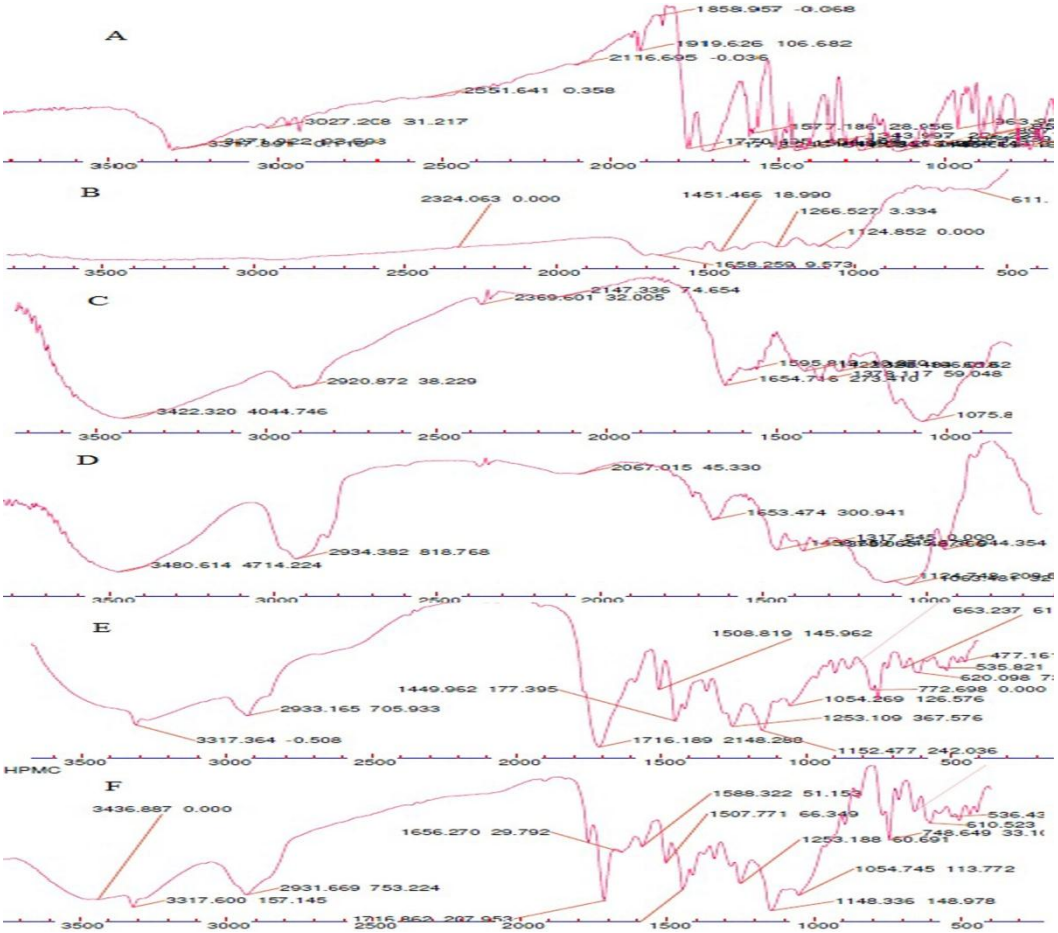

Figure (1) FTIR Spectra of A) Aceclofenac B) Guggle lipid C) Carbopol 934k D) HPMC E) Formulation G2 F) Formulation G6 .

Differential scanning calorimetry

The results of DSC studies are given in Figure 2. Pure aceclofenac showed a sharp endotherm at 157.2°C corresponding to its melting point/transition temperature.

There was no appreciable change in the melting endotherms of the physical mixture (aceclofenac + polymer) compared to pure drug. This observation further supports the IR spectroscopy results , when our optimised formulation G2 showed a sharp endothern on 154.7°C which indicated the absence of any interactions between drug and additives used in the preparation. However there was slight decrease in the melting point of the drug. It was also observed that there was a noticeable reduction in the enthalpy of the formulation with compare to aceclofenac formulation G2 showed -3.6j/mg.

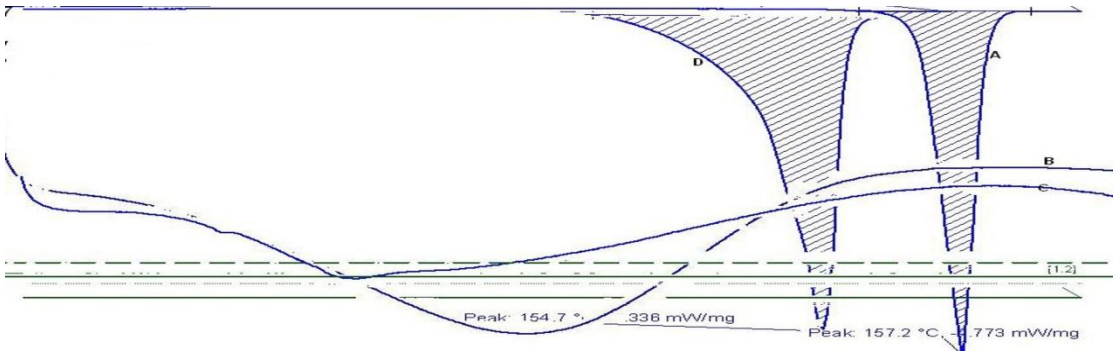

Figure 2: DSC Spectra of A) Aceclofenac B) Guggle lipid C) Carbopol 934k D) Formulation G2

Optical microscope observation and vesicular shape and surface morphology

The aceclofenac guggulusomes dispersion was spread on the glass slide using a glass rod. Formation of multi-lamellar vesicles was confirmed by examining the aceclofenac guggulusomes suspension under an optical microscope with the magnification power of 40x and 100 x. (Olympus CX41, Philippines) as showed in Figure 3. Photographs of vesicles were taken using Olympus camera. Surface morphology and three-dimensional nature of guggulusomes were further confirmed by SEM, justifying the vesicular characteristics possessed by this novel carrier Figure 4.

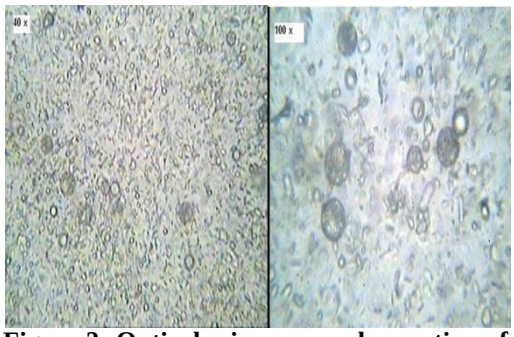

Figure 3: Optical microscope observation of guggulusome A) 40X B) 100X

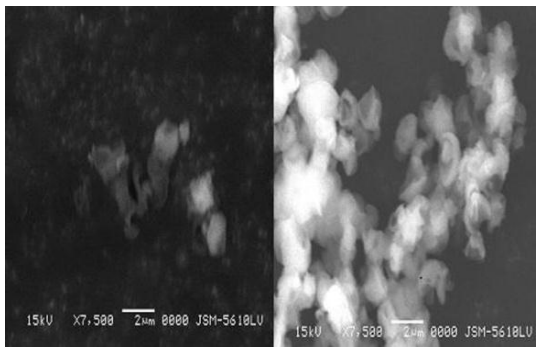

Figure 4: SEM of guggulusome formulation G2

Vesicle size and size distribution

Vesicular sizes are the basic parameter on the basis of which the formulations were optimized. The effect of guggul lipid and ethanol concentration on the size distribution of guggulusomes vesicles was investigated using dynamic light scattering (DLS) method. Formulation G 2 has concentration of guggul lipid 3% with carbopol 934k and G 6 have concentration of guggul lipid 3% with HPMC but ethanol concentration is same. The data shows narrow particle size distribution with an average vesicles size of 0.769µm of formulation G2 and formulation G6 particle size was shown 1.240µm. The size of the vesicles was increase with increasing guggul lipid concentration.

Determination of viscosity

Viscosity was measured by Brookfield viscometer for all the formulations of gel. A spindle (LV1) was rotated at 50rpm. Samples of gel formulations from G1 to G8 at the temperature 32°C±1° were taken as showed in (Table 1). Viscosity was determined from 7485±0.563 to 8760 ±0.316 as showed in (Table 1).

Determination of spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability.[12-15] The aceclofenac guggulusomes formulation G1 to G8 having optimum viscosity and spreadability as showed in (Table 1). The value is lie between 15.6±0.445 to 20.84±0.239.

Determination of pH and homogeneity

Accurately 2.5 gm of each gel formulation was weighed and dispersed in 25 ml of purified water. The pH of the dispersion was measured by using digital type of pH meter. [5,6] The guggulusomal gel formulation G1 to G8 pH lie between 6.2 to 6.7 and from the results it is concluding that all the gel formulation showed good appearance and homogeneity as showed in (Table 1).

Table (1) Composition and characteristics of aceclofenac guggulusomes gel

Composition in % (w/w)	G1	G2	G3	G4	G5	G6	G7	G8
Drug	1	1	1	1	1	1	1	1
Guggul Lipid	2	3	4	5	2	3	4	5
Ethanol (ml)	5	5	5	5	4	4	4	4
Propylene glycol	1	1	1	1	1	1	1	1
Liquid Paraffin	1	1	1	1	1	1	1	1
White Petroleum	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Carbopol 934K	1	1	1	1	-	-	-	-
HPMC 15cps	-	-	-	-	1	1	1	1
Water	q.s	q,s	q.s	q.s	q.s	q.s	q.s	q.s
Characterization								
% Entrapment efficiency	83.0± 3	93.2± 6	91.0± 8	89.5± 1	79.15±5	93.12±1	89.78±7	82.25±4
Vesicle size (µm)	-	0.769	-	-	-	1.240	-	-
Viscosity (cps)	7485± 0.563	8020 ± 0.746	8274± 0.131	8465 ± 0.487	8015± 0.431	8453± 0.869	8584± 0.351	8760 ± 0.316
Spreadibility	15.68± 0.949	17.36± 0.429	15.6± 0.445	16.16± 0.127	19.12± 0.854	20.84± 0.239	18.45± 0.45	19.15± 0.276
Homogeneity Gel strength (N/g)	++	++	++	++	++	++	++	++
pH	6.7	6.6	6.5	6.7	6.8	6.7	6.2	6.5
J (Flux) (µg/cm²/hr)	196.8	216.1	199.6	197.54	201.8	209.4	195.5	192.0
R(Regression coefficient)	0.9886	-	-	-	0.9464	-	0.991	-

Entrapment efficiency

The entrapment efficiency of guggulusomes was determined for all formulation from G1 to G8. Effect of ethanol concentration was observed on percent entrapment of guggulusomes. The entrapment efficiency was maximum for formulation G2 (93.26%) and minimum for formulation G5 (79.15%). Solubility of aceclofenac also increased when ethanol was used in concentration. Therefore, the drug also entrapped in the core of the vesicles. [5,16,17] The entrapment efficiency increased with an increase in concentration of guggul lipid but, above 3% of guggul lipid concentration there was no significant increase in percent entrapment. In formulations G1 to G4, percentage guggul lipid concentration was varied 2% to 5% and the concentration of ethanol is same that is 5%. Similarly in formulations G5 to G8, percentage guggul lipid concentration was varied 2% to 5% and the concentration of ethanol is same that is 4%. This indicates that guggul lipid play an important role in percent entrapment. It is shown that increase in the guggul lipid concentration increases entrapment but, above 3% guggul lipid concentration there was no significant increase in the entrapment efficiency. The results show that as the concentration of ethanol increased from the entrapment efficiency increased as showed in (Table 1) and (Figure 5).

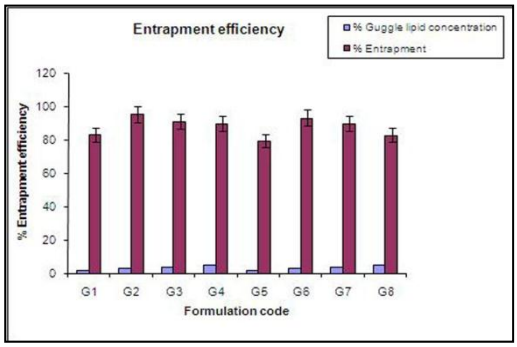

Figure 5: Percentage entrapment efficiency of guggulusome

In-vitro permeation study

Release study of different formulations was undertaken in a modified diffusion apparatus containing mouse skin. All the formulations had propylene glycol as a skin penetration enhancer. The *in vitro* drug releases of various formulations are shown in Figure 6.

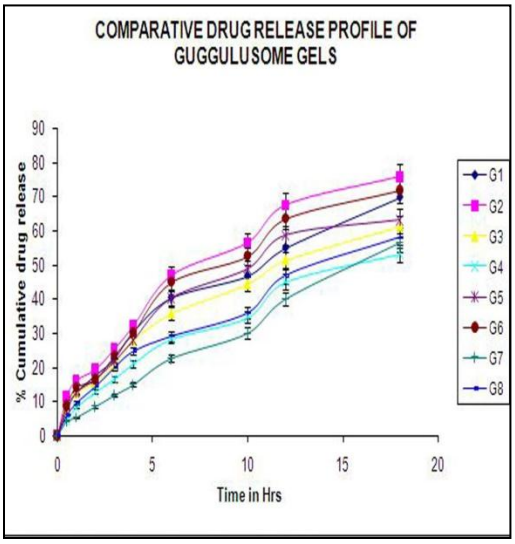

Figure 6: *In-vitro* drug release profile from guggulusomes formulation

The cumulative release of the guggulusomes gel (G2) was 75.8% in 18hrs, which is greater than that all the gel formulation. The formulation was having carbopol 935 as polymer from G1 to G4 and formulation G5 to G8 having HPMC as polymer. Ethanol provides the vesicles with soft flexible characteristics, which allow them to more easily penetrate into deeper layers of the skin. When guggul lipid concentration was increased, there was increase in drug permeation. Use of propylene glycol also influenced the amount of drug permeation. Propylene glycol also acts as permeation enhancer, which increases permeability of vesicle through biological membrane due to synergistic effect with ethanol on bilayer of the vesicles. Formulation (G1) containing 2% guggul lipid showed 69.7% drug release within 18h. Formulation (G2) containing 3% guggul lipid showed 75.81 % drug release within 18h. where as Formulation (G3 and G4) containing 4% and 5% guggul lipid showed 61.1 % and 52.8% drug release within 18h. In formulation G1 to G4 carbopol 934 used as base. Similarly Formulation (G5) containing 2% guggul lipid result showed 63% drug release within 18h. and formulation (G6) containing 3% guggul lipid result showed 71.8% drug release within 18h. where as Formulation (G7 and G8) containing 4% and 5% guggul lipid showed 56.4 % and 58% drug release within 18h.

In-vivo efficacy study

(Table 2) shows the results of paw edema and percentage inhibition of carrageenan-induced paw edema in rats treated with Aceclofenac guggulusomes gels and commercial formulation of aceclofenac gel (AC Rub). The control group consisted of rats treated with plain aceclofenac gel base. The results revealed a significantly higher inhibition of carrageenan-induced paw edema in rats treated with guggul lipid-loaded guggulusomes gels as compared with the control animals. However, an highly significant inhibition of carrageenan induced paw edema was observed in animals treated with commercial reference product (AC Rub) gel in comparison with the control during the entire 5h duration of the study. Further, the results of percent inhibition of paw edema produced by reference products were statistically higher as compared to the guggul lipid loaded guggulusomes gels. The study shows that the guggul lipid loaded guggulusomes gels possesses fair anti-inflammatory activity but it is not as good anti-inflammatory as the commercial product of aceclofenac. Thus, aceclofenac guggulusomes gels is a potential anti-inflammatory formulation**.**

Aceclofenac guggulusomes gels were devoid of any irritation potential and no edema formation was observed in any case. Irritation score [18,20] (primary skin irritation index) for aceclofenac guggulusomes gels was zero, which indicated its safety and acceptability for topical administration.

Stability study

The ability of vesicles to retain the drug was assessed by keeping the guggulusomes suspension at different temperature. Optimized guggulusomes formulation G2 was selected for stability studies of vesicles. The vesicular suspension was kept in sealed vials (10ml) at 4 ± 2°C and at room temperature for 45 days. Percent entrapment was determined at different time intervals. It was observed that the ethosomal system was more stable at 4 ± 2°C showed in (Table 3).

Table(2) Paw thickness and percentage inhibition (paw edema) in different groups at various time intervals.

Group	Treatment	Paw volume (mm)* (Percentage inhibition)				
		1 h	2 h	3 h	4 h	5 h
I	Control	5.37 ± 0.13	5.26 ± 0.19	5.42 ± 0.22	5.56 ± 0.12	5.43 ± 0.07
II	Standard	4.24 ± 0.37 (21.02%)	4.12 ± 0.43 (21.6%)	4.36 ± 0.32 (19.5%)	4.29 ± 0.17 (22.8%)	4.13 ± 0.28 (23.9)
III	G2	5.14 ± 0.14 (4.2%)	5.08 ± 0.13 (3.4%)	5.00 ± 0.24 (7.7%)	4.97± 0.15 (1%)	5.03 ± 0.11 (7.3)
IV	G6	4.68 ± 0.19 (1.2%)	4.72 ± 0.16 (1%)	4.88 ± 0.18 (9.9%)	4.80 ± 0.19 (1.3%)	4.73 ± 0.20 (1.2%)

Values are the mean ± SEM (n = 5)

Table(3)Stability study of optimized formulation (G2) aceclofenac guggulusomes

Time (days)	Percent entrapment (4 ± 2°C)	Percent entrapment (RT)
1	92.1±0.2	91.1±0.8
15	91.0±0.8	89.8±0.6
30	90.4±0.6	85.2±0.4
45	87.9±0.7	82.9±0.3

Mean ± SD, n = 3, RT = Room temperature

Conclusion

The result advocates the potential of guggulusomes gel formulation to treat rheumatic disease where facilitated penetration of the drug into muscle and synovial fluid is desirable. In light of the data obtained from experimental work we can expect the guggulusomes gel formulation to be safe and very efficient as a drug carrier for systemic as well as topical delivery of drug, holding future in effective transdermal delivery. It is envisaged that this particular formulation should be the basis of further studies in the clinically relevant environments.

Acknowledgement

Authors would like to thank to Prof. Aditya Shastri, Honorable V.C., Banasthali University, Rajasthan, India.

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