Formulation and Screening of Topical Herbal Liposomal Gel for Analgesic and Anti-Inflammatory Activity

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A B S T R A C T
Liposomal formulations have been successfully used in the treatment of a number of dermatological diseases. Various synthetic as well as herbal drugs are incorporated into liposome to improve its efficacy. Incorporation of herbal extract into liposome reduces side effects which are associated with the synthetic ones. Adhesiveness, stability and release of incorporated herbal extracts are the main features that influence the applicability of hydrogels for topical treatment, including the wound healing process. Adjustment of the textural properties of hydrogel should be conducted routinely. The texture analyzer measurements can provide deeper insight on gel adhesiveness. The gel properties are dependent on the polymer concentration and the pH. Carbopol hydrogels can take up to 15% (w/w) of liposomal dispersion, however, the stability of the liposomal gels need to be evaluated as well. Carbopol hydrogels can be used as advanced drug delivery systems. And the optimized formulation was selected by the basis of invitro drug release of the all the formulations.

Keywords: liposomal gel, hydrogel, liposomesm, polyherbal.

A R T I C L E  I N F O

1. Introduction
Liposomes are vesicles made of phospholipid bilayers. These phospholipid bilayers surround an aqueous core. Liposomal size is directly related to the method of preparation and can range from 50 nm to several microns. They form spontaneously when these lipids are dispersed in aqueous media. Vesicles can be constructed of natural constituents such that the vesicle membrane forms a bilayer structure which is principal identical to the lipid portion of natural cell membrane. Their ability to mimic the behavior...
of natural membranes and also to be degraded by the same pathways, makes vesicles a very safe and efficacious vehicle for medical applications. Vesicles can be composed even of entirely artificial components, chosen for their improved chemical properties (e.g. fatty acids, double chain secondary amines and cholesterol derivates). Moreover, liposomes may entrap both hydrophilic and lipophilic molecules; and be used as drug carrier for both types of drug molecules (Cevc, 2004). Vesicle membranes are semi-permeable membranes, in that the rate of diffusion of molecules and ions across the membrane varies considerably. For molecules with high solubility in both organic and aqueous media, a phospholipid membrane clearly constitutes a very tenuous barrier, but polar solutes and higher molecular weight compounds pass across the membrane only very slowly. Release rate of different types of drug molecules from liposomes is dependent on the type of drug applied (New, 1990).

The major obstacle for topical drug delivery is the low diffusion rate of drugs across the stratum corneum. Several methods have been proposed to increase the permeation rate of drugs temporarily. One of the most promising approaches is the application of drugs in vesicle-based formulations (Bouwstra and Honeywell-Nguyen, 2002). The present work is aimed at formulating poly herbal liposomal gels for the topical application. To study the effect of Drug polymer ratio or concentration of polymer on drug release. To study the effect of polymer, polymer grades on the parameters like duration of buoyancy and drug release. To determine the kinetics and mechanism of drug release.

2. Materials and Methods

**Gel:** Caropol Ultrez 10 NF was purchased from Noveon (Cleveland, USA); Triethylamine (TEA) was the product of Merck Schuchardt (Hohenbrunn, Germany); Acetic acid (glacial) was purchased from Merck KGaA (Darmstadt, Germany). **Liposomes:** chloroform, Methanol (HPLC grade) was the product of Merck KGaA (Darmstadt, Germany); cholesterol and lecithin. Others: Triglycerids (glacial) was purchased from Merck KGaA (Darmstadt, Germany); Triethylamine (TEA) was the product of Triethylamine (Barbuttel, USA); Acetic acid (Cleveland, USA); Triethylamine (TEA) was the product of Merck Schuchardt (Hohenbrunn, Germany); cholesterol and lecithin. Others: Triglycerids. 

**Methodology**

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the herbal liposomal gel extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids. as per the standard methods.

4.2 Experimental Animals and Housing of Animals

Albino Wistar rats weighing 150 ± 25 g of either sex were used for the study in different models. The animals were procured from National institute of Nutrition (hyderabad) at least 2 weeks prior to the study, so that animals could acclimatize to the new environment. Animals kept in well-maintained room under standard hygienic conditions. Commercial pellet diet and water were made available ad libitum. They were housed in propylene cages (32 x 24 x 16 cm) with stainless steel grill top, bedded with rice husk.

4.3 Preparation of Extract:

The leaves of both plants were dried under shade in room temperature for 3 days and powdered and the powder was used for preparation of methanolic extract. A 95% w/v methanolic extract was prepared by Soxhlet extraction method. The dried powder was extracted with 95% methanol for 12 h using Soxhlet apparatus. The combined extracts were concentrated at 40°C to obtain light brown residue. The yield obtained from above process was found to be 9.5%. The extract was preserved in a refrigerator.

**Selection of Doses and Preparation of Drug for Study**

Since the lethal dose was found at 2000mg/kg body weight, the 1/10th of the preceding dose i.e 100mg/kg body weight was taken as the testing dose for this study and the double of the dose i.e 200mg/kg body weight also tested to find out was there any dose dependent pharmacological effect or not.

**Screenings of anti-inflammatory activity**

*Carrageenan induced hind paw edema in rats*

Albino Wistar rats weighing between 150-200gms were divided into 5 groups of 6 rats each; three animals being housed in labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum.

**Grouping:**

**Group I:** Animals were administered 0.1ml saline p.o

**Group II:** Animals were administered 0.1ml saline p.o

**Group III:** Animals were administered standard (Indomethacin 10 mg/kg) p.o

**Group IV:** Animals were administered *Herbal liposomal gel* (100 mg/kg) p.o

**Group V:** Animals were administered *Herbal liposomal gel* (200 mg/kg dose) p.o

**Procedure:**

All rats of II, III, IV & V (except I group) groups were injected with 0.1ml of carrageenan (1%) in normal saline into sub planter area of right hind paw. All the drugs were given orally 1hr prior to carrageenan injection. Paw volume was measured by mercury plethysmograph at 0, 1, 2, 3, 6 hrs after the carrageenan injection.

**Screening of analgesic activity**

*a. Eddy’s hot plate method*

Albino Wistar rats weighing between 150-200gms were divided into 4 groups of 6 rats each; three animals being housed in labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum.

**Grouping:**

**Group I------** Animals were administered 0.1ml saline p.o

**Group II------** Animals were administered standard reference Pentazocin 10 mg/kg)i.p.

**Group III------** Animals were administered *Herbal liposomal gel* (100 mg/kg) p.o

**Group IV------** Animals were administered *Herbal liposomal gel* (200 mg/kg) p.o

**Procedure:**

The time for licking paws or jumping in hot plate was recorded as response, prior and 0, 30, 60, 90 120 min after administration of respective drugs.

**Statistical analysis:**

All the data’s were analyzed using One-Way ANOVA method followed by Dunnet’s / Tukey’s test. All values
Preparation of 1% Carbopol Gel: Carbopel resin 1gm was dispersed in distilled water 88 gm in which glycerol 10 gm was previously added. The mixture was stirred until thickening occurred and then neutralized by drop wise addition TEA until transparent gel appeared.

Incorporation of liposome in 1% Carbopol gel: Liposome containing herbal extracts was mixed in to 1% Carbopel gel by an electrical mixer 25rpm/2 min, with the concentration of liposome in hydrogel being 2.5% (w/w liposome suspension / total)

Gel characterization
The texture characterization of gels was performed on Texture analyzer TA.XT plus Texture Analyzer (Stable Micro Systems Ltd., UK). The following was studied:

- The correlation between the gel concentration and its texture
- Gel consistence and changes in texture as results of change in pH of a gel Changes in gel texture in relationship to gel stability

Texture analyzer:
Texture analyzer is an instrument that measures the response of a sample to:

- Tension
- Compression
- Penetration Bend

When the probe comes in contact with the sample, the instrument begins to measure the triggered force by test speed. The probe travels into the sample at the test speed until the specified force, distance or stain is reached. After that, the probe returns to the start at the post test speed. The probe continues moving upward and measures stickiness of the sample. The probe continues moving until it returns to the starting position.

Development of reproducible method for determination of hydrogel texture
As the texture analyzer was not previously employed to characterize texture properties of hydrogels in this laboratory, we needed to establish the reproducible method of measurement. The first focus of the method development was the measurement of gel hardness. All measurements were performed by three independent researchers and in triplicates. As the start, 0.5% Carbopol gel was used.

Influence of polymer concentration on gel properties
Three different gel concentrations, namely 0.2, 0.5 and 1% were prepared and their texture properties measured by using 40 mm diameter compression disc.

Influence of pH on gel viscosity
Three batches of two different gel concentrations, namely 0.2 and 0.5% were prepared and analyzed on texture analyzer. Each sample was measured 5 times.

Stability testing of hydrogels
Accelerated stability testing was used to study the influence of temperature on the gel stability. For that purpose, 0.5% Carbopol hydrogel with pH values of 4.2, 4.7, 5.8, 6.9, 8.2 and 10.4, respectively were kept in a thermostat at 40 °C. After one month period, the gels were analyzed on texture analyzer as previously described.

Preparation of liposomal hydrogels
Phosphatidylycholine, Cholesterol and herbal extracts were dissolved in chloroform/methanol (2:1, v/v) mixture and subsequently transferred into a pear-shaped flask connected to a Rotavapor (Büchi- type). Speed was maintained at 150 r/min, vacuum applied and the thin film was formed by slow removal of the solvents at 40°C. The lipid film was maintained under vacuum for 12hr in a desiccator to remove solvent traces and subsequently it was hydrated with a Saline Phosphate Buffer of pH 7.4 solution at 400C under continuous rotation of the flask until a dispersion was formed (about 1h). The final suspension consisted of multilamellar vesicles was subjected to vortexing for two 5-min periods and kept for 30 minutes. The formulation plan for the formation of liposome given table below.

Entrapment efficiency determination
Ultracentrifugation was applied as separation method. Liposomes were centrifuged in Beckman-L8-70M ultracentrifuge (CA/USA) at 10 °C, for 25 min period at 32000 rpm in order to separate unentrapped herbal extracts from liposomally entrapped extracts. Upon centrifugation, the pellet was resuspended in 1500 µl distilled water, and an aliquot (10 µL) further diluted and used in spectrophotometrical and HPLC analyses. An aliquot (30 µL) of the supernatant was further diluted with methanol and the choralamphenicol content determined both spectrophotometrically and by the HPLC analysis.

Spectrophotometrical analysis:
A stock solution of herbal extracts was made by dissolving 51.7 g of herbal extracts in 200 mL of methanol. Working solutions were prepared by diluting the stock solution to desired concentration with methanol and calibration curve prepared using Agilent technologies UV/Visible spectrophotometer, G1103A (CA/USA) at 268 nm wavelength.

Particle size analysis
Diameters of vesicles were measured by dynamic light scattering (DLS) on the NicompTM model 380 particle sizing system (USA) with software version C-370 V-1.51a, and equipped with a fixed 90° external fiber angle and a 632.8 nm, 5 mW He–Ne laser. In order to avoid any contamination with dust, sample preparation was carried out in a clean area using particle-free equipment. All handling was done in a laminar air-flow bench, test tubes were submersed in particle-free water and sonicated for 15 min in an ultrasonic bath and rinsed with freshly filtered (0.2 nm pore size syringe filter) water prior to use. The vesicle-dispersion was diluted empirically with freshly filtered medium until an intensity of 250–350 kHz was achieved (Hupfeld et al., 2006).

Incorporation of vesicles in hydrogels
Based on the evaluation of texture properties, 0.5% Carbopel gel was selected as the most suitable vehicle for liposome incorporation. Liposomes (either empty or in the presence of unentrapped extracts) were incorporated into Carbopel gels following the procedure described by Skalko et al. (1998). Briefly, liposomes were mixed into hydrogels by the help of hand mixing, with the concentration of...
lipoosomes in the gel being 5, 10 and 15 % (w/w, liposomal suspension/total), respectively.

**Characterization of liposomal hydrogels by texture analysis:** Empty 0.5% Carbopol hydrogel was examined again to assure that the measuring conditions are indeed the same.

**In-vitro release studies**

To study the release of herbal extracts from liposomal hydrogels, the *in vitro* release model for topical dosage forms (“Freiburger Schlangen Schnecke”) was used. Liposomal hydrogels were spread on the donor side of the release cells. Triglycerides (50mL) served as an acceptor phase in the experiment. The release was studied during 10hrs time period. Samples were taken at predetermined time intervals, namely at 1, 2, 4, 6, 8, 10. The concentration of herbal extract was determined by HPLC method. The procedure and the conditions were identical to the procedure described in entrapment efficiency method.

3. Results and Discussions

![Figure 1: Comparison of Average Particle Size and % drug entrapment Of Herbal Extracts Loaded Liposome](image1.png)

**In-vitro release studies**

The cumulative percent herbal extracts release for formulation batch F-7 was found to be higher i.e. 75.35% and formulation batch shows smaller %CDR i.e. 45.38 after 24 hours. Form the particle size measurement, percent herbal extracts entrapment study and in vitro herbal extracts released study i.e., %CDR, it was concluded that International Journal of Current Trends in Pharmaceutical Research formulation batch F-7 having particle size 5.40 µm, percent herbal extracts entrapment 61.70 and in vitro herbal extracts released study i.e., %CDR 75.35% after 24 hours shows good result as compared to other batches. Though the F-7 batch having herbal extracts content was low compared to F-8 batch but the particle size and % CDR was found to be higher. Hence the F-7 batch was considered for the further evaluation study.

**In-Vivo Studies:**

**Anti – inflammatory activity:**

*Carageenan induced paw edema in rats:*

![Figure 2: Anti-inflammatory effect of Herbal liposomal gel on carageenan induced paw edema in rats](image2.png)

**Analgesic activity**

![Figure 3: Effect of Herbal liposomal gel on reaction time (sec) in Eddy’s hot plate](image3.png)

**Discussion**

*Herbal liposomal gel* showed maximum analgesic activity at 60, 90 min for 100 and 200mg/kg dose. The reaction time in normal control group at 60, 90 min was found to be 3.52±0.002, 4.08±0.161 sec. The reaction time (paw licking / jumping response) in rats pretreated with lower dose of *Herbal liposomal gel* (100mg/kg), higher dose of *Herbal liposomal gel* (200mg/kg/day) and Pentazocine (10 mg/kg) at 60, 90 min were found to be 9.26±0.851, 7.16 ± 0.193, 9.82 ± 0.894 and 8.60 ± 0.992, 9.12 ±0.372, 14.12±3.182 respectively when compared to control group rats. The development of edema in the paw of the rat after injection of carageenan is a biphasic event. The initial phase of the edema has been attributed to the release of histamine and serotonin, the edema maintained during the plateau phase to kinin like substances and the second accelerating phase of swelling to the release of prostaglandin like substances. Inhibition of edema observed in various inflammatory
models induced experimentally in the present study may, therefore be attributed to the ability of the Herbal liposomal gel to inhibit various chemical mediators of inflammation like histamine and 5-HT during the initial phase. In the present study Herbal liposomal gel significantly increased the reaction time in hot-plate test suggesting its central analgesic activity; the probable mechanism could be by inhibition of prostaglandin synthesis. Prostaglandins play significant role in different phases of inflammatory reactions and elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli.

**Table 1: In-vitro release studies**

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**4. Conclusion**

Adhesiveness, stability and release of incorporated herbal extracts are the main features that influence the applicability of hydrogels for topical treatment, including the wound healing process. Adjustment of the textural properties of hydrogel should be conducted routineosly. The texture analyzer measurements can provide deeper insight on gel adhesiveness. The gel properties are dependent on the polymer concentration and the pH. Carbopol hydrogels can take up to 15% (w/w) of liposomal dispersions, however, the stability of the liposomal gels need to be evaluated as well. Carbopol hydrogels can be used as advanced drug delivery systems. And the optimized formulation was selected by the basis of invitro drug release of the all the formulations. The development of edema in the paw of the rat after injection of carageenan is a biphasic event. The initial phase of the edema has been attributed to the release of histamine and serotonin, the edema maintained during the plateau phase to kinin like substances and the second accelerating phase of swelling to the release of prostaglandin like substances. Inhibition of edema observed in various inflammatory models induced experimentally in the present study may, therefore be attributed to the ability of the Herbal liposomal gel to inhibit various chemical mediators of inflammation like histamine and 5-HT during the initial phase. In the present study Herbal liposomal gel significantly increased the reaction time in hot-plate test suggesting its central analgesic activity; the probable mechanism could be by inhibition of prostaglandin synthesis. Prostaglandins play significant role in different phases of inflammatory reactions and elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli.

**5. References**


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