

## DEVELOPMENT AND CHARACTERIZATION OF TOPICAL LIPOSOMAL GEL FORMULATION FOR ANTI-CELLULITE ACTIVITY

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### ABSTRACT

**Objective:** The present study was aimed at development and characterization of a novel liposomal gel formulation of caffeine that could reduce the cellulite depositions over human body. It is already reported that liposomes made up of phosphatidylcholine may reduce cellulites from fat deposition sites by emulsifying and breaking down the fat and cholesterol while caffeine facilitate the movement and removal of dissolved cellulite in blood circulation.

**Experimental work done:** For this study liposomes of phosphatidylcholine and cholesterol were prepared by thin film hydration technique. The prepared liposomes were characterized for vesicle size, shape, morphology, zeta potential, encapsulation efficiency, in vitro drug release and drug disposition in animal model.

**Results and Discussion:** The percentages of caffeine entrapped in all liposome formulations varied between 37 and 58%. Gel containing liposomal dispersion was prepared in carbopol 934 and characterized for gel strength, viscosity and drug deposition in the animal skin. Stability studies were performed for both liposomal dispersion and gel formulation for 3 months. Amongst different storage conditions, the liposomes stored at 2 to 8°C were found to be most stable, with only 5% drug loss over the storage period of 5 weeks. Significantly higher skin permeation of liposomal formulations has been achieved, as compared to plain drug solution and carbopol gel containing caffeine.

**Conclusion:** Preparation of liposomes containing caffeine and dispensing in the form of carbopol gel was found to be well suited and sound approach to obtain stable liposomal formulation. All the results obtained from various in vitro and in vivo studies concluded that liposomal gel containing caffeine is one of the most successful formulations for reduction of cellulite deposits over human body.

**Keywords:** Anti-cellulite activity, Caffeine, Liposomal gel, Novel drug delivery.

### INTRODUCTION

Cellulite deposition is one of the most common problems associated with a large population male and female person through out the world. It occurs mainly in the area of abdominal bulges, love handles, back fat, saddle bags, thighs (inner and outer), side of buttocks, lower buttocks, arms, and double chins, cheeks and jowls [1]. Approximately 85% of women over the age of 20 have some degree of cellulite. Cellulite deposition on skin causes dimpled, orange peel appearance of skin that usually affects buttocks and legs. The truth about cellulite is that it can affect even the slim and trim body. Regardless of age or weight, many people develop a dimpling of the skin, a problem so persistent that even a balanced diet and regular exercise cannot completely make it disappear. Once it occurs, cellulite is impossible to hide. Hence we are proposing a novel treatment for cellulite removal, which is based on liposomal technology to deliver anti cellulite substances to the body in topical gel formulation.

Liposomes are microscopic vesicular structures consisting of one or more concentric spheres of lipid bilayers, enclosing aqueous compartments [2]. They have become a valuable experimental and commercially important drug delivery system, owing to their biodegradability, biocompatibility, low toxicity and ability to entrap lipophilic and hydrophilic drugs. Unfavorable pharmacokinetic profile of a certain drug can be altered by entrapping the drug in liposomes. Liposomes have also been noted to be superior as drug carrier systems for topical treatment over conventional topical preparations [3]. Phospholipids, being the major component of liposomal systems, can easily get integrated with the skin lipids and maintain the desired hydration conditions to improve drug penetration and localization in the skin layers [4-5]. Phosphatidylcholine has also been reported as a useful agent in reducing cellulites from fat deposition sites inside body by emulsifying and breaking down the fat and cholesterol. However anti cellulite activity of liposomes further improved by incorporating caffeine in its aqueous core, which is also having a breaking down action on

certain fat-storing enzymes. Thus the system collectively allows the fat cells to become emulsified and soluble in the circulation where it is then removed permanently by the liver, bowels and kidneys. Thus, recognizing the need for topical delivery of anti cellulite agents and the promising potential of liposomes, it has been envisaged to entrap the drug into these carriers and deliver in a topical gel formulation [6].

### MATERIAL AND METHODS

#### Materials

Soy lecithin (PC; phosphatidylcholine) was procured from Sam Industries Pvt. Ltd. Indore, India as a gift sample. Caffeine (Caf), Cholesterol (CHL), Sephadex G-50 medium (bead size range 50-150 mm), Triton-X 100, Carbopol 934 and Triethanolamine were procured from Himedia, India. All other ingredients used in the study were of analytical grade. Double-distilled (DD) water was used throughout the experiments.

#### Methods

##### Preparation of liposome

Multilamellar liposomes were prepared employing thin film hydration technique [7-8]. A lipid phase was prepared by dissolving accurately weighed quantities of the Caf, PC and CHL (Table. 1) in the chloroform, in 250 ml round-bottom flask containing glass beads. For each lot, an amount of 60 ml of each liposome dispersion sample was prepared. The lipid mixture was dissolved in 60 ml of chloroform and vacuum-desiccated (500 psi, 65°C) using a rotary evaporator for 90 min. A formed thin film layer was flushed under a stream of nitrogen for 1 min. The thin film was re-suspended in 15 ml of PBS (pH 7.4). The dispersion was swelled by swirling in a water bath (80°C, 200 rpm) for 30 min. Small multilamellar vesicles were obtained following the sonication of large multilamellar vesicles for 10 min, using a microtip probe sonicator. The liposome dispersion samples were kept at 4 °C and protected from light, prior to use.

### Preparation of carbopol gel and incorporation of Caf loaded liposomes

As a vehicle for incorporation of liposomes for transdermal delivery, a carbopol gel was made. Carbopol 934 was dispersed in distilled water by stirring at 800 rpm for 60 minutes. Then, propylene glycol was added and the mixture was neutralized by drop wise addition of triethanolamine. Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 6 [9]. Liposomes containing caffeine (separated from the untrapped drug) were mixed into the 1% (w/w) Carbopol gel with an electrical mixer (25 rpm, 2 min), the amount of liposomes of optimized batch (added into the gel, such that the prepared gel have 2.5% w/w liposomes concentration (25 mg drug per 1gm of gel) [10].

### Characterization of liposomal gel formulation

#### Particle size and morphology of liposome

A small aliquot of liposome dispersion sample was used to characterize the particle size and size distribution, using Transmission electron microscopy (Morayagni 268D, Holland) at All India Institute of Medical Sciences, New Delhi. A drop of liposome dispersion sample was applied on a 300-mesh formvar copper grid on paraffin and left for 10 min, to allow some of the liposome powder to adhere on the formvar. The remaining dispersion was removed and a drop of 2% aqueous solution of uranyl acetate was applied for 5 min. The remaining solution was then removed and the sample was air dried, prior to measurement with a TEM, for morphology and lamellarity of liposomes [11-12].

#### Percentage drug loading

The percentage drug loading was determined by taking 1.0 ml of liposomal suspension and separating the untrapped drug via mini column (Sephadex G-50 pre-saturated column with empty liposomes) centrifugation at 2000 rpm for 3 min [13-14]. Elutes containing drug loaded liposomes were collected and observed under optical microscope to ensure the absence of untrapped drug particles. Appropriate amount of elute was digested with Triton-X-100 solution (0.1 % v/v) and the clear solution thus obtained was analyzed spectrophotometrically (U.V./Visible spectrophotometer, Shimadzu-1601, India) for the drug content estimation at a  $\lambda_{max}$  of 273 nm. Liposomes prepared without drug were treated in similar manner and served as blank for the above study. Studies were conducted in triplicate. Percent drug loading (PDL) for the prepared liposomes was calculated as in given equation-

$$PDL = \frac{\text{Entrapped drug (mg)}}{\text{Total drug added (mg)}} \times 100$$

#### Determination of pH of liposomal gel formulation

The pH was experimentally determined using a pH - meter.

#### The analysis of rheological properties

Rheological analysis of liposome loaded carbopol gels were performed using a stress control rheometer (Viscotech Rheometer , Rheological Instruments AB, Lund, Sweden), equipped with stress rheologic basic software, using cone-plate geometry with a diameter of the cone being 25 mm and a cone angle of 10, operating in the oscillation and static mode. Rheological analysis was performed at room temperature and parameters like oscillation stress sweep, frequency sweep and creep-recovery were carried out for rheology measurement [15-18].

#### Storage - stability studies

The ability of vesicles to retain the drug (i.e., drug retentive behavior) was assessed by keeping the liposomal suspensions and liposomal gel at two different temperature conditions, i.e., 4-8 °C (Refrigerator; RF), 25±2 °C (Room temperature; RT), for a period of 60 days. Samples were withdrawn periodically and analyzed for the

drug content and particle size for liposomal suspension and drug deposition for liposomal gel in the manner described under entrapment efficiency and particle size distribution studies [19].

#### In-vitro skin permeation studies

Skin permeation studies with Caf loaded liposomal formulations (liposome suspension and liposomes incorporated in Carbopol gel), were carried out using abdominal skin of albino rats, employing modified Franz-diffusion cells [20]. The results obtained were compared with that of non-liposomal formulations of Caf i.e., aqueous solution and Carbopol gel, containing equivalent amounts of Caf. Briefly, to obtain skin, animals were sacrificed after getting approval of the animal ethics committee of Vidyabharati College of Pharmacy, Camp, Amravati, MH India. Hairs on the dorsal side of the animal were removed with the help of a 0.1 mm animal hair clipper, in the direction of tail to head. Dermis part of the skin was wiped 3 to 4 times with a wet cotton swab soaked in isopropanol to remove any adhering fat material. Skin was mounted on the receptor chamber with cross-sectional area of 3.801 cm<sup>2</sup> exposed to the receptor compartment. Saline solution (35 ml, 0.9% w/v) was employed as receptor phase and the temperature was maintained at 37±2°C. Liposomal or non-liposomal Caf formulation (amount equivalent to 3 mg of drug) was applied uniformly on the dorsal side of rat skin [21]. Aliquots of 3 ml were withdrawn periodically and replaced with same amount of saline solution to maintain the receptor phase volume at a constant level. The samples were quantified spectrophotometrically at  $\lambda_{max}$  of 273 nm.

#### Determination of Caf liposome retention in skin

The ability of vesicles to help retain the drug within the skin layers rich in cellulites was investigated by determining the amount of drug retained in the skin samples employed in permeation studies. After completion of the permeation experiment, skin mounted on the diffusion cell was removed. The skin was cleaned with cotton dipped in saline solution and blotted with tissue paper to remove any adhering formulation. Subsequently, the skin sample was homogenized with 10 ml of chloroform for the extraction of Caf. Homogenate suspension thus obtained was filtered using membrane filter (0.45m) and quantified for the drug content [22-23].

## RESULTS AND DISCUSSION

### Preparation of drug loaded liposomal gel formulation

The preparation of Caf loaded liposomes was initiated by studying the influence of drug-lipid ratio on drug entrapment in vesicles. Various formulation variables like vacuum, speed of rotation, hydration media and hydration time were studied in order to produce Caf loaded liposomes with desired properties. Rotational speed of the flask markedly influenced the thickness and uniformity of the lipid film. The optimum speed was found to be 150 rpm, as the same yielded a uniform thin film on the flask and subsequently homogeneous population of liposomes [24]. Vortexing for two minutes was found to be appropriate to produce aggregate free liposomal suspension. With regard to the influence of formulation components on the PDL, different compositions with varying ratios of drug, PC and CHL were studied. Table 1 summarizes the influence of drug-lipid ratio and the effect of CHL on the PDL of Caf in the liposomes. The drug bearing capacity of liposomes (i.e., PDL) was found to be invariably dependent on drug-lipid ratio employed in the liposomal composition. In case of CHL free PC liposomes (formulation CL1 to CL4), maximum drug loading of 42.5 percent could be achieved using 1:10 w/w drug-lipid ratio during preparation. A further improvement of 10 to 15 % in the entrapment of CL3 liposomes was noted with the addition of 30 to 50% w/w of CHL (formulation CL5 to CL7, Table 1). Although the PDL was found to increase with CHL addition, however, the effective drug-lipid ratio in the liposomes decreased due to increase in the total amount of lipids. Based on the above findings, liposomal formulation CL7 was selected for further studies.

**Table 1: Effect of drug-lipid ratio and amount of cholesterol on the Caffeine loading in PC liposome**

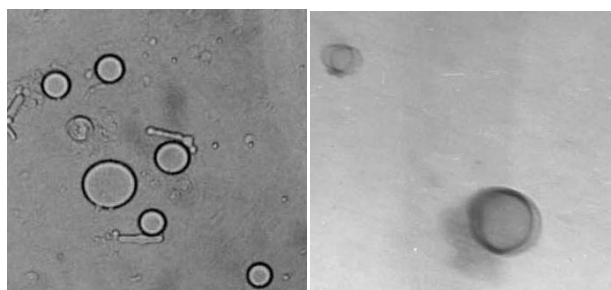
Formulation code	Caf: PC: CHL (Weight ratio)	Drug entrapped* (mg)	% drug loading	Vesicle size
CL 1	05:100:00	1.85±0.20	37.0	5.01 ± 1.38
CL 2	7.5:100:00	3.12±0.12	31.2	8.25 ± 1.52
CL 3	10:100:00	4.25±0.18	42.5	9.64 ± 1.56
CL 4	15:100:00	3.80±0.12	38.0	14.18 ± 1.28
CL 5	10:100:30	5.16±0.11	51.6	9.46 ± 1.42
CL 6	10:100:40	5.45±0.21	54.6	10.54 ± 1.66
CL 7	10:100:50	5.78±0.15	57.8	10.02 ± 1.34
CL 8	10:100:60	5.61±0.14	56.1	14.56 ± 1.64

\*Value indicates mean ± S.D. (n=3)

### Characterization and stability profile of liposomes

#### Particle size and morphology of liposome

The size range of various Caf liposome formulations was found to be 5 to 15 µm, with 90% of the liposomal population equal or below 10 µm. The Optical photomicrograph of CL7 liposomes (Fig. 1A) obtained at suitable magnification (65X) confirms the micron size of the vesicles. The reproducibility of the liposomal formulation with respect to size was confirmed by preparing the formulation three times, but the statistical analysis was avoided, as the particle size data was highly reproducible each time. The transmission electron micrographs of Caf loaded liposomes showing the morphology and lamellarity of liposomes (Fig. 1B).



**Fig. 1: A and B: Photomicrograph of drug loaded liposomes (65x), TEM photograph of caffeine loaded liposomes**

#### Percentage drug loading

Determination of PDL is an important parameter in case of liposomes as it may affect the drug release and skin deposition. In the present study, the observed PDL for different batches of CL7 formulation was in the range of 37 to 56%. Incorporation of CHL in PC liposomes enhanced the percent entrapment of Caf, owing to its cementing effect on the membrane packing. The same would prevent drug leakage from the bilayer membranes leading to enhanced drug retention in liposomes. According to the size and PDL, small sized liposomes which can cover the skin surface more compared to larger particle size were selected as optimized batch for incorporation in to gel formulation [25].

#### pH determination

The pH of the experimental gels with different percentage of carbopol 934 was found in between 6.6-7.0 (Table 2). This pH was found to be close with the pH of human skin and hence it can be

assumed that no skin irritation will occur after application of gel containing liposomal drug.

#### Rheological study

During stress sweep, it was observed that there was linearity between stress and strain produced all over the applied stress range, which indicate that system was working in correct range. It was clear that gel containing 2% of carbopol 934 had shown low dependency on frequency for viscous moduli and phase degree. The value of phase degree for 2% was well in visco-elastic region. Texture profile analysis measures the mechanical properties of a gel like hardness, cohesiveness and adhesiveness. They are determined with the help of QTS-25 Texture Analyzer. Maximum hardness and adhesiveness were exhibited by gel prepared with 2% carbopol 934 were 15.00±0.51 g and -45.03±5.02 g, respectively. Cohesiveness of carbopol gel containing liposomal dispersion was found to be 1.10±0.05 (Table 2). Cohesiveness reduced with increase in amount of mucoadhesive agents. With increase in amount of liposomal dispersion, semisolid nature of the gels increased, which caused the gel to become less coherent.

#### Stability studies

Responses obtained for different parameters for liposomal dispersion and liposomal gel during stability period are as shown in Table 3. From results it can be concluded that at room temperature and freeze temperature there was slightly but insignificantly decrease in % entrapment efficiency and increase in particle size for liposomal batch. Result suggests that keeping the liposomal product in refrigeration conditions minimizes stability problems of liposomes.

#### In-vitro skin permeation studies

Results of in-vitro drug permeation studies conducted with different formulations of caffeine are shown in Table 4. Significant augmentation in the skin permeation of caffeine has been observed with liposomal formulations and drug in carbopol gel. The amount of caffeine permeated in eight hour was found to be 36.16% and 34.32% from liposomal suspension and liposomal gel, respectively, whereas only 28.43% of the drug permeated in case of plain Carbopol gel preparation. Higher values of flux obtained with liposomal suspension 0.268µg/cm<sup>2</sup>/h and liposomal gel 0.212 µg/cm<sup>2</sup>/h, comparative to those obtained for carbopol gel 0.124µg/cm /h, which clearly vouch for the permeation enhancing effect of vesiculation on the drug. Results of this study clearly depict that the amount of drug retained in the skin was considerably higher in case of liposomal preparations, than with non-liposomal (Fig. 2).

**Table 2: Evaluation of carbopol 934 Gels**

Formulation Code	Carbopol 934	pH	Hardness (g)	Adhesiveness	Cohesiveness
F1	1%	7.0	21.34±0.88	-51.32±4.78	0.96±0.03
F2	2%	6.6	15.00±0.58	-45.03±5.02	1.10±0.05
F3	3%	6.8	27.20±0.32	-76.25±3.56	1.02±0.11

Value indicates mean ± S.D. (n=3)

Table 3: Effect on entrapment efficiency and vesicle size for liposomal dispersion from liposomal gel during stability

No. of days	Entrapment efficiency %		Vesicle size ( $\mu\text{m}$ )	
	4-8°C	Room Temp.	4-8°C	Room Temp.
0	57.8	57.8	10.02	10.02
30	56.2	55.6	10.88	11.45
60	54.7	52.5	11.24	13.68

Table 4: In-vitro skin permeation and skin retention of Caf from different formulations

Caffeine formulation	Mean cumulative % drug permeated ( $\pm\text{SD}$ )	Permeation flux $\mu\text{g}/\text{cm}^2/\text{h}$	% drug retained in skin
Plain drug solution	12.78 $\pm$ 1.42	0.068	3.56
Drug in carbopol gel	28.43 $\pm$ 2.40	0.124	7.42
Liposomal Suspension	36.16 $\pm$ 1.34	0.268	16.04
Liposomal Gel	34.32 $\pm$ 1.68	0.212	13.36

Value indicates mean  $\pm$  S.D. (n=3)

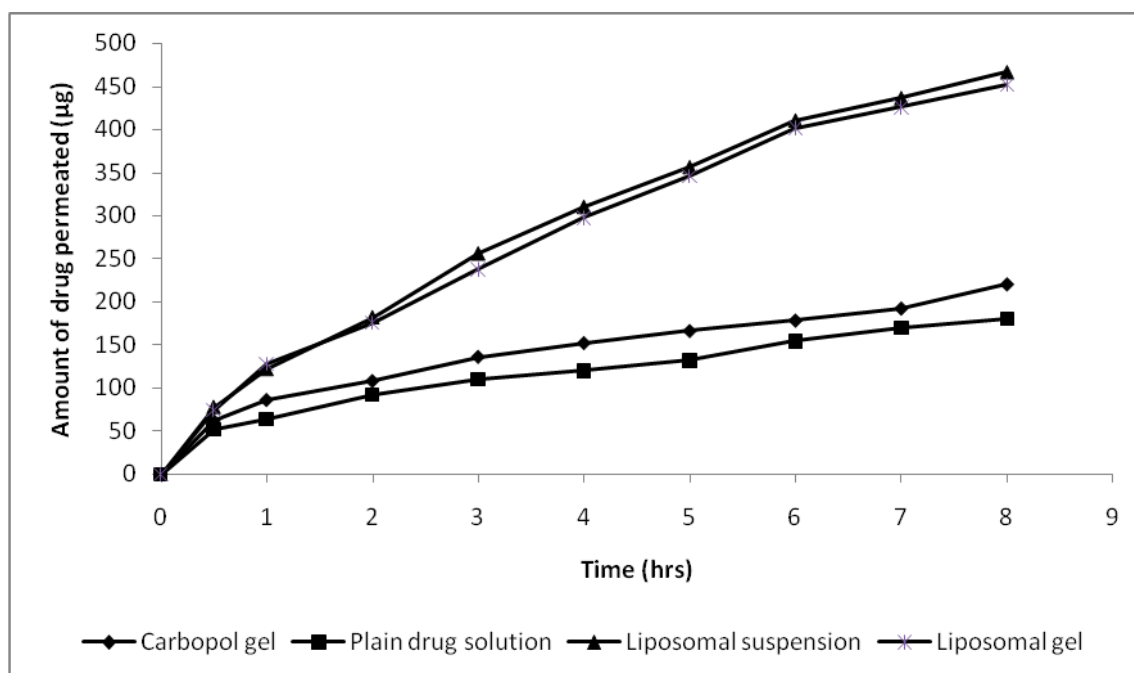


Fig. 2: Permeation profile of different caffeine containing systems

Above studies depicts the utility of liposomes which could not only enhance the penetration of drug molecules but also helped in localization of the drug in skin tissues. Improved skin permeation of drug coupled with its enhanced retention in the skin tissues with liposomal formulation can be ascribed to the higher lipo-dissolve activity of caffeine molecules. The liposomal phospholipids (also one of the natural constituent of skin lipids) helped generating and retaining the required physico-chemical state of the skin for enhanced permeation of the caffeine. Further, the phospholipid-rich domains of vesicles might have helped to produce the depot effect for drug molecules. The latter has been reflected as higher amount of drug retention within the skin layers in case of liposomal formulations.

Thus, the liposomal gel formulation, with desired characteristics for lipo dissolve activity, could be successfully prepared. The formulated Caf liposomes have shown an appreciably enhanced skin permeation as well as retention of drug molecules in the skin. The skin tightens over the treated area as the fat is removed, so that there is no looseness of skin post-treatment. Furthermore, liposomal gel formulation also improved the appearance of the surrounding cellulite in the treated area along with the reduction in the appearance of stretch marks, although the results from this are usually variable.

## CONCLUSION

Preparation of liposomes containing caffeine and dispensing in the form of carbopol gel was found to be well suited and sound approach to obtain stable liposomal formulation. Variables in liposomal formulation such as amount of phospholipid, amount of stabilizer have a profound effect on the vesicle size and entrapment efficiency. Rheological studies of all liposomal gels prepared with 1% w/w carbopol gave a clear idea of concentration of carbopol required. Liposomal dispersion and gels were found to increase the skin permeation and deposition compared to control. Stability studies performed for liposomal gel indicates the prepared liposomes have more stability at cold temperature than that of room temperature. Caffeine molecules could be successfully entrapped in liposomes with reasonable drug loading. Hence from results obtained it can be concluded that liposomal gel containing caffeine has potential application in reduction of cellulite deposits from the skin.

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