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FORMULATION AND CHARACTERIZATION OF TOPICAL LYCOPENE PHYTOSOMES FOR IMPROVED PERMEATION

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ABSTRACT

Objective: An attempt was made to develop topical delivery of lycopene for its beneficial use as an anti-oxidant by phytosome technology. **Material & Method:** Phytosomes were prepared by refluxing or dissolving different ratios of lycopene in chloroform or hexane and Phospholipid in dichloromethane involved precipitation sonication method. 1% equivalent complex was placed in three different formulations of Cream and hydrophilic gel bases made out of steric acid and Carbopol respectively for better utility. Formulations were evaluated for appearance, pH, viscosity, Spreadability, Extrudability, washable property and *in vitro* diffusion to check for its stability. **Results & Discussion:** 1: 1 molar ratio was found to be the best phytosomal complex with entrapment efficiency of 83.22±0.54 % and physicochemical parameters showed acceptable results for both creams and gels for pH in range of 6.4±0.043 to 6.95±0.041 compatible to skin pH with less irritation. Viscosity 3.28±44.04 kP for cream and Gel 5.8±56.88 kP, Spreadability 14.66±0.04 g*cm/sec for cream and gel 36.22±0.04 g*cm/sec and % decrease Extrudability for cream 99.17±0.05 and gel 99.84±0.04 respectively showed inter related parametric performance for better results. *In- vitro* studies were found to be fare release up to 95% for creams and gels at the end of 5 h. Stability studies was carried out at two different temperature at 30 ^{o}C ±0.5^{o}C and 65% RH±2 RH and 4 ^{o}C ±0.5^{o}C and 75%RH for 90 days and found to be stable.

KEYWORDS: Lycopene, Phytosomes, Spreadability, Extrudability, Skin irritation.

INTRODUCTION

Life style modulation has encouraged the use of tomato or tomato products as a dietary supplement for the intake of Lycopene for its anti-oxidant property which has shown its effect on the treatment of not only major chronic diseases[1] but externally as anti-aging or anti-wrinkle agent as carotenoids of tomato deposits in the skin and protect form sun burn[2] etc.,

The Molecular size and hydrophilic nature of flavonoids or carotenoids limits the absorption when taken orally or passive diffusion when applied externally resulting in poor lipid solubility limiting its ability to pass across the lipid-rich outer membranes resulting poor bioavailability of drugs. The effective level of delivery of the phyto constituents is based on effective level of herbal drugs. These can be overcome by suitable incorporation of novel drug delivery technology. The solution for this is phytosome technology where the phytoconstiuents complexes with Phosphotidylcholine or soya lecithin which provides amphiphilic environment for better absorption and improved bioavailability.[3]

The draw back through use of chemical like di and triethanolamine in personal care products causing carcinogenic effect;[4] use of natural ingredients by pharmaceutical approach of delivering the phytoconstiuents targeting skin for improving the beauty and skin tone is solution. ROS (reactive oxygen species) induced skin damage can be improved by topical supplementation with antioxidants is regarded as a useful strategy that may improve skin antioxidant capacity.[5] More than β Caro-tene lycopene can prevent oxidative damage more efficiently.[6] Lycopene being very lipophilic its skin permeation is limited from conventional topical formulation.

MATERIALS AND METHODS

Standard Lycopene was procured from Hisar Phytochemicals Delhi India, Soya Lecithin was obtained from Hi media, aprotic solvent, such as dioxane or acetone, n-hexane, ethanol were obtained by Merck chemicals. Animal Ethical clearance by: IAEC Srinivas College of Pharmacy, Mangalore – 574 143, Approval Number: SCP/TAEC/F150/P144/2018 dated 15.12.2018.

Extraction and Formulation of Lycopene Phytosomes

Extraction and Preparation of Lycopene Phytosomes was done as per (Aghel N *et al.*, 2011) and (Jain S *et al.*, 2019) respectively.[7, 8]

Formulation of O/W Topical cream and Gel

The preparation of the topical cream was done as per (Chinmoy B et al., 2019) Table 1 and Gel was prepared as per (Amudha S et al., 2018) using carbopol 940 as the gelling agent. The prepared phytosomes were incorporated into the gel, and thus, the phytosomal gel was obtained. Table 2.

Fig. 1: Lycopene Phytosomal Cream C1.

Table 1: Composition of Lycopene Phytosome Topical Cream.

Sl.No	Ingredients for Cream	C1	C2	C3
	Equivalent Lycopene Phytosome (1:1)	**1%**	**1%**	**1%**
1	Glyceryl monostearate (g)	4	3	2
2	Isopropyl palmitate (ml)	1	1	1
3	Light liquid paraffin (ml)	10	10	10
4	Emulsifying wax (g)	1.5	1.5	1.5
5	Stearic acid (g)	2	3	4
6	Cetyl alcohol (g)	0.1	0.2	0.3
7	Glycerin (ml)	5	5	5
8	Triethanolamine (ml)	0.1	0.2	0.3
9	Methylparaben (g)	0.125	0.125	0.125
10	Propylparaben (g)	0.125	0.125	0.125
11	Water q.s	50 ml.	50 ml.	50 ml.

Table 2: Composition of Lycopene Phytosome Topical Gel.

Sl.No	Ingredients for Gel	G1	G2	G3
1	Equivalent Lycopene Phytosome (1:1)	1%	1%	1%
2	Carbopol 934	1 g	1.25 g	1.5 g
3	Methyl paraben (0.5%)	0.05	0.05	0.05
4	Propylparaben (0.2%)	0.02	0.02	0.02
5	Propylene glycol 400 (5%)	0.5 ml	0.5 ml	0.5 ml
6	Triethanolamine	1.2 ml	1.2 ml	1.2 ml
7	Water	100 ml	100 ml	100 ml

Fig. 2: Lycopene Phytosomal Gel G1.

Physical evaluation of cream and gel[4]

Preliminary evaluation was carried out as per (Julie M J et al., 2018):

pH

pH of all the Samples was measured using Systronic Digital pH meter and was calibrated using standard buffer solution such as pH 4 and 7. About 0.5 g of the cream / Gel was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.[9]

Homogeneity

The formulations were tested for the homogeneity by visual appearance (Kumari A et al., 2015).

Viscosity: The measurement of viscosity of the sample was done using Brookfield Viscometer (DV - E Model). The required quantity of cream or Gel was placed in a small volume holder and the spindle used was LV4 – 64 at 0.3 (rpm) rotations speed per minute. The corresponding % Torque value and cp (centipoises) was noted.[10]

Spreadability

Two glass slides of 20 cm × 20 cm were selected. A small amount of sample was sandwiched between the two glass slides. A 50 g weight was placed on the upper

slide so that the cream or Gel between the two slides was pressed uniformly to form a thin layer. The time taken for the cream/gel to spread was noted using a stop clock by placing weight tied to the upper plate. This parallel plate method is the most widely used method for determining and quantifying the Spreadability of semisolid preparations.[11] Simplicity and relative lack of expense are the advantages of this method. The following equation was used for this purpose:

$$S = m \times L/T$$

Where

S - Spreadability

m - Weight tied to the upper slide L - Length of the glass

T - Time taken in seconds.

Fig. 3: Apparatus showing Spreadability test for Phytosomal Cream and Gel.

Extrudability[11]

It is an empirical test to measure the force required for the cream to extrude out from the tube. The prepared cream was filled into a collapsible tube and it was sealed and the weight of the tube was recorded. Placed a 100 g weight on the tube and the amount of cream that extruded out was collected and weighed. Then, the percentage of cream extruded was calculated. The packing of creams has gained a considerable importance in the delivery of desired quantity of cream; therefore, measurement of Extrudability has become some important criteria for creams.

Following equation was followed

% of decrease Wt. (Extrude) = Total Wt. in tube – Wt. of Extrude / Total Wt. X 100

Fig. 4: Extrudability Study of Phytosomal Cream C1.

Fig. 5: Extrudability Study of Phytosomal Gel G1.

Wash ability: The weighed quantity of cream (1gm) was taken and spread on the hand and washed under running water for 1 min.

***In-vitro* drug diffusion studies of the Lycopene Phytosomal Topical Cream / Gel[12,13]**

The diffusion medium used was phosphate buffer 6.8 pH. Assembly of the diffusion cell for *in–vitro* diffusion studies was done as per the dimensions. The diffusion cells were placed on the magnetic stirrers. The outlet of the reservoir was maintained at 37±0.5^{o}C and was connected to water jacket of diffusion cell using rubber latex tubes. The receptor compartment was filled with phosphate buffer 6.8 pH. Then the prepared cellophane paper (Boiling with phosphate buffer 6.8 pH) was mounted on the cell carefully so as to avoid the entrapment of air bubble under the paper. Intimate contact of paper was ensured with receptor fluid by placing it tightly with clamp. The speed of the sitting was kept content throughout the experiment. With the help of micropipette 5ml of sample was withdrawn first at 30 min. and further at a time intervals of one hour from sampling port of receptor compartment and same volume was then replaced with receptor fluid solution in order to maintain sink condition. The samples were appropriately diluted and the absorbance was measured at 471 nm using Jasco UV-VIS spectrophotometer.

RESULTS

Physicochemical parametric evaluation studies

Physicochemical parameters such as pH, viscosity, homogeneity, spreadability, extrudability and washability of lycopene phytosomal creams and gels were found out, and it was found that C1 and G1 formulation showed optimum value.

Physicochemical parameter evaluation of antiaging cream

pH measurement

It was previously reported that, for creams and gels to be non-irritant and safe for topical application, their pH has to fall in the physiologic accepted range for topical preparations, i.e., pH 6–7 units. Table 3 shows that pH of various cream & gel formulations ranged from 6.4 to 6.95 which lies in the normal physiologic range and thus produces no skin irritation.

Viscosity

The prepared creams and gels were formulated using emulsion base and Carbopol 940 respectively (Fig 3 & Fig 4), Table 4 shows that the viscosity of various cream and gel formulations ranged from 28.8 to 32.8 and 10.8 to 58.0 kP respectively.

Spreadability

The spreadability is an important criterion for uniform and ease of application of topical preparations. It also plays a major role from patient compliance point of view. Application of the formulation to the skin is more comfortable if the base spreads easily, exhibiting

maximum "slip" and "drag." Spreadability of creams and gels are measured in terms of average diameter of the spread circle. (Fig 5) Table 3 shows that the Spreadability values for all prepared cream & gel formulations ranged between 14.07–15.85 and 15.22 – 36.22 cm.g/ sec respectively.

Extrudability

It is an empirical test to measure the force required for the cream or gel to extrude out from the tube. For topical preparations, it is an important criterion to check the easiness of the cream or gel to extrude out from the tube (Fig 6 & Fig 7). Table 3 shows that the % decrease extrudability values of cream and gel are ranged from 99.11 – 99.17 % and 99.68 – 99.84 % respectively

***In-vitro* Diffusion Studies**

The percentage release of the drug during diffusion studies showed more than 95% of release within 5 hours among all the formulations creams and Gels as showed in Fig 8 & 9 respectively.

Table 3: Physicochemical parameter evaluation of Cream and Gels.

Code	C1	C2	C3	G1	G2	G3
pH	6.4	6.6	6.8	6.5	6.8	6.95
Wash ability	Washable	Washable	Washable	Washable	Washable	Washable
Spreadability gm-cm/ sec	15.85	14.66	14.07	36.22	19.30	15.22
Extrudability % Decrease	99.11	99.15	99.17	99.68	99.76	99.84

Table 4: Viscosity of various Phytosomal Cream / Gel

Code	**Sample**	**Spindle used**	**RPM**	**% Torque***	**kP (Kilopoises)**
C1	Phytosomal Cream	64	0.3	14.4	28.8
C2	Phytosomal Cream	64	0.3	18.4	30.6
C3	Phytosomal Cream	64	0.3	20.2	32.8
G1	Phytosomal Gel	64	1.0	18.0	10.8
G2	Phytosomal Gel	64	0.3	21.6	43.2
G3	Phytosomal Gel	64	0.3	29.0	58.0

*% Torque should be more than 10.

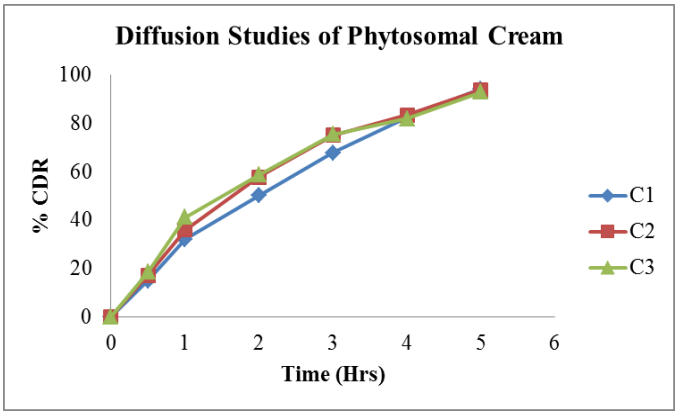


Fig 6: *In-vitro* Diffusion studies of the Lycopene Phytosomal Cream.

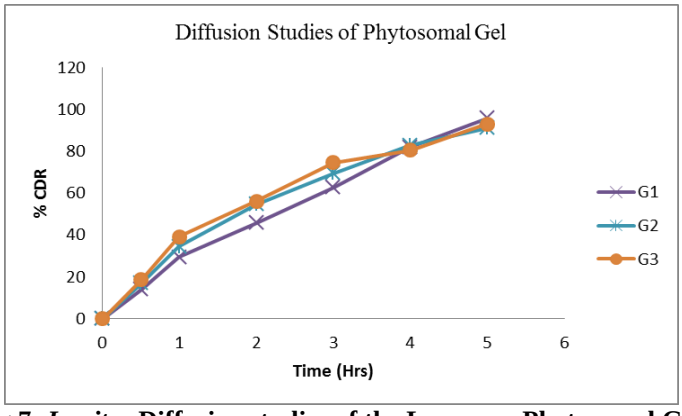
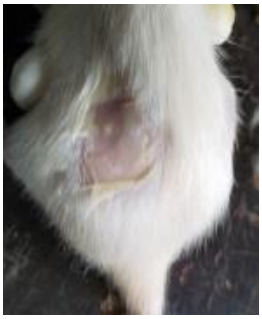


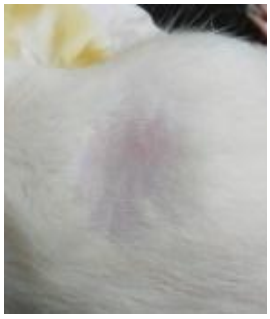
Fig 7: *In-vitro* Diffusion studies of the Lycopene Phytosomal Gel.

Rat skin irritation test

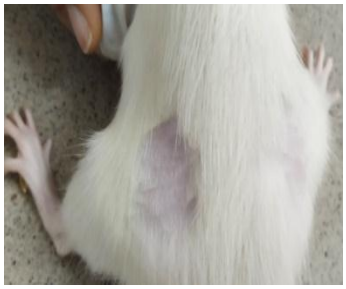
The tests were carried out by employing OECD guidelines. Approximately 24 h before the test, fur was shaved from dorsal area at different site of the trunk and the herbal ointment (500 mg) was applied on one site and cleaned after one hour. A separate untreated site was used as a control. Observation of the sites was done at 24 h after application, and repeated at 48 h, 72 h, 7th day and 15th day thereafter. The reactions, defined as erythema and edema, were evaluated according to skin reactions scoring system Fig 10.



a)Photo of Rat skin showing application of cream



b)Photo of Rat skin after 1 hour of removing the test cream



c)Photo of Rat skin after 24 hours of removing the test cream

Fig 8: Rat Skin Irritation Test

Table 5: Stability Studies of Lycopene Cream C3.

Code C3	At 25±2° C / 65±5% RH* and At 40±2° C / 75±5% RH** for 6 months			
Month	pH	Viscosity (kP)	Spreadability gm-cm/ sec	%CDR at the end of 12 h
1	6.8*	32.8*	14.07*	92.8*
	6.5**	30.6**	15.21**	91.0**
2	6.8*	32.8*	14.10*	92.2*
	6.6**	30.6**	15.20**	91.0**
3	6.8*	32.8*	14.11*	92.0*
	6.5**	30.5**	16.20**	90.58**
4	6.8*	32.8*	14.02*	91.00*
	6.5**	30.45**	15.80**	90.22**
5	6.8*	32.8*	14.26*	91.12*
	6.5**	30.4**	15.84**	89.56**
6	6.8*	32.8*	14.02*	91.11*
	6.5**	30.4**	15.61**	89.11**

Table 6: Stability Studies of Lycopene Gel G3.

Code G3	At 25±2° C / 65±5% RH* and At 40±2° C / 75±5% RH** for 6 months			
Month	pH	Viscosity (kP)	Spreadability gm-cm/ sec	%CDR at the end of 12 h
1	6.95*	5.8*	15.22*	93*
	6.8**	5.68**	16.22**	91**
2	6.90*	5.8*	15.22*	92.5*
	6.8**	5.69**	15.92**	91**
3	6.90*	5.8*	15.12*	92.5*
	6.8**	5.7**	16.22**	90.2**
4	6.90*	5.8*	15.22*	92.14*
	6.8**	5.65**	16.72**	89.6**
5	6.90*	5.8*	15.42*	92.10*
	6.8**	5.63**	16.12**	89.20**
6	6.90*	5.8*	15.22*	92.0*
	6.8**	5.63**	16.42**	89.10**

CONCLUSION

The present study has been a satisfactory attempt to formulate phytosomal topical dosage form for enhanced delivery of Lycopene using polymer Soya Lecithin. From the reproducible results of the executed experiments, it can be concluded that:

The lycopene phytosomal topical dosage form can be prepared by best use of emulsified and gel base. pH of the topical formulations was found to be equal to skin pH i.e., 6.8. Viscosity of topical creams and gel were found to be satisfactory, different concentration of cream base and gelling agent showed noticeable change in viscosity which can be compared the activity of Spreadability and Extrudability of topical creams and gel indicating the ease with which cream and gel are spreadable by the amount of shear applied and force applied. Wash ability of both creams and gels were satisfactory washable within a minute under running water. In-vitro diffusion studies of creams and Gels were found to be more than 95% within 5 hours. Stability studies showed

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