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FORMULATION AND DEVELOPMENT OF LIPOSOMAL DELIVERY OF ANTICELLULITE

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Keywords:

Phosphatidylcholine, Rotavapor, Anitcellulite, Stearic acid, Percent drug entrapment

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ABSTRACT:

Aim: Cellulite treatment incorporates iso-slim complex to reload the skin with minerals and trace elements to achieve absolute effectiveness in treating cellulite condition. The revitalizing effect of anticellulite helps to refine and reshape the silhouette and give the body a more toned, defined and firmer appearance. Aim of present work was to develop and investigate a novel delivery system for the fatty skin in the form of liposome delivery system.

Method: Phosphatidylcholine, Cholesterol and iso-slim complex were dissolved in chloroform/methanol (2:1, v/v) mixture and subsequently transferred into a pear-shaped flask connected to a Rotavapor (Buchi-type). Rotary evaporation method was used for the formulation of liposomes.

Result: Liposome prepared was evaluated for particle size measurement, percent drug entrapment, formulation of dosage forms, Stability Study and in vivo study. Optimized batch had particle size 18.62 um, percent drug entrapment 96.70%. Hence, optimized was batch further evaluated for *in vivo* study and stability study.

Conclusion: The present study has been a satisfactory attempt to formulate and evaluate liposome of iso-slim complex and liposomal gel with a providing sustained delivery of drug. The liposome made by iso-slim complex with lecithin and stearic acid found better effect on the skin. Drug loaded liposome have great penetration power and deposited in deeper layer of the skin. The main aspect for this preparation is to reduce the fat from the skin.

INTRODUCTION: 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. And, once it occurs, cellulite is impossible to hide [1].

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There's simply no way to hide those lumpy ripples on thigh, buttocks, upper part of arms, knees and more rarely the lower part of the legs and the back of the neck. That's why millions of dollars are spend every year on liposuction surgery to rid our bodies of this unsightly phenomenon. But there is another way to get rid of unwanted fat. Cellulite treatment incorporates iso-slim complex to reload the skin with minerals and trace elements to achieve absolute effectiveness in treating cellulite condition. High quality of compounds helps to stimulate removal of excess fluids, eliminating toxins, rebalance the metabolism and break down cellulite.

The revitalizing effect of anticellulite helps to refine and reshape the silhouette and give the body a more toned, defined and firmer appearance. With time the skin looks smoother and firmer, and the silhouette remodeled. But before adding these active directly into the product it must be core with liposome technology, because it transport 100% active to the skin and increase the stability into the product over a long period of time. Also since no much more has been done on incorporation of active agent loaded liposome into cosmetics products, the effects are made to evaluate its efficacy [2].

Today's sedentary life style contributes to the aggravation of cellulite leading to a decrease in muscle mass and an increase in fatty mass. The factors such as overweight, pregnancy, aging poor blood circulation., poor lymph drainage, lack of exercise, high fat and sugar consumption, sun exposure, etc. have the direct impact onto the condition of the skin, the skin looks loose, wrinkled, bulgy, dimpled, and dull.

So the use of anticellulite agent like isoslim complex was our key object besides improving the texture of skin. Beside it, one of our main objective of using novel system of transporting the key ingredients in appropriate amount, affected site and for requisite time period, it is necessary for us to incorporate the active in cream and gel [3].

Iso-Slim Complex:

The new dimension for your slimming and anti-cellulite cosmetic [4]: Iso-Slim Complex is a new and highly effective anti-cellulite formula based on a combination of actives that work in a synergistic way:

***Spirulina platensis*:** Rich in minerals and polysaccharides:

1) Protection against oxidative stress
2) Skin moisturization and stimulation of lipolysis

Caffeine and Carnitine:

1) Inhibition of the enzyme phosphodiesterase -> stimulation of lipolysis
2) Activation of cell metabolism

A human study with Iso-Slim Complex delivered the following impressive results after just 6 weeks of treatment:

Genistein: The biologically active form of soy isoflavones (Genistein) improves the collagen content in the skin and in doing so, strengthens the connective tissues.

Further-more Genistein inhibits the formation of new adipocytes and this in turn will lead to the breakdown of fat. Consequently, this will result in:

1) An increase in skin firmness
2) A reduction of adipose tissue

Cellulite Development: Cellulite is the dimpled and pitting skin that tends to predominately appear on buttocks, thighs and legs. This is something that affects almost 90% of women, yet is found much less often in men. One of the reasons for this is the difference in the fibers that anchor the skin to the muscles between the two genders; whilst the fibers in the skin of women run only in one direction, those of men are tight and criss-crossed, effectively creating a net to keep the fat firmly in place. In addition, adipocytes in women are aligned directly under the dermis whereas mal fat cells are embedded under a thicker and more elastic skin layer. The depositing of fat will result in the expansion of the female fat cells. This causes a bulging in the dermis and the epidermis and leaves the skin resembling the texture of an orange peel.

The skin strengthening qualities of Genistein will restrict the formation of new adipocytes: Iso-Slim Complex, containing the soy isoflavone Genistein, will successfully fight cellulite in the following two ways:

1. Its skin thickening and strengthening activity will enhance the firmness of the skin.

2. Genistein is an inhibitor of phospho-diesterase and as such, it acts directly on the fat metabolism. Phosphodiesterase is an enzyme that inactivates cyclic AMP (cAMP), which itself stimulates the enzyme lipase that breaks down fat. Therefore, the overall reduction of fat is made possible and the amount of fatty tissue will decrease.

The bioactive Aglycone Form: There are a large number of soy isoflavone products available on the markets that are either sold as dietary supplements or cosmetic raw materials. However, in the majority of cases, these products contain the isoflavone glycosides, which are biologically inactive. The oral application of such natural occurring isoflavone glycosides will lead to the active form upon hydrolysis in the gastrial tract. However, critically, the topical application on the skin will not result into active isoflavones (D. Schmid/F. Zülli) for this reason, we at Mibelle AG Biochemistry decided to develop a process of manufacturing a pure isoflavone a glycone preparation that is suitable for topical applications. The reaction showing the conversion of isoflavone glycosides to isoflavone aglycone is shown in **fig. 1**.

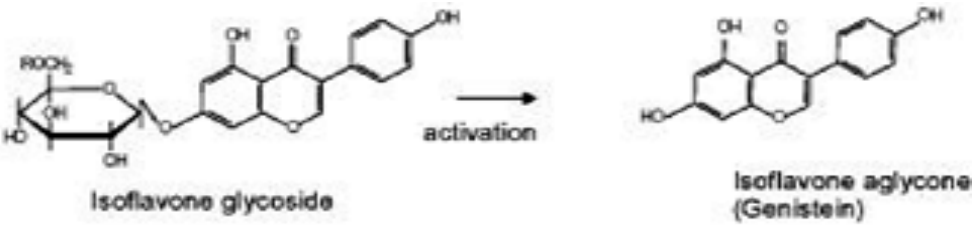


FIGURE 1: REACTION SHOWING THE CONVERSION OF ISOFLAVONE GLYCOSIDES TO ISOFLAVONE AGLYCONE

Spirulina: *Spirulina platensis* (**figure 2**) is classified as blue - green algae. It is a simple, single cell form of algae that lives in warm, alkaline fresh waters. The word "spirulina" is derived from the Latin word for "helix" or "spiral". This denotes the physical configuration of the organism when it forms swirling, microscopic strands. In Japan, Spirulina is a popular food supplement and is marketed as a nutritional supplement in many other countries. It has a very impressive ability to synthesize high-quality concentrated nutrients. Spirulina is a rich source of both proteins and rhamnose sugars (complex natural plant sugars). It also contains chlorophyll, carotenoids, minerals, gamma linolenic acid (GLA) and some unique pigments. Spirulina is one of the few plant sources of vitamin B12 and is usually only found in animal tissues.

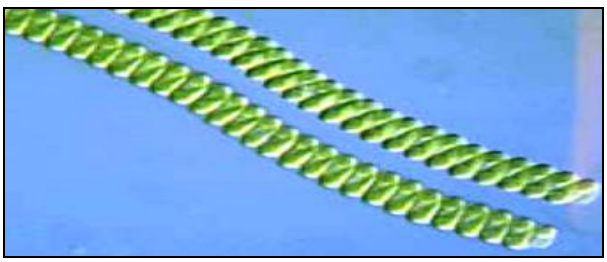


FIGURE 2: SPIRULINA

The impact of Caffeine and Carnitine: Caffeine is a well know natural substance that can be used to combat cellulite. Caffeine engenders a lipolytic effect: Like Genistein, it is able to block the enzyme phosphodiesterase, which is responsible for the destruction of cAMP, a molecule that is required to ensure the breakdown of triglycerides. This results in an increased lipolytic activity. Caffeine also contains vasodilator properties that help to increase the blood flow. Consequently, it successfully creates both a lipolytic and a venotonic effect. L-Carnitine is an amino acid that contributes to the oxidation of fats and removal of toxic ketones and it is another significant factor in fighting cellulite. Renowned for enhancing triglycerides mobility, Carnitine will successfully accelerate their breakdown. Iso-SlimComplex INCI/CTFA-Declaration:-Spirulina Platensis Extract (and) Soy Isoflavones (and) Caffeine (and) Carnitine (and) Polysorbate 80 (and) Phenoxyethanol (and) Alcohol (and) Aqua/Water.

MATERIAL AND METHOD: Isoslim complex was received as a gift sample from Arihant Chemical, Mumbai, Soya lecithin (Phosphatidyl choline) was received as a gift sample from Phospholipid GmbH Nattermannallee, Germany, stearic acid and Carbopol 940 were purchased from S.D. Fine Chem., Ltd., Mumbai. All other reagents used were of analytical reagent grade.

1) Liposome Preparation [5, 6]:

Preparation of Liposomes of soya Lecithin and stearic acid: Different batcher of liposomes was prepared using fixed concentration of soya lecithin and variable concentration of stearic acid. The formulation was prepared by taking 5gm of mannitol powder (sieved with 120 mesh) was

placed in 100ml around bottom flask which was held at 60-70^{o}C temperature and the flask was rotated at 80-90 rpm. The rotating flask was kept into the water bath at 70-80 rpm and mannitol was dried under vacuum for 30 min. After drying, the temperature of the water was adjusted to 20-30^{o}C.

Then in beaker soya lecithin 2gm and 0.8gm stearic acid is taken. In that the add a mixture of organic solvent i.e. chloroform: methanol, 8:2 v/v; After the formation of one phase solution with the help of syringe of 0.5ml add the solution into the flask containing mannitol powder, 0.5ml solution was added at the interval of 10 min up to the solution get finished. After last loading, flask was kept in desiccators for overnight or in sunlight to remove the traces of solvent. After it get complete dry pass, from the sieve. The collected powder was transfer into the glass bottle and get stored. Formula was developed as shown in **table 1**.

TABLE 1: PREPARATION OF LIPOSOMES OF SOYA LECITHIN AND STEARIC ACID

Formula	1	2	3	4
Soya Lecithin	1%	2%	3%	4%
Stearic Acid	0.2%	0.4%	0.6%	0.8%

2. **Optimization of Liposomes (Unloaded): Optimization of liposomes:** Although the general procedure for the preparation of soya lecithin liposomes has been essentially the same in almost all the batches. Variation in formulation condition has been tabulated in **table 1**. The data reveal the variation in concentration of stearic acid mixing. Various batches of liposomes were prepared by varying the parameter under the study. Keeping all the other factor same instead of RPM. RPM should vary in the batches of liposomes and evaluated with respect to particle size.

Lecithin and stearic acid complex liposomes: The batches of cross linked liposomes prepared using different concentration of soya lecithin and stearic acid with methanol, chloroform combination ratio.

Chloroform: Methanol - 8: 2

Particles with wide range of size distribution occurred. Hence, more batches are prepared using different concentration of stearic acid [0.2, 0.4, 0.6, and 0.8]. The concentration of 1%/0.4% have good liposome size distribution. Also these batch show increased batch yield as compared to other batcher [5].

Parameters of optimization of method of preparation: Factors affecting the formulation process:

1) **Temperature:** As the temperature increases the particle size reduces but yield was found to be low.

2) **Mixing**: The homogenous dispersion of the phospholipids alone with stearic acid in chloroform: Methanol mixture is required. The homogeneous mixture was obtained by using magnetic stirrer.

3) **Time**: Time factor is most important in the preparation of Liposome. But for liposomal preparation, time factor was constant.

4) **Storage**: Storage is important to the stability of liposomes**;** Powder form has good stability than liposomal suspension form.

Preparation of active loaded liposomes of soya lecithin and Stearic acid: Different batches of active loaded liposome with variation in concentrations of active, shown in **table 2**.

TABLE 2: PREPARATION OF ACTIVE LOADED LIPOSOMES OF SOYA LECITHIN AND STEARIC ACID

Formula	1	2	3	4
Lecithin	1%	1%	1%	1%
Stearic acid	0.2%	0.4%	0.6%	0.8%
Active	1%	2%	3%	4%

In this study, various batches were taken for different concentration of active.

Procedure: The formulation was prepared by taking 5gm of mannitol powder was placed in a 100ml round bottom flask which was held at 60-700^{o}C temperature and the flask was rotate at 80-90 rpm. The rotating flask was kept into the water bath at 70-80 rpm and mannitol was dried under vacuumed for 30 min. After drying, the temperature of the water bath was adjusted to 20-300C. Active means iso-slim complex, soya lecithin and stearic acid were mixed in organic solvent i.e chloroform: methanol, 8:2 v/v.

After formation of one phase solution with the help of syringe the 0.5ml was added in RBF containing mannitol the flask was in rotating motion. After the formation of loaded liposome, it was kept in desiccators for overnight, after it gets complete dry, it was passed from mesh, and the collected powder was transfered into glass bottle and stored [6].

3. **Product Formulation and Development**

- Preparation of Cream
- Preparation of Gel

A. Preparation of Cream:

a) Formulation of cream: Shown in **table 3.**

TABLE 3: FORMULA FOR CREAM PREPARATION

Ingredients	Quantity for 100 gm		
	1	2	3
Stearic acid	8%	7%	6%
Cetyl alcohol	3.5%	3.5%	3.5%
Glycerine	5%	6%	7%
Mineral oil	12%	12%	12%
Tea	0.5%	0.5%	0.5%
Perfume	q.s.	q.s.	q.s.
Vit. E	0.4%	0.4%	0.4%
Almond oil	0.4%	0.4%	0.4%
Methyl paraben	q.s.	q.s.	q.s.
Propyl paraben	q.s.	q.s.	q.s.
Water	69.7%	69.7%	69.7%

b) Variation in formulation: Shown in **table 4.**

TABLE 4: VARIATION IN FORMULATION

Ingredients	ACC-1	ACC-2	ACC-3
Stearic acid	8%	7%	6%
Glycerine	5%	6%	7%

c) Incorporation of liposome loaded with iso-slim complex in base: Shown in **table 5**.

TABLE 5: INCORPORATION OF LIPOSOME LOADED WITH ISO-SLIM COMPLEX IN BASE

Ingredients	Quantity for 100 gm		
	1	2	3
Stearic acid	8%	7%	6%
Cetyl alcohol	3.5%	3.5%	3.5%
Glycerine	5%	6%	7%
Mineral oil	12%	12%	12%
Tea	0.5%	0.5%	0.5%
Perfume	q.s.	q.s.	q.s.
Vit. E	0.4%	0.4%	0.4%
Almond oil	0.4%	0.4%	0.4%
Methyl paraben	q.s.	q.s.	q.s.
Propyl paraben	q.s.	q.s.	q.s.
Water	69.7%	69.7%	69.7%
Active isoslim complex	2%	3%	4%

Procedure: Separately oil phases and water phases were weighed and then both phases were heated at 80^{o}C. Oil phase was added to the water phase and thus emulsification process was carried out with the help of mechanical stirrer.

When temperature lowers down to 40^{o}C then perfume and color was added. The cream was stored for further study [7].

B. Formulation of Gel:

a) **Carbopol with desired property:** Shown in **table 6**.

TABLE 6: CARBOPOL WITH DESIRED PROPERTY

Number	Chemical form	Viscosity	Neutralized clarity	Effect of temperature of viscosity	0.5% Brookfield viscosity, cps
934	Acid	High	Good	Second best	34,500
940	Acid	High	Good	Best	44,000

Acidic Carbopol derivatives were neutralized with different kind of alkalis and amount of respective alkalis required to bring 1 part Carbopol, either type, to a pH of approximately 7.0 were as follows.

b) **Preparation of Gel:** Gel is aqueous system comprising more than 80% water. Gel is an aqueous system comprising more than 80% water. Aqueous gel preparation more effective than oily form. Shown in **table 7**.

TABLE 7: FORMULA FOR GEL

Ingredients	Quantity for 100 gm		
	ACG-1	ACG-2	ACG-3
Carbopol 940	0.8%	1%	1.2%
Glycerine	2%	2%	2%
Propylene glycol	4%	4%	4%
EDTA di.sod. salt	0.1%	0.1%	0.1%
Methyl paraben	0.2%	0.2%	0.2%
Triethanolamine	0.9%	1.1%	1.3%
Distilled water	92%	91.6%	91.2%

ACG = Anti Cellulite Gel

Procedure: Carbopol 940 was dispersed in half quantity water. Then disodium EDTA salt was added in half of water. Glycerin, propylene glycol and preservative were mixed well in another beaker. Bothe quantities was mix in one beaker and the TEA added. pH 7 was adjusted. The gel was stored in suitable container and use for further study [7].

c) **Optimization of Gel:** A formulation of optimum characteristic was selected. The desired characteristics were checked by varying the concentration of various ingredients. Variation in formulation is tabulated below **Table 8**.

d) **Incorporation of Liposome loaded with iso-slim complex in Gel:** Shown in **table 9.**

TABLE 8: FORMULATION OF GEL

Ingredients	ACG-1	ACG-2	ACG-3
Carbopol 940	0.8%	1%	1.2%
TEA	0.9%	1.1%	1.3%

TABLE 9: INCORPORATION OF LIPOSOME LOADED WITH ISO-SLIM COMPLEX IN GEL

Ingredients	Quantity for 100 gm		
	ACG-1	ACG-2	ACG-3
Carbopol 940	0.8%	1%	1.2%
Glycerine	2%	2%	2%
Propylene glycol	4%	4%	4%
EDTA di.sod.salt	0.1%	0.1%	0.1%
Methyl paraben	0.2%	0.2%	0.2%
Triethanolamine	0.9%	1.1%	1.3%
Distell water	92%	91.6%	91.2%
Active	2%	3%	4%

Evaluation of Liposome:

1) **Particle size determination**: Light microscopy was been utilized to examine the gross size distribution of large vesicles. The size of the liposomes was characterized with a stage micrometer and an eyepiece micrometer. The eyepiece micrometer was calibrated using stage micrometer. The sizes of around 100 particles were measured and their average particle size was determined [8].

2) **Entrapment efficiency:** 25 ml of iso slim complex liposome was weighed and suspended in 10 ml of the ethanol, as the drug is freely soluble in ethanol. Only the drug which was present in vesicles got extracted in the ethanol. The suspension was shaken vigorously for 1 hour and kept for few hours with intermittent shaking. After this the suspension was taken and centrifuged for 15 to 20 minutes at 2000 rpm and supernatant was collected and analyzed at max 255nm [9].

Entrapment efficiency was calculated by the formula.

Entrapment efficiency =

$$\frac{\text{Total amount of the drug} - \text{Free drug}}{\text{Total amount of the drug}} \times 100$$

3) **Determination of pH:** The formulation of cream is meant for topical applications so their pH should be similar to that of the skin. The skin has an acidic mantel and the pH of skin creams as per standards should be in the range 5.0-9.0. To ensure the required shelf life of skin cream, chemical inertness is essential i.e. it should neither be too acidic nor too alkaline. Base on the above points it was thought that the standard pH of skin creams should be in the range of 5-6.5 [9].

Apparatus used: Digital pH- meter.

Procedure: Take 5 gm of cream sample in a beaker and add 45 ml of distilled water to it, mix it properly until the whole cream is dissolve in water; then note the pH of sample mixture by using pH meter.

4) **Determination of spreadability:**

Apparatus: Petri-dish.

Procedure: 1 gm of cream sample was placed on outer side of Petri-dish and place second dish on it, pressed a little, kept aside for a minute then diameter of sample in cm was noted [9].

5) **Determination of thermal stability:-**

Apparatus: Beaker, oven.

Procedure: Spread 20mm broad and 5mm thick strips from the cream sample to be tested on the internal walls of beaker of 100ml capacity in its total weight; keep the beaker for 8 hours at room temperature and also in oven at 45^{o}C. If there is no separation of oil phase & water phase, then it passes the test.

6) **Determination of viscosity:**

Apparatus: Brookfield viscometer.

Procedure: The viscosity of cream was determined by spindle no. 4 using Brookfield viscometer then all the operating condition was set up. Then five readings were taken at different RPM and average of these will be the final readings. Viscosity was measured directly at various RPM in cps.

7) **Determination of total fatty matter:-**

Reagent: Reagent: Dilute hydrochloric acid, ethyl ether, sodium sulfate.

Methyl orange indicator solution: Dissolve 0.1gm of methyl orange in 100 ml of water.

Procedure: 2gm of sample was weighed in to conical flask, about 25 ml of dilute HCl was added, and reflux condenser fitted into the flask and the content of the flask was boiled until the clear solution is obtained. The content of the flask was rinsed with 50 ml of ethyl ether in portions of 10ml. content of flask was poured in separating funnel, the separating funnel was shake well and kept aside in order to separate the layer of oil. Washed out with 50ml portions of ether twice, all the ether extract was combine and washed them with water until free of acid then the extract was filtered through the filter paper by using sodium sulfate. The filtrate was dried at 60+-20^{o}C to a constant mass.

$$\text{Total fatty substance \% by mass} = \frac{M1}{M2} \times 100$$

Where; M1 = Mass in gm of residue and, M2 = Mass in gm of material taken for the test.

8) **Determination of water content:**

Reagents: Toluene – treated with excess of water and distilled.

Procedure: About 200ml of toluene and dry pumice stone was added. The apparatus was connected and the receiving end of the tap was filled with toluene poured through the top of the condenser. The flask was heated gently for 15minutes and when the toluene begins to boil it was reflux at the rate of 2drops/second until the most of water passed over. The rate to about 4drops/sec. was increased when all the water had apparently distilled over, the inside of the condenser tube was rinsed with toluene while brushing down the tube with the tube brush attached to a copper wire and saturated with toluene. The distillation was continued for 5min, then the source of heat was removed and the receiving tube was allow to cool at room temp, the water is collected in receiving tube. The water in toluene had separated, and then volume of water was read.

9) **Stability Study:** Stability may be defined as the ability of drug to retain its properties within specified limits throughout its shelf life. Improper storage of cosmetic product leads to their physical detoreriation and chemical degradation resulting in reduced activity and occasionally in the form of toxic degradation product. So stability studies are carried out for each product. The present stability are carried out according to guidelines given by international council of harmonization (ICH guidelines) [10, 11].

10) ***In vivo* studies:** The gel containing active loaded liposome was subjected to *in-vivo* evaluation on the human volunteers. The patch test and photographic evaluation was carried out for the *in-vivo* studies.

A) **Patch test:** Patch test was performed on sensitive part of skin, e.g. bend of elbow, popliteal space of the skin behind the ears. The prepared cosmetic formulation to be tested was applied to an area of 1 sq. inch of the skin; control patches were also being applied. The site of patch was inspected after 24 hrs. There was no reaction and then test was repeated once more on the same site. Still no reaction was there, then he test was repeated a third time. No reaction was observed on third application, the person may be taken as not hypersensitive.

Photographic evaluation: To study whether the finished products were really effective, formulated fat reducing products have been subjectively studied. Human volunteers requested to assist in this research study and requested to apply this newly formulated fat reducing product for 30days on thigh, buttock, and stomach etc. area of body in this study thigh area was selected and the steps included applying the products as follows;

Steps:

1. Clean the thigh area by removing the hair and wash with water before applied the product.
2. Measure the thigh area with measuring tape note down reading before applying the product and take photo of that area.
3. Applied the product daily at least two times to thigh area.
4. After 15 and 30 days measure the thigh area with measuring tape note down the reading after applied the product and take photo of that area.
5. Calculate the reading before applied and after applied and note down the final result.

All experimental procedures were reviewed and approved by the Institutional Ethics Review Committee, Vidya Bharti Mahavidhyalaya, Amravati, Maharashtra (Reg. No.- 1504/PO/ 15/CPCSEA, dated: 23/09/2011).

RESULT AND DISSCUSION:

A. Preparation of Liposomes of soya Lecithin and stearic acid

Formula	1	2	3	4
Soya Lecithin	1%	1%	1%	1%
Stearic Acid	0.2%	0.4%	0.6%	0.8%

After evaluation study, formula 2 was found be stable batch, so it was selected for loading the active. Note: It is unloaded batch of liposomes.

B) **Optimization of Liposomes (Unloaded):** Shown in **table 10**.

C) **Optimization of liposome (loaded with isoslim complex):** Shown in **table 11**.

TABLE 10: OPTIMIZATION OF LIPOSOMES (UNLOADED)

Batch No.	Name	Parameter	Level	Remark
1	SLL-1	Temperature	60°C	Large size
2	SLL-2	Temperature	30°C	Optimum size
3	SLL-3	Time	15 min	Large size
4	SLL-4	Time	half hour	Optimum size
5	SLL-5	Time	45 min	Large size
6	SLL-6	Time	1 hour	Optimum size
7	SLL-7	Storage	30°C	Large size
8	SLL-8	Storage	4°C	Optimum size

TABLE 11: OPTIMIZATION OF LIPOSOME (LOADED WITH ISO SLIM COMPLEX)

Batch No.	Name	Parameter	Level	Remark
1	SV-1	Temperature	60°C	Large size
2	SV-2	Temperature	30°C	Optimum size
3	SV-3	Concentration of isoslim complex	1 ml	not satisfied
4	SV-4	Time	2ml	Optimum size
5	SV-5	Time	15 min	Large size
6	SV-6	Time	30 min	Optimum size
7	SV-7	Storage	30°C	Large size
8	SV-8	Storage	4°C	Optimum size
9	SV-9	Time	45 min	Large size
10	SV-10	Time	1 hour	Optimum size
11	SV-11	Storage	30°C	Large size
12	SV-12	Storage	4°C	Optimum size

D. **Determination of Particle size:** Determination of particle size is the most important evaluation parameter for iso-slim complex liposome.

E. **Particle size determination of unloaded liposome:** Shown in **table 12**.

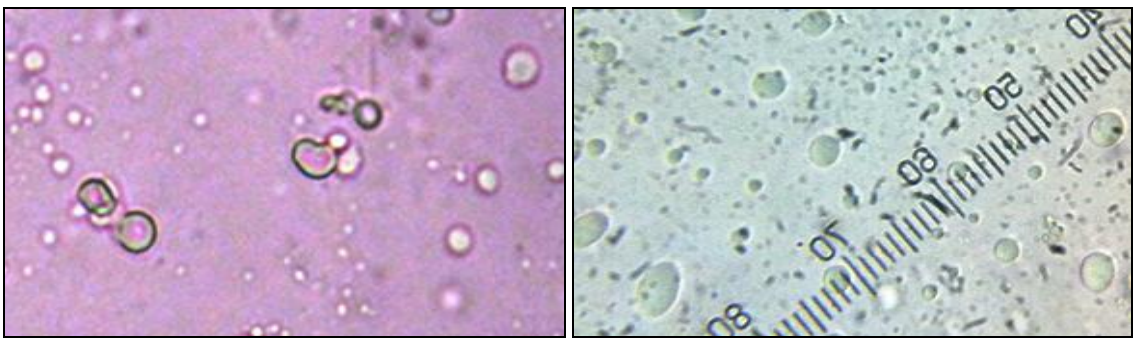
TABLE 12: PARTICLE SIZE DETERMINATION OF UNLOADED LIPOSOME

Sr. No.	Concentration of active in µgm / ml	Maximum drug loading capacity	% yield
1	0	0	0%
2	100	0.044	75.11%
3	200	0.083	75.73%
4	300	0.132	94.40%
5	400	0.142	96.70%

ULL = Unloaded liposome

I. **Particle size determination of loaded liposome:** According to the above table for the preparation of loaded liposome mean diameter was selected i.e. 18.62 um.

F. **Photomicrography Evaluation on iso-slim complex Liposomes:** Photomicrography of iso-slim complex liposomes loaded with active iso slim complex and observed for their characteristic such as shape, size etc. Shown in **fig. 3**.



(A) **(B)**

FIGURE 3(A): ACTIVE LOADED LIPOSOME AND (B): UNLOADED LIPOSOMES

G. **Solubility of Liposome in Different Solution:** Solvent Solubility

- Deionised water - Dispersible suspension
- 100% ethanol - Insoluble
- 25 : 75 alcohol : water - Dispersible suspension
- 50 : 50 alcohol : water - Dispersible suspension
- 75 : 25 alcohol : water - Insoluble
- Corn oil - Insoluble
- Olive oil - Insoluble
- Mineral oil - Insoluble
- Butelyne glycol - Insoluble
- Propylene glycol - Partially soluble
- Silicon oil - Insoluble
- Inonony isononanoate - Insoluble
- SLES surfactant - Dispersible suspension

H. **Entrapment efficiency:** For maximum drug loading capacity of iso-slim complex liposome: Shown in **table 13** and its graphical representation in **fig. 4**.

TABLE 13: MAXIMUM DRUG LOADING CAPACITY OF ISO SLIM COMPLEX LIPOSOME

Sr. No.	Concentration of active in u gm / ml	Maximum drug loading capacity	% yield
1	0	0	0%
2	100	0.044	75.11%
3	200	0.083	75.73%
4	300	0.132	94.40%
5	400	0.142	96.70%

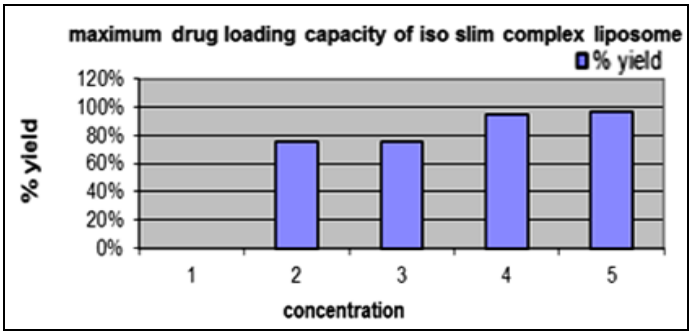


FIGURE 4: GRAPHICAL REPRESENTATION OF MAXIMUM DRUG LOADING CAPACITY

I. **Determination of pH:** Shown in **figure 5, 6 and table 14, 15**.

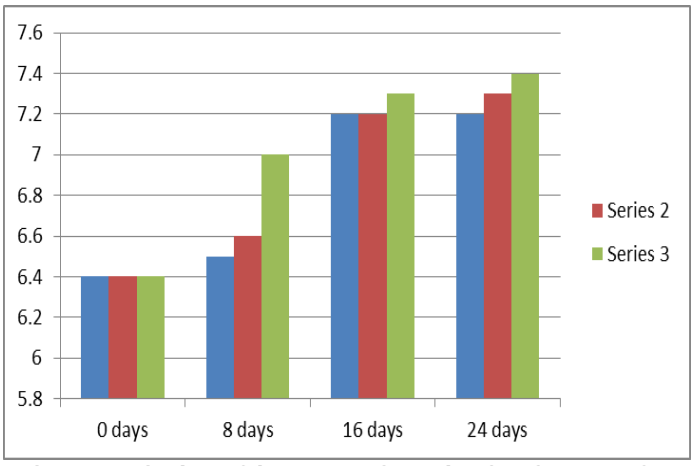


FIGURE 5: GRAPHICAL REPRESENTATION OF EFFECT OF pH OF ANTI CELLULITE CREAM CONTAINING ACTIVE

TABLE 14: pH OF CREAM

Sr. No	Days	F-1 1%	F-2 2%	F-3 3%
1	0 day	6.4	6.4	6.4
2	8 day	6.5	6.6	7
3	16 day	7.2	7.2	7.3
4	24 day	7.2	7.3	7.4

TABLE 15: pH OF GEL

Sr. No.	Parameter	Time interval / Cream base	F-1 1%	F-2 2%	F-3 3%
1	Spreadability (d)	Initial	7.8	8.2	7.6
2		After 10 days	7.6	8.1	7.5
3		After 20 days	7.5	8.1	7.3

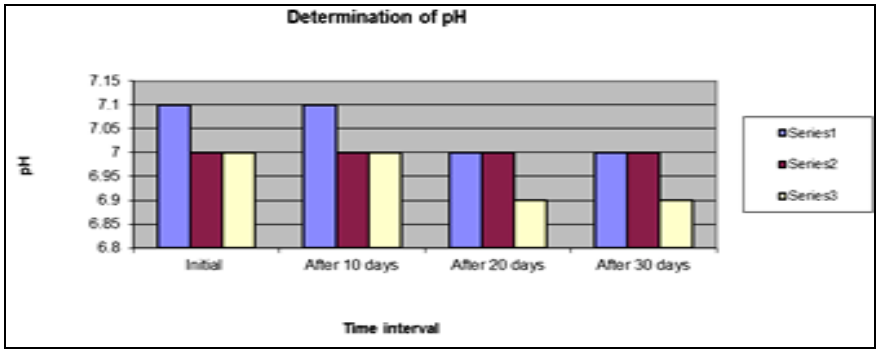


FIGURE 6: GRAPHICAL REPRESENTATION OF pH OF ANTI CELLULITE GEL CONTAINING ACTIVE

J. Determination of Spreadability: Shown in **table 17**.

K. Determination of Thermal Stability: Shown in **table 18**.

L. Determination of Viscosity: Shown in **table 19** and its graphical representation in **fig. 7**.

TABLE 16: SPREADABILITY OF CREAM

Sr. No.	Parameter	Time interval	F-1	F-2	F-3
		Cream base	1%	2%	3%
1		Initial	7.8	8.2	7.6
2	Spreadability	After 10 days	7.6	8.1	7.5
3	(d)	After 20 days	7.5	8.1	7.3

TABLE 17: THERMAL STABILITY

Sr. no	Parameter	Cream containing active		
		F-1 1%	F-2 2%	F-3 3%
1	Thermal stability	Passes	Passes	Passes

TABLE 18: VISCOSITY OF CREAM

Sr. no	Time interval	Cream containing active		
		F-1 1%	F-2 2%	F-3 3%
1	0 days	42331	42650	42770
2	15 days	42435	42748	42844
3	30days	42464	42843	42899

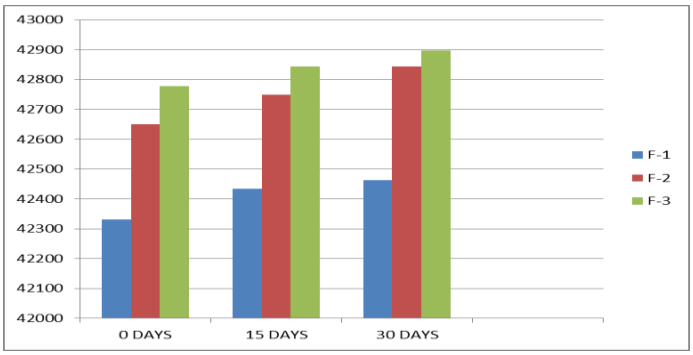


FIGURE 7: VISCOSITY OF ANTI CELLULITE CREAM CONTAINING ACTIVE

The viscosity of gel was measured by using spindle number 6 using Brookfield viscometer**.**

TABLE 19: VISCOSITY OF GEL IN CPS

Parameter	Final Formulation		
	F-1	F-2	F-3
Viscosity	103055	103650.0	103030.0

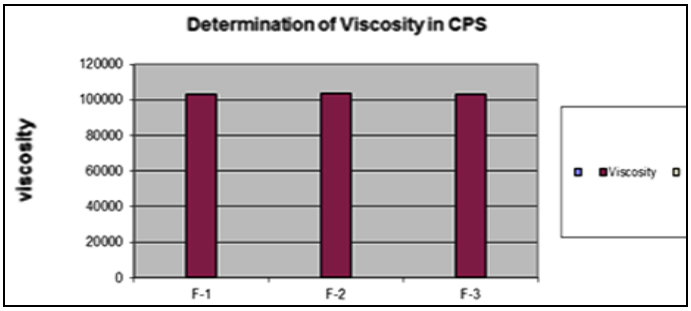


FIG. 8: GRAPHICAL REPRESENTATION OF VISCOSITY OF ANTI CELLULITE GEL CONTAINING ACTIVE

M. Determination of Total Fatty Matter: Shown in **table 21** and its graphical representation in **fig. 9**.

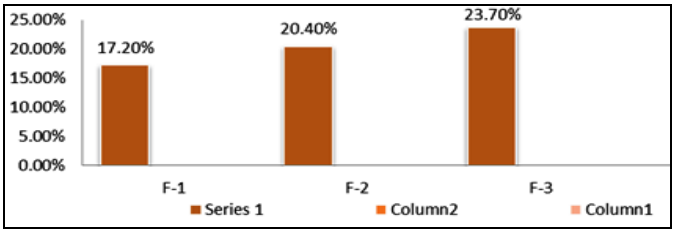


FIG. 9: GRAPHICAL REPRESENTATION OF TOTAL FATTY MATTER OF ANTI CELLULITE CREAM CONTAINING ACTIVE

TABLE 20: FATTY MATTER

Sr. no.	Parameter	Cream containing active		
		F-1 1%	F-2 2%	F-3 3%
1	Determination of total fatty matter	17.34%	20.40%	23.70%

N. Determination of Water Content: Shown in **table 22** and its graphical representation in **fig. 10.**

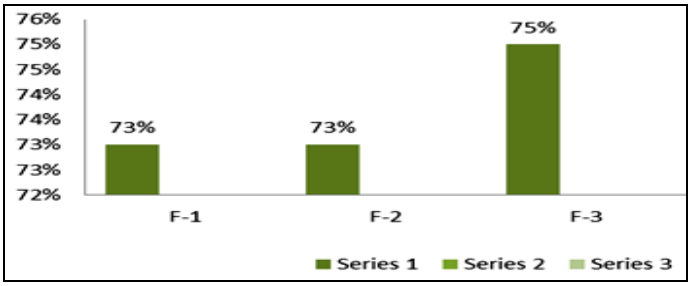


FIG. 10: GRAPHICAL REPRESENTATION OF WATER CONTENT OF ANTI CELLULITE CREAM CONTAINING ACTIVE

TABLE 21: WATER CONTENT

Sr. No.	Parameter	Formulations		
		F1 1%	F2 2%	F3 3%
1	Water content)	73%	73%	75%

D. Determination of Physical parameter:

TABLE 22: PHYSICAL PARAMETER

Physical Parameter	Final Formulation		
	F-1	F-2	F-3
Appearance	Transparent	Transparent	Turbid
Color	White	White	White
Consistency	Spreadable	Spreadable	Spreadable
Spreadability	Very good	good	good
Oily feel	No	No	No

Procedure: Small amount of sample should be visualized to determine the physical parameter.

E. Stability Study: Stability study of liposome: Shown in **table 24**.

TABLE 23: STABILITY STUDY OF LIPOSOME

Physical Parameter	Liposome containing active		
	F-1	F-2	F-3
Appearance	Dry powder	Dry powder	Dry powder
color	White	White	White
odour	odourless	odourless	odourless

Stability study of Cosmetic Product:

A. **Stability study of Anticellulite cream:** Stability studies for creams were carried out according to ICH guidelines. The cream samples were kept on the 5°C, room temperature, and 40°C. The changes in the physical appearance, color, odour etc. and chemical changes such as change in pH, viscosity and separation were checked and thus the formulation of cream was optimized, shown in **table 25**.

TABLE 24: STABILITY STUDY OF ANTICELLULITE CREAM

Physical Parameter	Final Formulation		
	ACC-1 2%	ACC-2 3%	ACC-3 4%
Appearance	Cream like	Cream like	Cream like
Color	White	White	White
Consistency	Semi-solid	Semi-solid	Semi-solid
Spreadability	Good	Very Good	Very Good
Oily feel	No	No	No

B. **Stability study of Anticellulite Gel:** Stability studies for Gel were carried out according to ICH guidelines. The Gel samples were kept on the 5°C, room temperature, and 40°C. The changes in the physical appearance, color, odour etc. and chemical changes such as change in pH, viscosity, and separation were checked and thus the formulation of Gel was optimized, shown in **table 26**.

TABLE 25: STABILITY STUDY OF ANTICELLULITE GEL

Physical Parameter	Final Formulation		
	ACG-1 2%	ACG-2 3%	ACG-3 4%
Appearance	Transparent	Transparent	Transparent
color	White	White	White
Consistency	Spreadable	Spreadable	Spreadable
Spreadability	Very Good	Good	Good
Oily feel	No	No	No

C. **Accelerated stability studies at various temperatures:** Procedure: The accelerated stability formulation was check at room temperature, 45 ± 2°C and freeze thaw cycles, shown in **table 27**.

TABLE 26: ACCELERATED STABILITY STUDIES AT VARIOUS TEMPERATURES.

Sr. No.	Parameter	Room Temp.			45 C			freeze thaw cycle		
		ACG-1	ACG-2	ACG-3	ACG-1	ACG-2	ACG-3	ACG-1	ACG-2	ACG-3
1	Colour	NC	NC	NC	NC	NC	NC	NC	NC	NC
2	Consistency	NC	NC	NC	NC	NC	NC	NC	NC	NC
3	Spreadability	NC	NC	NC	NC	NC	NC	NC	NC	NC
4	Appearance	NC	NC	NC	SC	SC	SC	NC	NC	NC
5	Feel	VG	VG	VG	G	G	G	VG	VG	VG

* NC = No Change * G = Good * SC = Slight Change * VG = Very good

From above formulation observed that F-1 was not change its physical parameter except for spreadability at 45±2°C. Hence, it was stable. F-1 was selected for above subjective evaluation.

D. Patch Test and Photographic Evaluation :-

- Material: product: containing active.
- Prep of sample: sample of the product containing active was prepared with known concentration.
- Selection of volunteers: the healthy subjects were selected as per procedure given in material and method section.
- Mode of execution: The test was performed as follows: different areas are marked on skin and labeled as controlled product. Sample is applied daily twice and observations are noted.

The test was performed as follows: different areas were marked on skin and labelled as controlled product. Sample is applied daily twice and observations are noted.

Result of stability studies: Shown **in table 27**.

TABLE 27: RESULT OF STABILITY STUDIES

Physical Parameter	Final Formulation		
	F-1	F-2	F-3
Appearance	Milky	Milky	Milky
color	White	White	White
odour	Colourless	Colourless	Colourless

E. ***In-vivo* studies:** Determination of moisturizing activity of anticellulite cream formulation by Mechanical Method: Shown in **table 29** and its graphical representation in **fig. 11**.

TABLE 28: DETERMINATION OF MOISTURIZING ACTIVITY OF ANTICELLULITE CREAM FORMULATION BY MECHANICAL METHOD

Sr. No.	Days interval	Anti-Cellulite cream containing Active			
		Control	ACC-1	ACC-2	ACC-3
			2%	3%	4%
1	0 days	0	0	0	0
2	5 days	0	4	7	8
3	10 days	0	6	6	7
4	15 days	0	6	7	7
5	20 days	0	6	8	8
6	25 days	0	7	8	9
7	30 days	0	7	8	10

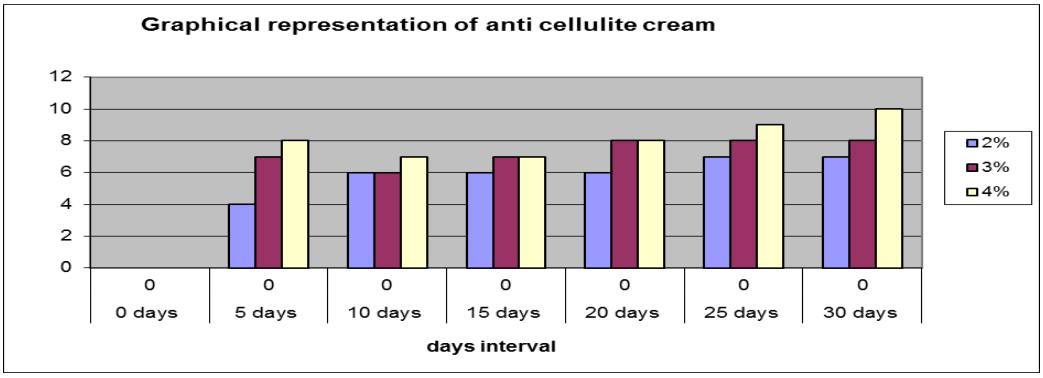


FIG. 11: GRAPHICAL REPRESENTATION OF ANTI CELLULITE CREAM

Determination of moisturizing activity of anticellulite Gel formulation by Mechanical Method: Shown in **table 30** and its graphical representation in **fig. 12**.

TABLE 29: MOISTURIZING ACTIVITY OF ANTICELLULITE GEL FORMULATION BY MECHANICAL METHOD

Sr. No.	Days interval	Anti-Cellulite Gel containing Active			
		Control	ACG-1	ACG-2	ACG-3
			2%	3%	4%
1	0 days	0	0	0	0
2	5 days	0	8	9	10
3	10 days	0	6	7	8
4	15 days	0	9	10	11
5	20 days	0	9	10	11
6	25 days	0	11	12	13
7	30 days	0	11	12	13

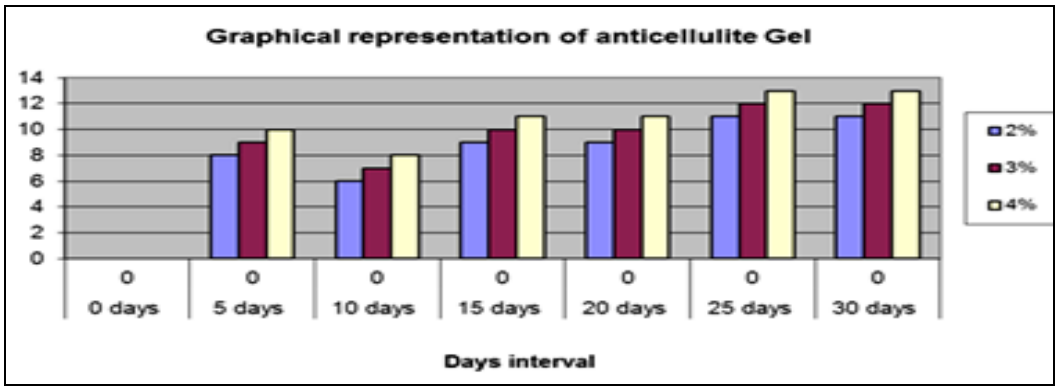
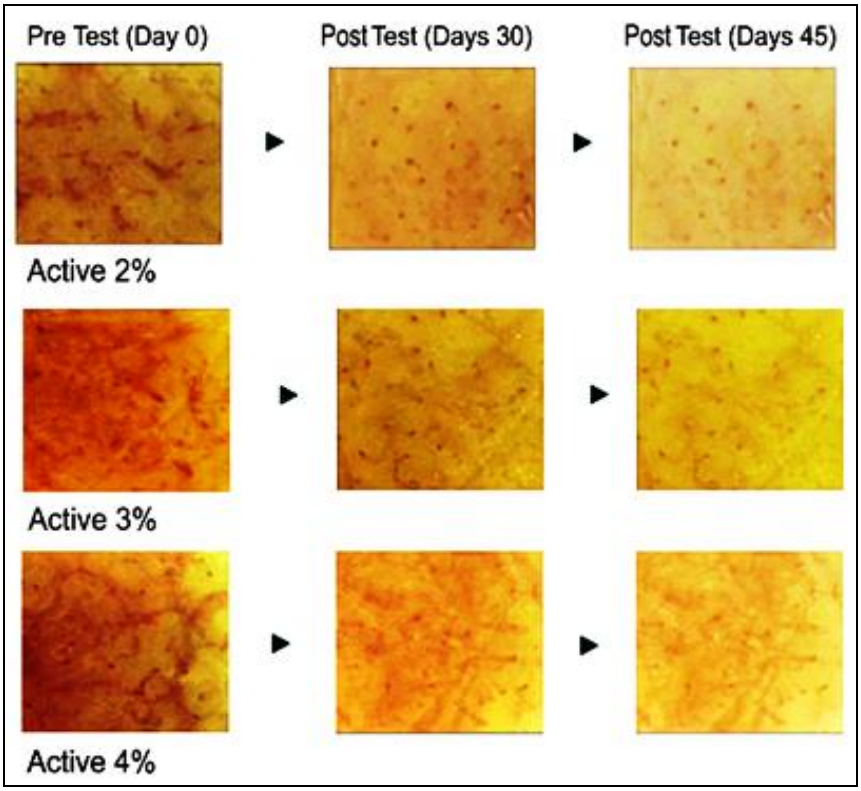


FIG. 12: GRAPHICAL REPRESENTATION OF ANTI CELLULITE GEL



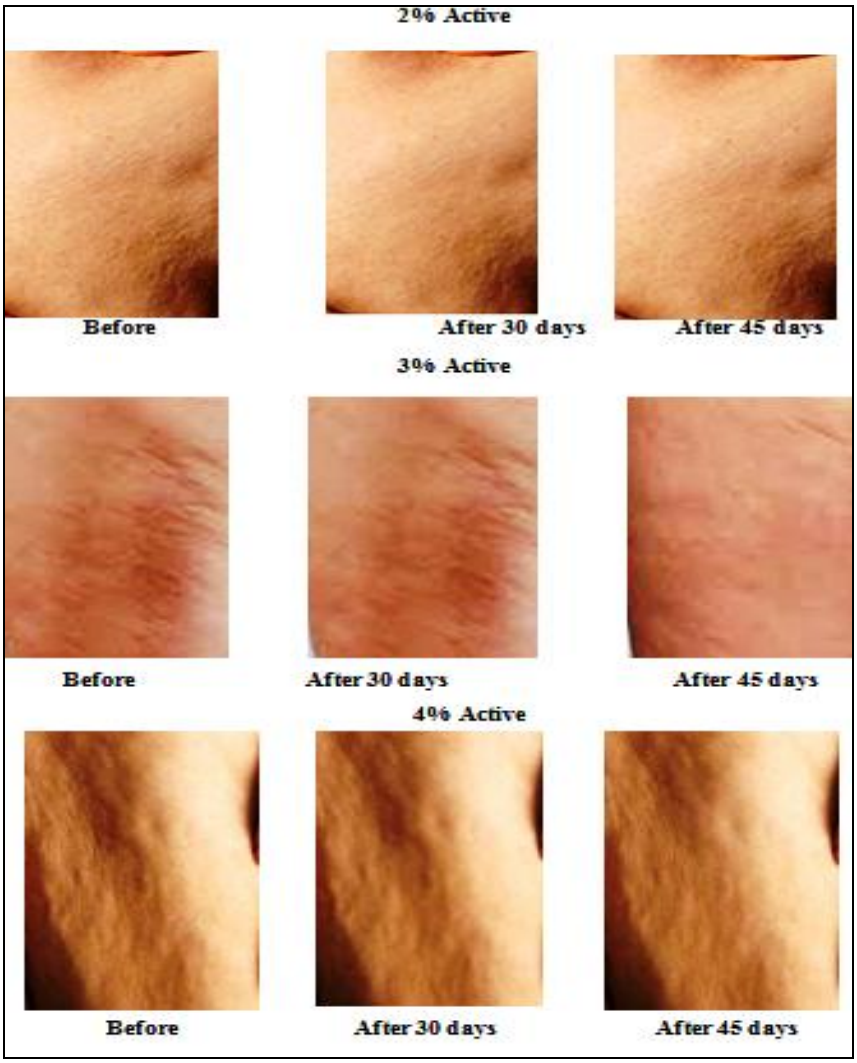


FIGURE 13: *IN-VIVO* TESTING FOR ANTI-CELLULITE PROPERTY – FOR CREAM

SUMMARY: Aim of the present work was to develop and investigate novel delivery system for the fatty skin in the form of liposome delivery system. Thus, one of the objectives of present work was to increase the drug stability of the active, for that novel polymer was aid to achieve the goal.

The present day research in the field of skin care products aim to select active ingredients loaded with liposome to reduce and prevent the appearance of cellulite. This is necessary because synthetic agent may lead unwanted side effect and adverse reaction on skin which may accentuate skin, structure delivery system show greater selectivity and consequent increasing beneficial effect.

There are various active ingredients which are available to reduce the cellulite like retanol, coffine, iso-slim complex etc. But iso-slim complex show excellent result in skin care. These active drugs which get completely inoculate in the liposome. Those liposomes which get incorporate into the various cosmetic products. Due to this we can get absolute, result for dissolving the fat from the body. The liposome loaded with iso-slim complex get easily and directly penetrated in subcutaneous layer.

Therefore, products were subjects for evaluation 15 days and were studied for its effects. The liposome made by iso-slim complex with lecithin and stearic acid gives better effect on the skin. Because active loaded liposome have great penetrating power due to this property active were loaded into deeper layer of the skin. The main aspect for the preparation is to reduce the fat from the skin.

CONCLUSION: Liposome represents a model for biological membrane in biological and medicinal research, which contains a lipid composition. The liposome made by iso-slim complex with lecithin and stearic acid showed better effect on the skin. Because active loaded liposome has great penetrating power, due to this, active was loaded into deeper layer of the skin. The main aspect of this preparation was to reduce the fat from the skin.

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