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FORMULATION AND EVALUATION OF TOPICAL CREAM OF PIPERINE FOR VITILIGO

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ABSTRACT

The main interest of the investigations gathered here was in pursuit of developing a topical dosage form of a suitable antivitiligo agent. In the light of reported works on antivitiligo activity of piperine, this project was aimed to bring a ray of hope to the "down casted" by a dosage form incorporated with the bioactive compound piperine, as the API (active pharmaceutical ingredient) for antivitiligo. The formulation must be able to achieve the drug to penetrate the stratum corneum and get lodged in the dermal layers without further trespassing into the systemic circulation. Thus the tissue bioavailability of the drug in the dermal region must be maximized where the melanocytes proliferency and formation of melanocytic dendrites can be achieved. The formulation and development of conventional topical preparations-cream and ointment incorporated with piperineis also carried out with a NDDS known as phytosomes.

KEYWORDS: Piperine, Vitiligo, Ointment, Phytosme, Creams.

1. INTRODUCTION

Vitiligo also known as Leukoderma is a chronic skin disease. This is caused by the loss of pigment, resulting in irregular pale patches of skin. It is reported that Vitiligo is a disorder in which the body destroys its own pigment cells, melanocytes (autoimmune) in various parts of the skin. In affected areas, the pigment gradually disappears. Vitiligo is classified according to the distribution, pattern and extent of depigmentation. There are many reports on classification. However, most investigators distinguished two large subtypes of Vitiligo, segmental Vitiligo(S) and non-segmental Vitiligo(NS). According to another classification proposed by Norlund and Lerner, three types are identified i.e., localized, generalized and universal Vitiligo. Localized Vitiligo is further classified into focal and segmental:

generalized into acrofacial, vulgaris and mixed subtypes.

The researchers found that piperine as antivitiligo agent compared the effects of Piperine and its analogues tetrahydro Piperine (THP), cyclohexyl analogue of Piperine (CHP) and reduced CHP (rCHP) when applied to the skin of mice, either alone or followed by UVR.

2. MATERIAL AND METHODS

Pepper fruits (berries) were collected from various organic farming from locations in Karoor, Chalakudy, Thrissur Dist. Kerala, South India and authenticated. Piperine was isolated and standardized in SRGI Faculty of Pharmacy, Jabalpur and also procured from Sigma-aldrich INC, USA. Phosphatidyl choline was a generous gift sample from Lipoid GMBH, Ludwigshafen, Germany. Poloxamer 407 was procured from Spectrum chemicals Mfg Corp, Gardina, CA, USA. Tri ethanol amine and 4-Hydroxyanisole were procured from Universal lab and chemicals, Penang, Malasia. Glyceryl monooleate was obtained from Himedia Pvt. Ltd. Sagar. The following chemicals were purchased from SD Fine-chem Ltd., Sagar: Carbopol 934, Stearic acid, Hard paraffin, Cetostyryl alchohol, Glycerine, Beeswax, Lanolin, Ethanol, KOH, NaOH, Acetone, Hexane, Toulene. Silica gel 60 F 254 and Ethyl acetate were received from Merck specialties Pvt. Ltd. Mumbai. Tween 80 and Span 80 were procured from Accord labs, Secunderabad. 4-Hydroxyanisole was provided by Hychem Laborotaries, Sagar. All reagents used were of high quality analytical grade.

2.1. Extraction, isolation, standardization and preformulation studies on piperine

As represented in the figure 24a, extraction of piperine was carried out by using two separate methods soxhlation and reflux method. In both extraction methods was different but the isolation procedure was kept same. A 50gm, aliquot of powdered black pepper was taken and extracted with 500ml of 95% ethanol in Soxhlet apparatus/reflux for 3 hrs. The solution was filtered and concentrated under vacuum in a water bath at 60˚C by solvent recovery method either by distillation or by using rotary evaporator. Alcoholic potassium hydroxide 50ml was added to the concentrate and the solution was stirred continuously for 30min. The obtained solution was heated and water was added drop wise until yellow precipitate was formed. Water was added until no more precipitate appeared to form and this was allowed to settle overnight. Needles of Piperine were observed to be separated out. The solid was collected and washed with cold ether 2-3 times. It was recrystallized by using acetone. For this, dissolve solid in acetone and filter it to remove extraneous matter and keep the filtrate aside

for 24hrs so that crystals of piperine are formed. Yellow coloured rod shaped crystals were recrystallized after 24 hrs.

2.2. Construction of standard graph of piperine in ethanol and phosphate buffer(pH 6.6)

Piperine, 10ml was dissolved in 10ml of ethanol (1mg/ml). From this, 1ml was taken and made upto 10ml with ethanol. From this 0.5, 1, 1.5, 2, 2.5, 3ml were taken and made up with ethanol to 10ml separately to produce 5, 10, 15,20,25,30 µg/ml respectively. The solution was suitably diluted and absorbance was taken at 344nm.

10mg of Piperine was dissolved in little of ethanol and then the volume was made upto 10ml with buffer (1mg/ml). From this, 1ml was taken and made upto 10ml with buffer. From this 0.5, 1, 1.5, 2, 2.5, 3ml were taken and made up with buffer to 10ml separately to produce 5, 10, 15, 20, 25, 30 µg/ml respectively. The solutions were suitably diluted and the UV spectral absorbance were taken at 344nm. The equation used was Y = mx + c; where *Y*= Absorbance, m = slope**,** x = Concentration, ***c*** = Intercept.

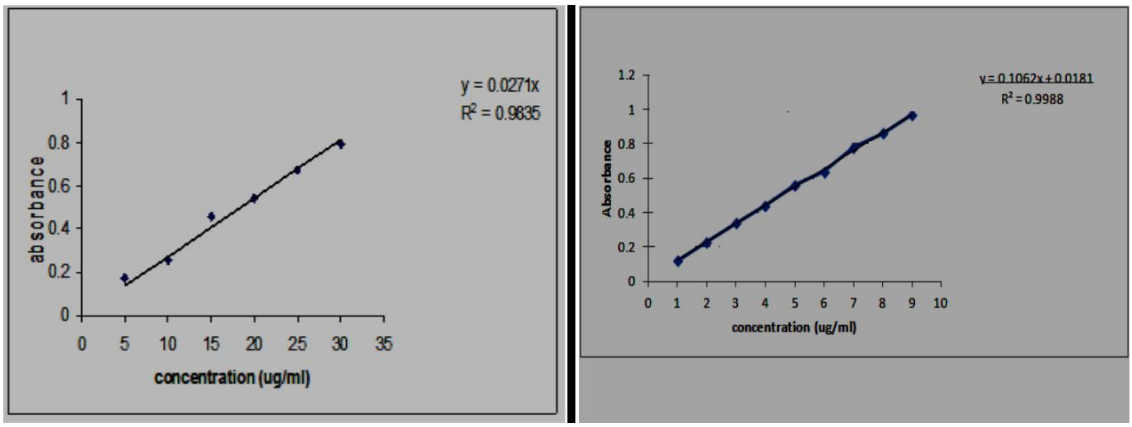

Figure 1: Standard calibration curves for Piperine at λ_{max} 344nm in ethanol, (left) and right (buffer pH 6.6) n=6.

2.3 Formulation of cream incorporated with 1% piperine- Formula to prepare 25g cream incorporated with 1% piperine was calculated as follows. Beeswax (12.5 g), Lanolin (2 g) and stearic acid (2.5 g) were taken in one beaker. In another beaker, Piperine (1%) was dissolved in ethanol by sonication and introduced into glycerine (6.25 g), water (7.26 g), Triethanolamine (0.44 g). Both the beakers were maintained at 60°C and all the ingredients were melted. Then oily phase was added to aqueous phase and stirred continuously.

2.4 Formulation of ointment incorporated with 1% Piperine

Ingredients were calculated for 15g ointment. Ointment was prepared by using fusion method. Hard paraffin (50g) was taken in a china dish along with cetostearyl alcohol 50g and was allowed to melt. Wool fat 50g and white soft paraffin 850g were also added after sometime. After the excipients were melted 1% Piperine was incorporated. Stirred well at reduced maintained temperature.

2.5 Characterization and evaluation of ointment and cream

(i) Drug content uniformity- Drug content uniformity of the formulation in ethanol as solvent was performed. For 1g of cream, 9750µg of Piperine was found. So for 25 gms, the amount of drug is 243.750mg which is almost equal to the total amount taken i.e., 250mg establishing pass test for drug content uniformity.

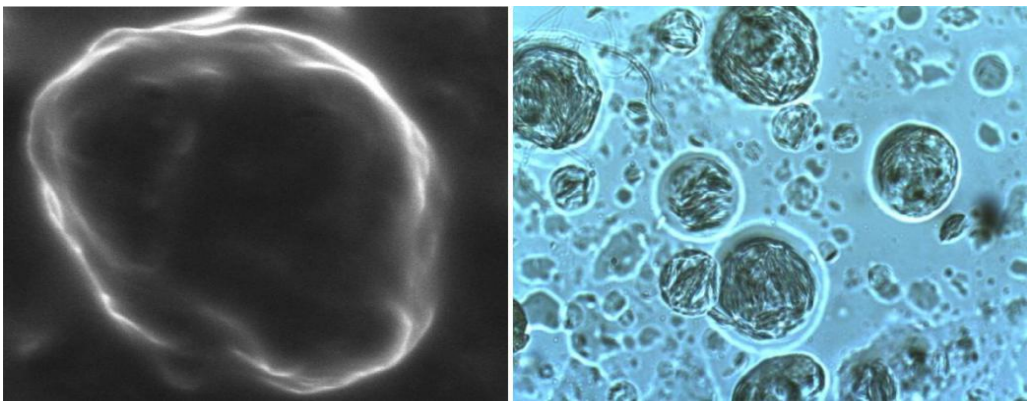

Figure 2. SEM of cream (left) and phase contrast microphotograph (200X) of ointment (right).

(ii) Organoleptic characters and consistency

Organoleptic characteristic studies of the cream were conducted. The cream was light yellow colour and final preparation was found to be free from gritty nature with light yellow colour and peppermint odour.

(iii) Ex-vivo permeation and tissue bioavailability a. Preparation of the tissue

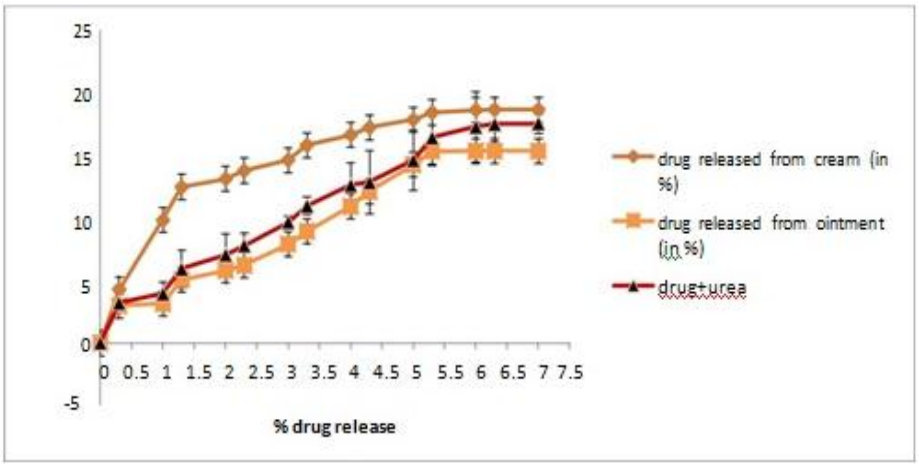

Figure 3. Drug diffusion profile from cream and ointment with and without urea into buffer pH6.6 which was detected at 344 nm.

(iv) Globule size of cream under 10 x observed periodically as a part of stability assessment

Table no 1.

Globule size	1st week (µm)	2nd week (µm)	3rd week (µm)	4th week (µm)
Min size	4.5	4.6	4.7	4.72
Max size	52.6	52.7	52.75	52.75
Average size	28.55	28.65	28.67	28.67

(v) Fabrication of phytosomes

The phytocomplex (phytosomes) was prepared by considering three drug-phosphatidyl choline (PC) ratios of 1:1, 1:3, 1:5. The required amounts of piperine and phospholipids were dissolved in anhydrous ethanol (30ml) and refluxed for 2 hrs while stirring with a magnetic stirrer. Later the solution was transferred into a 100 ml round-bottom flask and ethanol was evaporated under vacuum at 40^0C using rotary evaporator. The complex was washed with n-hexane, dried residues were gathered and placed in desiccators overnight, then sieved through a no. 100 mesh. The resultant extract–phospholipid complex was transferred into a glass bottle and stored in the room temperature for further evaluations.

(vi) Composition and preparation of 1 % cream (25gm)-For a scope of comparative study with the previously reported work, phytosomal complex was incorporated with a cream of same composition. Emulsion system was prepared based on the procedure mentioned under the formulation of piperine 1% cream. Briefly, beeswax, lanolin and stearic were taken in one beaker. Glycerine, water, triethanolamine were taken in another beaker. Phytosome complex

was dissolved in ethanol by sonication and maintained at 40^0c. Both the beakers were maintained at 60°C and all the ingredients were melted. Then oily phase is added to aqueous phase and stirred continuously. The phytosome complex was added to the mixture when the temperature dropped to 40^0C. Creams of three phytosomal complexes (1:1, 1:3, 1:5) and with pure drug were coded as P_1, P_2,P_3 and P_0 respectively.

RESULT AND DISCUSSION

Evaluation of phytosomes and phytosomal cream

(i) Fourier Transform Infrared Spectroscopy (FT-IR)

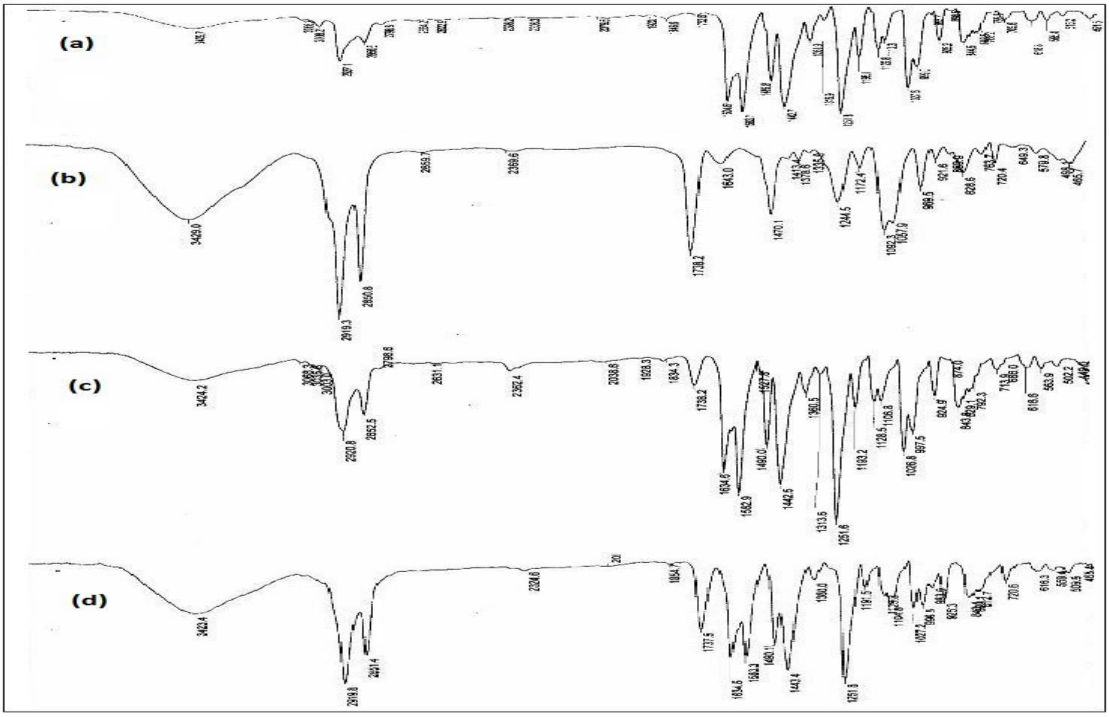

Figure 4: FTIR reports: (a) piperine (b) phosphatidyl choline (c) physical mixture (d) complex.

Table no 2 FTIR Peaks and functional group.

Sr. No.	Wave No.	Functional group
1	2919.3	CH2
2	1738.2	C=O
3	1244.5	C – O
4	1092.3	C-N
5	3429	OH
6	3008.2cm-1	Un saturation
7	2937cm-1	CH2-CH2 –CH3
8	1634 cm-1	C=O stretching
9	1027cm-1	C-N stretching
10	1251.8cm-1	C-C-O

(ii)Average particle size of phytosomes

was found to be 30.6 microns. From the drug content uniformity, the drug concentrations found in P1, P2, P3 formulations are 247.36mg, 239.42 mg and 239.42mg/ml respectively.

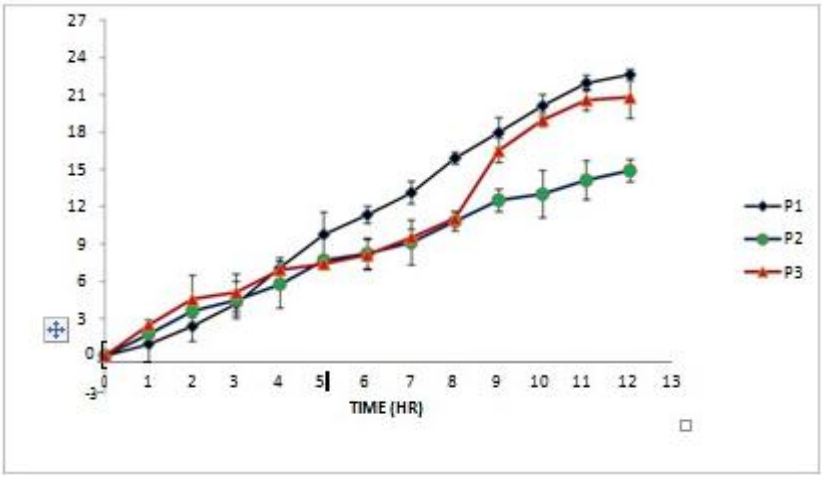

Figure 6: Cumulative drug release profile of piperine from phytosomal cream formulations (P1, P2, P3) diffused into buffer solution of pH6.6 detected at 344 nm. ***Results are expressed as mean ± SD when= 6.***

(iii) Curve fitting analysis

Table no 3. Drug release kinetic model fitting from phytosomes.

Sr.no.	Phytosome code	Zero	First	Higuchi	Peppas	n
1	**P1**	0.993752	0.878752	0.899746	0.9949	1.2994
2	**P2**	0.991302	0.863181	0.949023	0.9692	0.9582
3	**P3**	0.951157	0.894835	0.84127	0.9252	0.992

(iv) The pH- of the formulation F1 was found to be 6.2 which denotes the cream is slightly acidic in nature.

(v) The globule size of the Formulation P1 has shown no significant change even after 4 months.

Table no 4. The globuler size of formulation.

Sr. no.	Globule size	1st Month (µm)	2nd Month (µm)	3rd Month (µm)	4th Month (µm)
1	Min size	5.4	5.4	5.42	5.42
2	Max size	55.81	55.8	55.75	55.75
3	Average size	30.6	30.6	30.58	30.55

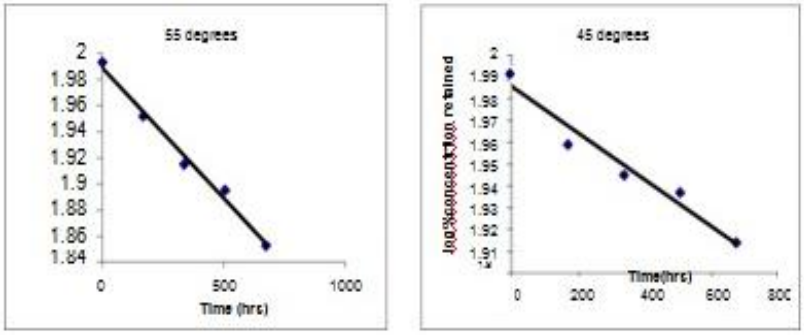

Figure 7: Plots of log % drug concentration V_S time for different temperatures.

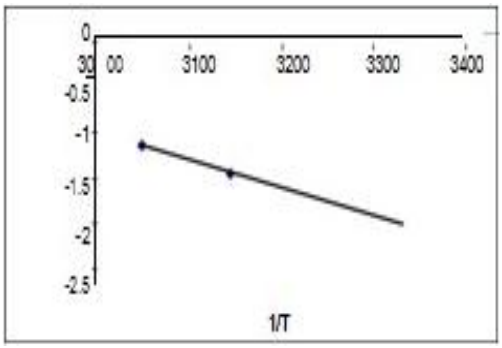

Figure 8: Plot of log k V_s 1 / T to determine k at T25°C.

1 / T values of 45°C and 55°C in Kelvin are 3.048×10^{-3} and $3.144 \times 10^{-3.}$

A graph is plotted between the 1 / T values and log k values and the k value obtained by extrapolating the curve at 27 °C is 0.006 / months.

DISCUSSION

The crystals isolated from piperine was the long needle shaped with the average size of 59.8 µm was able to bring down to 13.77µm. This truly facilitated the drug incorporation in both ointments and creams. When urea as a permeation enhancer was used, the drug diffusion into the buffer was increased by 0.87% which ironically reflected less tissue drug bioavailability. Drug tailoring to the dermis was augmented by 0.6% which was negligible. Minimum, maximum and average globule size of cream was found to be 4.72µ 52.75µ and 28.75 µ respectively while the average size of piperine was in the range of 50-60 µm. Crystal growth should be more cautious than the sedimentation, since the former ruptures the lamellar structure. Sedimentation can be overcome by simple physical agitation. Thus the optimised formulation (TS1) was showing better tissue bioavailability (75.25%±1.72) than the previous formulation (phytosomes having 72.07 ±0.01) which clearly indicates TS are persuasive enough to be considered.

Drug release kinetics are greatly influenced by crystallinity, solubility, particle size amount of API (Active Pharmaceutical Ingredients) etc. A Water soluble API homogenously distributed in a matrix system was mainly released by diffusion. At the same time, liphophilic drugs, the mechanism was based on self erosion. In this case the API was liphophilic.

Comprehensive discussion on drug release kinetics of all formulations

Table 16: Drug release kinetics of cream and ointment.

Sr. no.	Formulation	Zero	First	Higuchi	Peppas	n
1	**Cream**	0.777322389	0.51691858	0.947135035	0.505880848	0.6286
2	**Ointment**	0.962658117	0.837180238	0.93231288	0.739151109	0.7695
3	**Drug+urea**	0.975245	0.800999799	0.960843795	0.721089635	0.7679

Table 17: Drug release kinetics of phytosomal formulation.

Sr no	Phytosome code	Zero	First	Higuchi	Peppas	n
1	**P1**	0.993752	0.878752	0.899746	0.9949	2994
2	**P2**	0.991302	0.863181	0.949023	0.9692	9582
3	**P3**	0.951157	0.894835	0.84127	0.9252	.992

Formulation P1 best fit into Korsmeyer-Peppas model with R^2 value 0.9949, then zero order followed by higuchi equation. Formulations P2 and P3 best fit into zero order with R^2 value > 0.9511, then Korsmeyer-Peppas followed by higuchi equation.

CONCLUSION

The main interest of the investigations gathered here was in pursuit of developing a topical dosage form of a suitable antivitiligo agent. As many as 65 million world population suffers from this skin disease. In Vitiligo, white blood cells attack and destroy pigment cells in discrete areas of the skin. It is called an auto-immune disorder, because the pigment cells become regarded by the body as "foreign" and are therefore rejected. Attempts in classifying Vitiligo has two approaches. In one approach Vitiligo is classified as segmental Vitiligo and non-segmental Vitiligo.

Phytosomes were considered because of its unique ability of bonding together with the phyto-constituent as its integral part. Such a complex is obtained by reaction of stoichiometric amounts of phospholipid and the substrate in an appropriate solvent. Phytosomal cream was prepared and slight increase in the tissue retention of piperine in the dermis when compared to cream was observed.

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