FORMULATION AND EVALUATION OF TOPICAL SOY-PHYTOSOME CREAM

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ABSTRACT

The bioavailability and absorption of water soluble phyto constituents is erratic due to poor solubility of these constituents in gastrointestinal tract. This can be overcome by a novel delivery system known as phytosome technology in which water soluble phyto constituents are allowed to react with phospholipids. For better and improved bioavailability, natural phyto constituents must have a good balance between hydrophilicity (helps in dissolution in gastro-intestinal fluids) and hydrophobicity (helps to cross lipid rich cell membranes). This is achieved through phytosome technology. Phospholipids have a dual solubility and acts as an emulsifier. Phytosome technology acts as a bridge between novel and conventional delivery systems. Many products are available in the market based on this phytosome technology which includes popular herbal extracts such as Ginkgo biloba, Silybum marianum, grape seed, olive oil flavonoids etc.

Keywords: *Phytosomes, Liposomes, Phytoconstituents, Bioavailability,Phospholipid complex.*

INTRODUCTION

Phytosome is a patented technology developed by a leading manufacturer of drugs and nutraceuticals, to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, called as phytosomes and so vastly improve their absorption and bioavailability.

The term "phyto" means plant while "some" means cell like. Phytosomes are one of the novel drug delivery systems containing hydrophilic bioactive phyto-constituents of herbs surrounded and bounded by phospholipids. The phytosome technology produces a little micro sphere or little cell, which protects the plant extract or its active constituent from destruction by gastric secretion and gut bacteria due to the gastro-protective property of phosphatidylcholine. This phyto-phospholipid complex resembles a little cell which exhibits better pharmacokinetic and pharmacodynamic profile than the conventional herbal extract resulting in better bioavailability.

The phytosome process has also been applied to many popular herbal extracts including *Ginkgo biloba*, grape seed, hawthorn, milk thistle, green tea, and ginseng. The flavonoid and terpenoid components of these herbal extracts lend themselves quite well for the direct binding to phosphatidylcholine. Phytosome is produced by binding individual components of herbal extracts to phosphatidylcholine, resulting in a dosage form that is better absorbed and thus, produces better results than the conventional herbal extracts. The results indicate that the absorption of silybin from silybin phytosome is approximately seven times greater compared to the absorption of silybin from regular milk thistle extract. Drugs can be embedded or dissolved in nano particles and can also be adsorbed or coupled on the surface. Encapsulating drugs within NPs can improve the solubility and pharmacokinetics of drugs, and, in some cases, enable further clinical development of new chemical entities that have stalled because of poor pharmacokinetic properties. The major carrier materials of nano particles are synthetic biodegradable high molecular polymer and natural polymer. The former usually includes poly-α-cyanoacrylate alkyl esters, polyvinyl alcohol, polylactic acid, and polylacticglycolic acid, etc. The latter is usually divided into two classes: proteins (albumin, gelatin and vegetable protein) and polysaccharides (cellulose, starch and its derivatives, alginate, chitin and chitosan, etc.) [1-3].

MATERIALS AND METHODS

Stearic acid, Cetyl alcohol, Liquid Paraffin (Mineral oil), Triethanolamine (TEA), Glycerin, Soy-phytosome (ratio 1:1), Preservative (Methyl Paraben), Perfume (Lemongrass oil), De-ionized water and other chemical and solvents were of analytical grade/IP/equivalent gradeand procured from laboratory.

Formulation of topical soy-phytosome cream

The formulation of soy-phytosome topical cream consists of two stages:

1. Preparation of cream base (oily phase) and aqueous phase.
2. Formulation of soy-phytosome cream.

Formulation Design

For preparation of soy-phytosome topical cream, it is proposed to use the following formulation composition in Table 1.

PROCEDURE

According to above formulation composition all substances were weighed accurately. Then stearic acid, cetyl alcohol and liquid paraffin (oily phase) were mixed in separate beaker and melted at water bath on temperature range of 60-70ºC, similarly remaining all substance except perfume were mixed in another beaker and heated on water bath at temperature range of 50-60ºC. Further molten mass of oily phase was added into other (aqueous) phase with continuous stirring and perfume was added and then cooled down to an emulsified cream. This procedure was followed for preparation of all 5 batches including control batch (without active ingredient).

Evaluation of topical soy-phytosome cream

Various evaluation tests were performed on prepared formulations to evaluate various physicochemical and biological parameters, as follows:

1. Appearance
2. Determination of pH
3. Extrudability
4. Rheology (Viscosity)
5. Spreading Coefficient (Spread ability)
6. Diffusion studies
7. Skin irritancy test

APPEARANCE

By visual inspection color, presence of foreign particles and homogeneity of formulations were evaluated and summarized in Table 2.

DETERMINATION OF Ph

The pH of the prepared topical phytosome cream was determined by using a digital pH meter. 1 gm of the cream was stirred in distilled water, until a uniform dispersion was formed. It was kept aside for 2 hours. The volume was then made up to 100 ml. Then, the pH was measured. The test was performed in triplicate using a digital pH meter and the mean value ± SD was calculated. The results are shown in Table 3.

Extrudability: Extrudability test is based upon the determination of the weight required extruding 0.5 cm ribbon of cream in 10 seconds from lacquered collapsible aluminum tube. The results were shown in Table 4.

Extrudability (%) = (X÷Y) ×100

Where; X= Weight of extruded cream from tube; and Y= Weight of cream in tube before extruding.

Rheology (viscosity): The viscosity of the formulated batches was determined using a Brookfield Viscometer with spindle 95. The formulation whose viscosity was to be determined was added to the beaker and was allowed to settle down for 30 min. at room temperature before the measurement was taken. Spindle was lowered into the centre of formulation, taking care that spindle does not touch bottom of the beaker and rotated at a speed of 10, 20, 50 rpm. The viscosity reading was noted down and the averages of three readings were taken. Determined viscosity for prepared cream represented in Table 5.

Spreading coefficient (Spread ability): Spreading coefficient was determined by the apparatus suggested by Multimer *et al* (1956). It consists of a wooden block, which is attached to a pulley at one end. Spreading coefficient was measured on the basis of 'slip' and 'drag' characteristics of cream. A ground slide was fixed on the wooden block. An excess of cream (about 2 gm.) under study was placed on this ground slide. Phytosome cream preparation was then sandwiched between this slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 1 kg was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of phytosome cream between the two slides. Measured quantity of weight was placed in the pan attached to the pulley with the help of hook. The time (in sec) required by the top slide to move a fixed distance was noted. A shorter interval indicates better Spreading coefficient. It is calculated by using the formula:

[S = M.L / T]

Where, **M** = weight tied to upper slide; **L** = length of glass slides/distance of travel; and **T** = time taken to travel a fixed distance.

Diffusion Study: Franz diffusion cell (with effective diffusion area 3.14 cm^2 and 15 ml cell volume) was used for the drug release studies. Egg membrane was used for the release study. The egg membrane was obtained from egg by keeping an egg in a 10% HCl solution. It was kept until the egg shell dissolved completely in HCl solution. Further, the egg was punctured and egg yolk was discarded and egg membrane was obtained, washed completely with de-ionized water and used. The membranes were previously treated with ethanol: water (70:30) solvent system and soaked overnight in this solvent system at refrigeration temperature.

The phytosome cream (1gm) was applied onto the surface of egg membrane evenly. The membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with solvent system to solubilize the drug. The receptor chamber was stirred

magnetically. The samples (1 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV visible spectrophotometer at 281.4 nm after appropriate dilutions. Cumulative amount of drug release at each time interval was computed. The cumulative amount of drug released across the egg membrane was determined as a function of time. The drug release profile data is presented in Table 7 and in Fig. 2.

Skin Irritancy Test: Approval for the use of animals in the study was obtained from the institutional animal ethics committee (Reg.No.541/02/C/CPCSEA). For this study, rabbits (1250-1500g) of either sex were used. The animals were maintained on standard animal feed and had free access to food and water. The day before the experiment the hair from the back of the rabbits were carefully removed with a hair removing cream and a razor without breaking the skin. An area of 6.25 cm^2 was marked on both the groups of control and test animals. The phytosome cream formulation $F_5$was applied (1000 mg/rabbit) once a day for 24 hours and the site was observed for any sensitive reaction if any, and graded as 0, 1, 2, 3 for no reaction, mild, moderate and severe types of erythema respectively.

Stability testing of optimized soy-phytosome cream based on ICH Q_1 guideline: Stability of medicinal products may be defined as the capability of a particular formulation in a specific container to remain within its physical, chemical, microbial, therapeutic and toxicological specification, i.e. stability of drug is its ability to resist detoriation. 90% of labeled potency is generally recognized as the minimum acceptable potency level. Detoriation of drug may take several forms arising from changes in physical, chemical and microbiological properties. The changes may affect the therapeutic value of preparation or increase its toxicity.

Accelerated Stability Testing: Protocol for accelerated stability testing is shown in Table 8.

Among the formulations, formulation F_5 had good spread ability value, extrudability, so F_5 was selected for stability study. The formulation (F_5) was packed in the collapsible tube and stored at 40±2˚C/75±5% R.H. for 4 weeks in stability chamber. Samples were withdrawn at 7 day time interval and evaluated for physical stability. Physical stability study data is shown in Table 9 and *in vitro* release from the developed topical phytosome cream formulation F_5 during stability is shown in Table 10 and Fig. 4.

RESULTS AND DISCUSSION

All formulated batches of phytosome cream were white in color and free from foreign particles. But in homogeneity all batches were having some differences as $F_1/F_3/F_4$ batches were homogenous but are greasy in nature whereas F_2 batch was less homogenous and also greasy in nature. Among all batches F_5 batch was having good homogeneity and non-greasy nature when applied. This difference in appearance can occurred due to improper mixing of ingredients during formulation stages.

In this study, the pH of freshly prepared base and formulations were 5.48 to 5.92, respectively, which is within the range of skin pH. As the pH of Human skin typically ranges from 4.5 to 6.0 and 5.5 in considered to be average pH of the skin. Therefore, the formulations intended for application to skin should have pH close to this range.

The extrudability of the prepared phytosome cream was found to vary from (4.96±0.15) to (5.48±0.21)%. There was significant difference ($P \leq 0.05$) in extrudability value of different formulations. This result is showing as we increase concentration of phytosome in formulations there is easiness in expulsion of cream from tube.

The viscosity of the prepared phytosome cream was found to decrease in general with increase in concentration of phytosome. The effect of rpm on viscosity is depicted in Fig. 1. It is seen that there is a shear thinning effect, that is, if we decrease the rate of shear it increases the viscosity of cream. Viscosity of creams is inversely proportional to rate of shear (rpm) as results shown in Table 5.

The spread ability indicates the ease with which cream is spreadable by the amount of shear. The spreading coefficient for the prepared Phytosome-cream formulations found was in the ascending order from $F_2<F_3<F_4<F_5$ except in formulation F_1 which is not consisting phytosome (control batch).

The release of the soy-phytosome through egg membrane from cream formulation can be ranked in the following descending order: $F_5>F_4>F_3>F_2$ where the cumulative amounts of drug released after 4 hours were 54.96±6.11%, 46.31±5.27%, 39.00±6.33% and 34.67±7.17 respectively. It is seen that with increase in the concentration of soy-phytosome there is a significant ($p \leq 0.05$) increase in the release and permeation across the egg membrane.

Various drugs, when applied topically, might elicit primary skin irritation. This irritation might vary with the ability of the agent to cross the SC barrier and subsequently interact with the viable cells of the epidermis and dermis. The skin irritation study conducted on the rabbits by application of the phytosome cream for 24 hours did not show any sign of irritation (all samples showed zero of the erythema score), as shown in Fig. 3.

The selected optimized formulation F_5 showed good physical stability, as there was no significant difference ($p \leq 0.05$; t- Test: Paired two samples for means) in pH, extrudability, spread ability and appearance of the formulation while there

was a significant difference ($p \leq 0.05$; t- Test: paired two samples for means) in globule size and viscosity at 10/20/50 rpm between the initial (0 week) and fourth week results. However, all the test results were within the acceptance criteria. Formulation release profile at 4th week was compared to the profile at 0 week release profile by calculating similarity factor f_2 (Table 10); the values of $f2 > 50$, which indicates similarity of 4th week profile to 0 week release profile. Further, there was no phase separation and microbial contamination in the product for the period under study.

Table 1: Formulation composition of Soy-phytosome topical cream

Ingredients (% w/w)	F1	F2	F3	F4	F5
Stearic acid	7	7	7	7	7
Cetyl alcohol	2	2	2	2	2
Liquid Paraffin (Mineral oil)	20	20	20	20	20
Triethanolamine (TEA)	2	2	2	2	2
Glycerin	10	10	10	10	10
Soy-phytosome (ratio 1:1)	-	2	5	7.5	10
Preservative (Methyl Paraben)	0.5	0.5	0.5	0.5	0.5
Perfume (Lemongrass oil)	1	1	1	1	1

Table 2: Visual Inspection of prepared topical soy-phytosome cream

Sr. No.	Parameter	Observation				
		F_1	F_2	F_3	F_4	F_5
1.	Color	White	White	White	White	White
2.	Foreign Particles*	(-)	(-)	(-)	(-)	(-)
3.	Homogeneity**	(+)	(±)	(+)	(+)	(++)

*(-) showing absence of foreign particle. **(±) showing average homogeneity. (+) showing good homogeneity. (++) showing excellent homogeneity.

Table 3: pH determination of phytosome cream

Parameter	Observation				
	F_1	F_2	F_3	F_4	F_5
Mean pH ± S.D.*	5.48± 0.11	5.63± 0.07	5.71± 0.11	5.77± 0.09	5.92± 0.05

* Data indicates mean pH ± standard deviation of triplicate determinations

Table 4: Extrudability study of phytosome cream

Parameter	Observation				
	F_1	F_2	F_3	F_4	F_5
Mean Extrudability ± S.D.*	4.96±0.15	5.29±0.27	5.08±0.18	5.48±0.21	5.23±0.13

* Data indicates mean± S.D. of triplicate determinations

Table 5: Rheology (viscosity) of phytosome cream

Formulation Batch	Viscosity(cps)* at rpm		
	10	20	50
F_1	1784±14	1326±19	1104±12
F_2	1803±11	1358±13	1029±16
F_3	1726±15	1264±21	983±14
F_4	1597±7	1146±11	914±18
F_5	1419±13	1075±23	863±17

***** Data indicates mean± S.D. of triplicate determinations.

Table 6: Spreadability study of phytosome cream

Parameter	Observation				
	F_1	F_2	F_3	F_4	F_5
Mean Spreadability ± S.D.*	15.63±0.43	13.71±0.39	14.57±0.61	16.36±0.47	17.94±0.54

* Data indicates mean ± std. deviation of triplicate determinations.

Table 7: *In-vitro* drug release of phytosome cream

Time (Min)	% Cumulative drug release*				
	F_1**	**F_2**	**F_3**	**F_4**	**F_5**
15	11.54±2.17	12.32±4.13	11.17±2.33	19.57±3.38	22.08±4.17
30	14.79±5.17	13.85±4.22	14.84±3.67	21.27±3.83	24.98±3.31
45	18.83±4.27	17.76±3.12	18.52±2.67	24.74±4.11	28.71±2.33
60	23.96±4.83	22.10±4.33	21.25±5.67	26.91±5.17	31.58±4.13
90	26.67±6.17	24.26±4.67	26.57±6.17	31.07±3.31	38.40±5.11
120	30.17±7.31	27.12±5.33	31.43±5.33	35.92±6.17	43.12±3.12
180	36.79±6.11	30.23±6.67	35.07±5.67	42.63±5.33	50.11±5.67
240	41.24±8.67	34.67±7.17	39.00±6.33	46.31±5.27	54.96±6.11

***** Data represent mean ± S.D. **batch without addition of drug

Table 8: Protocol for accelerated stability testing of the developed soy-phytosome cream based on ICH Q1 Stability testing guidelines

1	Objective	To ensure whether the developed soy-phytosome topical cream has adequate physical stability.		
2	Selection of batches	Optimized formulation- F_5, Number of batches- 3		
3	Container and closure	collapsible aluminum tube		
4	Specification	Parameter	Acceptance criteria	Test method
		pH	4.5 to 6.0	Digital pH meter
		Spreadability ($gm.cm.sec^{-1}$)	8.50 to 11.50	Modified apparatus containing glass slides with pulley system
		Extrudability (%)	5 to 8	Load on collapsidal tube
		In vitro drug release at 240 minute (%)	up to 58	Franz diffusion cell
		Viscosity (cps) At 10 rpm At 20 rpm At 50 rpm	 1348±58 1075±37 851±44	Brookfield viscometer
		Globule Size (µm)	56.50± 12.45	Microscope with eye-piece micro-meter
5	Testing frequency	5 sample at 0,1,2,3 and 4 week		
6	Storage condition	40±2°C and 75±5% RH		

Table 9: Physical stability study of optimized batch F_5 at accelerated testing condition

Parameter	**Time Interval (in days)**				
	0	**7**	**14**	**21**	**28**
Appearance	White	White	White	White	White
Color					
Foreign particle*	(-)	(-)	(-)	(-)	(-)
Homogeneity**	(+)	(+)	(+)	(+)	(+)
pH	6.0±0.11	5.9±0.05	5.87±0.15	5.87±0.12	5.9±0.11
Viscosity (cps)					
At 10 rpm	1348±58	1234.3±40	1293.3±41.7	1304.6±31.5	1315.3±13.4
At 20 rpm	1075±37	985±7.50	1219.3±21.5	1234±34.22	1227.3±21.5
At 50 rpm	851±44	836.7±14.2	824.6±21.5	839.4±21.9	804.6±33.6
Spreadability (gm.cm/sec)	17.94±0.54	17.46±0.55	17.96±0.61	17.56±0.65	17.71±0.6
Diffusion study (%)	54.96±0.22	53.85±0.08	53.02±0.27	52.56±0.18	52.65±0.37
Phase-separation***	(-)	(-)	(-)	(-)	(-)
Globule size (µm)	56.50±12.45	58.66±15.58	57.95±14.63	52.66±9.30	49.33±8.23

*(-) represents 'no foreign particle'; **(+) represents 'homogenous consistency'; and ***(-) represents 'no phase separation'.

Table 10: In vitro release from the developed phytosome cream formulation F_5 subjected to accelerated stability storage condition

Time (min.)	Cumulative drug release (%)*				
	0 week	1st week	2nd week	3rd week	4th week
0	0.00	0.00	0.00	0.00	0.00
15	22.08±0.10	21.84±0.23	22.03±0.19	21.62±0.22	21.39±0.15
30	24.98±0.29	22.99±0.35	22.46±0.16	22.75±0.17	22.49±0.38
45	28.71±0.23	24.85±0.13	24.40±0.09	24.26±0.29	23.58±0.44
60	31.58±0.37	28.87±0.17	28.46±0.13	28.49±0.19	28.01±0.18
90	38.40±0.16	36.52±0.28	36.09±0.27	35.94±0.21	35.43±0.12
120	43.12±0.29	41.72±0.21	41.05±0.22	40.73±0.34	39.98±0.27
180	50.11±0.19	48.78±0.35	47.78±0.12	47.40±0.14	45.35±0.33
240	54.96±0.22	53.85±0.08	53.02±0.27	52.76±0.18	52.65±0.37
f_2		93.56	82.87	71.78	68.45

Table 11: Evaluation of prepared soy-phytosome topical cream

Parameter	Batch				
	F_1	F_2	F_3	F_4	F_5
Appearance	White	White	White	White	White
Color					
Foreign particle*	(-)	(-)	(-)	(-)	(-)
Homogeneity**	(+)	(±)	(+)	(+)	(++)
pH	5.48±0.11	5.63± 0.07	5.71± 0.11	5.77± 0.09	5.92± 0.05
Viscosity (cps)					
At 10 rpm	1784±14	1803±11	1726±15	1597±7	1419±13
At 20 rpm	1326±19	1358±13	1264±21	1146±11	1075±23
At 50 rpm	1104±12	1029±16	983±14	914±18	863±17
Spreadability (gm.cm/sec)	15.63±0.43	13.71±0.39	14.57±0.61	16.36±0.47	17.94±0.54
Extrudability (%)	4.96±0.15	5.29±0.27	5.08±0.18	5.48±0.21	5.23±0.13
Diffusion study at 240 min (%)	41.24±8.67	34.67±7.17	39.00±6.33	46.31±5.27	54.96±6.11

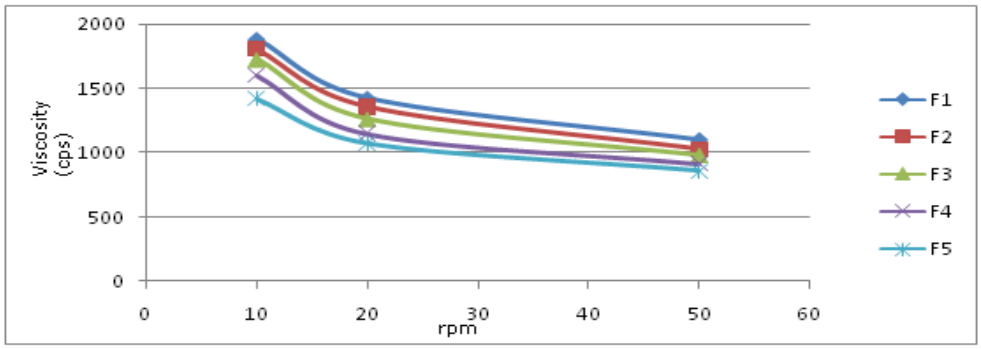

Fig. 1: Effect of rpm on viscosity

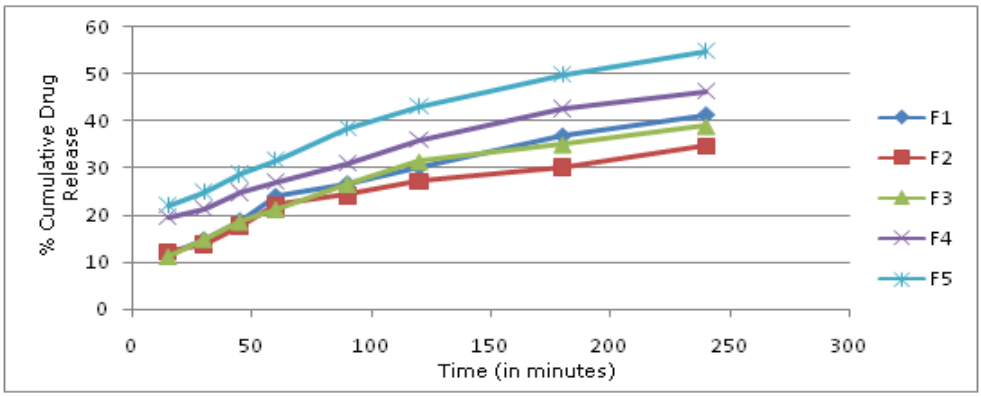

Fig. 2: *In-vitro* drug release of prepared phytosome cream formulations across egg-membrane

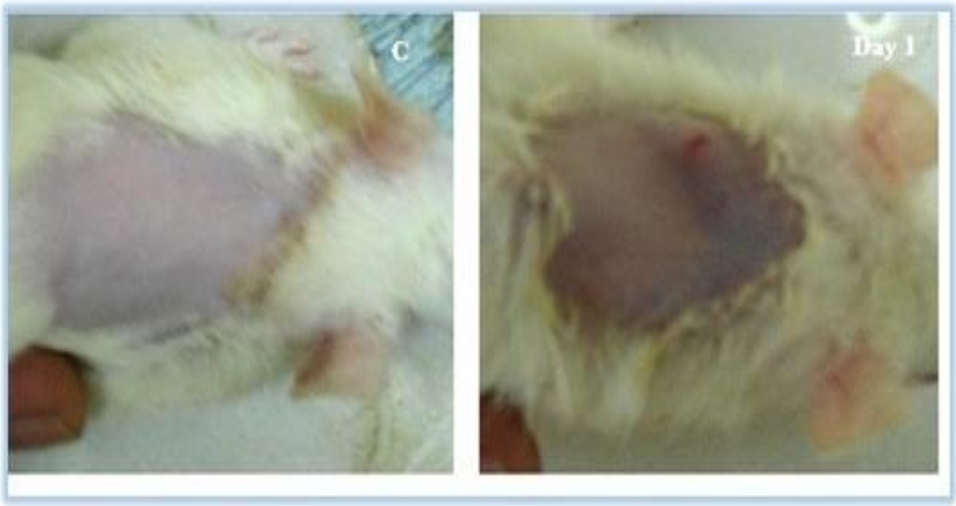

Fig. 3: Skin irritancy test for prepared soy phytosome cream

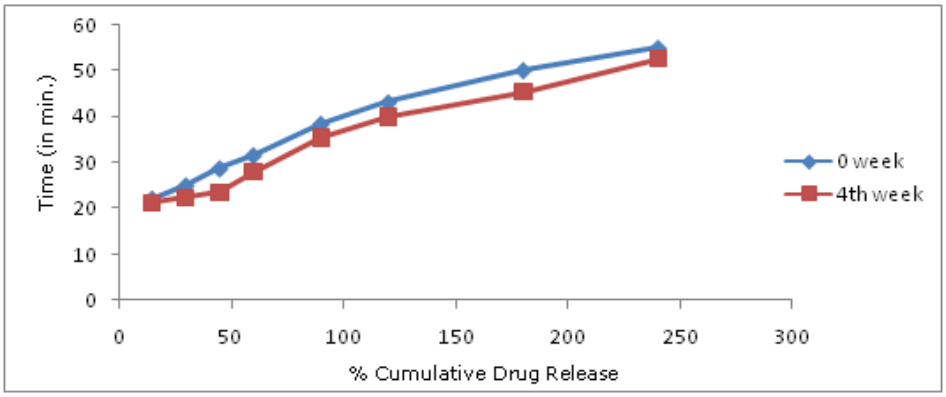

Fig. 4: *In vitro* release from developed soy-phytosome cream formulation F_5 stored under accelerated stability condition

CONCLUSION

For topical application, phytosomes were incorporated into semisolid dosage form i.e. cream. Various batches of phytosome cream were prepared and evaluated. The results of evaluated parameters of phytosome cream are summarized in Table 11.

F_5 batch (10% concentration) was selected for physical stability study. Stability study was carried out at 40ºC/75% RH for 4 weeks. The data of zero week and fourth week were compared and t-test was applied to assess the differences, if any. The cream was found to comply with the acceptance criteria. Further, skin irritation was checked using rabbit for one day and there was no reaction found onto the skin of the animal.

Soy phytosomes could successfully be prepared with 1:1 ratio of SE: PL using solvent system of ethanol: water (70:30) by spray drying technique.

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REFERENCES

1. Ajazuddin SS. Application of novel drug delivery system for herbal formulation. Elsevier- Fitoterapia 81 (2010).
2. Kareparamban JA. Phytosome: A Novel Revolution in Herbal Drugs. International Journal of Research in Pharmacy and Chemistry 2012; 2(2).
3. Suryawanshi JAS. Phytosome: An emerging trend in herbal drug treatment", Journal of Medical Genetics and Genomics 2011; 3(6).
4. Mohd AA. Commercially bio available proprietary technologies and their marketed products. Elsevier- Drug Discovery Today 2013. Available online at www.drugdiscoverytoday.com.
5. Bombardelli E. Phytosome: New Cosmetic Delivery System. BollChim Farm. 1991; 130:431-38.
6. Bombardelli E, Spelta M. Phospholipid-Polyphenol Complexes: A New Concept in Skin Care Ingredients. Cosm. & Toil.1991; 106:69-76.

7. Murray D. Phytosomes- Increase the Absorption of Herbal Extract 2008. (Online available at- www.doctormurray.com/articles/silybin.htm).
8. Choubey A. Phytosomes: A Novel Approach for Herbal Drug Delivery. International Journal of Pharmaceutical Sciences and Research 2011; 2(4): 807-816.
9. Chowdhury RS. Phytosome: An Emerging Technology in Phytomedicine. International Research Journal of Pharmacy 2012; 3(10): 60-64.
10. Jain N. Phytosome: A Novel Drug Delivery System for Herbal Medicine. International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(4): 224-228.
11. Vangapelli S. Phytosome: A Novel Drug Delivery System for Improving Bioavailability of Herbal Medicine. International Journal of Pharmaceutical Research and Development 2011; 3(6): 174-184.
12. Tripathy S. A review on Phytosomes, Their characterization, advancement and potential for transdermal application. Journal of Drug Delivery and Therapeutics2013; 3(3): 147-152.
13. www.drugbank.ca/drugs/DB01645.
14. www.soyconnection.com_sites_default_files_Soy-Lecithin-Fact-Sheet.
15. Rathore P, Swami G. A potential phyto-phospholipid carrier for the bioavailability enhancement of herbal extracts. International Journal of Pharmaceutical Sciences and Research (IJPSR) 2012; 3: 3.
16. http://en.wikipedia.org/wiki/Precipitation.
17. http://www.freezedrying.com/freeze_drying_principles4.html.
18. Swarbrick BJ. Spray drying and spray congealing of pharmaceuticals encyclopaedia of pharmaceutical technology. Marcel Dekker. 1992, 207-221.
19. He P, Davis SS, Illum L. Chitosan microspheres prepared by spray drying. International journal of pharmacy 1999, 53–65.
20. Saha S. Phytosome: A Brief Overview. Scholars Academic Journal of Pharmacy (SAJP)2013; 2(1):12-20.
21. Agarwal VK. Improvement in Bioavailability of Class-III Drug: Phytolipid Delivery System. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4 (1): 37-42.
22. Babitta R. Phytosomes: A potential carrier for herbal drugs. International Journal of Research and Reviews in Pharmacy and Applied Science (IJRRPAS); 2(3), 566-577.
23. Haijun H. Phytosomes: an effective approach to enhance the oral bioavailability of active constituents extracted from plants. Journal of Chinese Pharmaceutical Sciences 2013; 385-08.
24. Khan J. Recent advances and future prospects of phyto-phospholipid complexation technique for improving pharmacokinetic profile of plant actives. Journal of Controlled Release (JCR) 2013; 168: 50-60.