SCREENING, DESIGN AND CHARACTERIZATION OF ALGAE-BASED TOPICAL DOSAGE FORM AS A REMEDY FOR DIGITAL AGEING

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ABSTRACT

Objective: To design and characterize algae-based topical dosage forms as a remedy for digital aging and to assess the compatibility and safety of the developed topical formulation through *in vitro* studies.

Methods: A Central Composite Design (CCD) was employed to systematically investigate the impact of stabilizer concentration, sonication amplitude, and time on the particle size and zeta potential of the formulations. Fourier Transform Infrared Spectroscopy (FTIR), Sun Protection Factor (SPF), and antioxidant activity of the drug were initially assessed. Different concentrations of phospholipid and ethanol were used to create the ethosomes. Size, surface charge, shape, and Entrapment Efficiency (EE) were used to develop and characterize the drug-containing ethosomes. After that, the improved ethosomes were added to create a topical gel formulation. It was tested for stability over three months by looking at its organoleptic, pH, viscosity, and Sun Protection Factor (SPF) activity.

Results: The drug showed 78.94% efficacy in scavenging free radicals. The optimized ethosomes had a spherical form, particle size of 221.8 nm, surface charge is-28.21 mV, and a notable 99.36 % entrapment efficiency. The maximum Sun Protection Factor (SPF) activity was observed in the ethosomal gel EG2. Furthermore, the chosen topical gel held up well during the investigation.

Conclusion: The objective of the study was to develop a stable and functionally effective gel by using suitable ingredients. The evaluation study of the developed gel showed good pH, antioxidant activity, and sun protection activity.

Keywords: Digital aging, Marine algae, Phycocyanin, Ethosomes, Antioxidant activity

INTRODUCTION

Nowadays, the term "Digital Ageing" is most commonly used to describe the accelerated aging of the skin due to exposure to dangerous blue light that comes from the sun or, more commonly, our electronic devices, such as desktop computers, laptop screens, and cell phones [1].

Exposure to blue light can cause DNA damage, Reactive Oxygen Species (ROS) production, and skin cell malfunction. Overexposure of ROS to skin cells can cause melasma, hyperpigmentation, aging, and damage to the skin barrier [2].

Free radicals are important contributing factors to the development of a number of skin conditions, such as skin cancers, wrinkles, and aging of the skin. Ultra-violet (UV) light primarily generates Free Radicals (FR/ROS) by interacting with endogenous photosensitizers. This process results in cumulative physiological and structural changes, as well as progressive alterations in each layer of the skin and changes in the look of the skin [3]. Reference added from Asian Journal of Pharmaceutical and Clinical Research.

Certain cosmetic products, such as gels, creams, sprays, lotions, and ointments, have been proven to be effective in either avoiding or lowering the skin's production of free ROS [1]. Natural ingredients are being used as nanoformulations and act as an efficient delivery system for the skin [4]. Algae are the naturally occurring component that provides photoprotection. It is produced by secondary metabolites of living organisms that exist spontaneously [5]. One interesting photoprotective molecule is phycocyanin. The blue color of this pigment protein combination is a characteristic of cyanobacteria. Strong anti-inflammatory and antioxidant qualities as well as defence against Ultra-Violet B (UVB) induced apoptosis have been reported for phycocyanin. Due to its low toxicity profile, it is suitable for cosmetics, but its ability to absorb light makes it an excellent sunscreen candidate [6]. Cosmetics containing nanotechnology is the newest field of study. Nanomaterials are used by cosmetic businesses to promote skin penetration, texture, and UV protection [4]. As a non-invasive drug delivery technique, ethosomes are designed. These are flexible, soft vesicles [7]. The novel application of spirulina as an ingredient in dermo-cosmetic products. They demonstrated how the microalgae enhances the structure of the epidermis and functions as a moisture booster, improving the function of the skin barrier, especially in terms of skin protection and antiaging [8]. Reference added from International Journal of Applied Pharmaceutics.

Certain bioactive materials in the environment absorb UV radiation, shielding the skin from their damaging effects. Biologically active chemicals have gained popularity in cosmetic formulations recently due to their safety, lack of unpleasant reactions, absence of hazardous chemical components, and environmental integrity. The antimutagenic, anticancerous, and non-toxic properties of phytoconstituents are making them more popular in cosmetics than synthetic photoprotective compounds, which are more potentially harmful [9].

A novel approach was developed to address the shortcomings of the conventional formulation method, including the optimization technique known as experimental design, also referred to as experimental design. In order to determine the best formula based on the evaluation data of the prepared product, optimization is necessary during the preparation and formulation process. In certain circumstances, optimization can be defined as a technique for achieving the ideal combination of process or product features [10].

As novel photoprotective agents, a variety of systemic agents, including antioxidants, vitamins, and minerals, have been dubbed "systemic sunscreens." Sunscreens' primary objective is to scavenge ROS. The main goal of sunscreen is to protect against UVB and UVA radiation at long wavelengths, activate cellular repair systems, and repair DNA [11]. Subheadings in introduction are removed; sunscreens are primarily used to shield skin from the immediate and

long-term impacts of UV radiation. A minimum of 30 SPF, is considered to be the best sunscreen. Unlike traditional sunscreens, which leave a typical white, chalky film on the skin, sunscreen cosmeceuticals' nanoparticles work differently [12]. Numerous external factors can impact the skin and cause premature aging, such as UV radiation, weather, air, and environmental pollutants (like tobacco smoke). Prolonged exposure to these factors can result in oxidative stress, which is a result of an imbalance between antioxidants and oxidants and affects the health of the skin. In biological systems, ROS and nitric oxide (NO_2) are by-products of regular cellular metabolism. They can function as both harmful and helpful species. Proteins, DNA, lipids and membranes, and other cell components can sustain major damage as a result of oxidative stress, which is brought on by an excess of ROS.

Skin cancer is caused by UV radiation, which is extremely hazardous to the skin. Sunburn, photoaging, and even skin cancer can all be avoided and protected against by using photoprotective products containing UV filters. UV-absorbing bioactive substances can prevent UV-induced skin aging and shield human cells from UV-induced cell death. Macroalgae have evolved defense against UVB and UVA radiation, such as the production of phenolic or carotenoids, which are screen pigments [13].

Thus, the goal of the current study was to formulate an ethosomal formulation of Phycocyanin algae that would allow for transdermal distribution. The study compares the UV radiation absorption capacities of phycocyanin and phycocyanin ethosomes and looks at the size and appearance of the resulting phycocyanin ethosomes. Furthermore, a xanthan gum gel containing phycocyanin-containing ethosomes was developed and its capacity to absorb UV light at various UVB wavelengths was evaluated. Sunscreen chemicals must stay on the skin's surface without penetrating or absorbing into the underlying tissues to be safe.

MATERIALS AND METHODS

Materials

Phycocyanin was purchased from HM Phytochem, and Soya phosphatidylcholine (L alpha phosphatidylcholine) was purchased from Sisco Research Laboratories Pvt. Ltd. Taloja, Maharashtra, India. Propylene glycol and Ethanol were used as analytical reagents. (S. D. finechem. Pvt. Ltd. Mumbai, India.) Xanthum gum was collected from Molychem.

Methods

Pre-formulation studies

Absorption maxima for phycocyanin

An accurately weighed quantity of Phycocyanin (100 mg) was dissolved in 100 ml of distilled water in a 100 ml volumetric flask. This yielded a stock solution I with a concentration of 1000 µg/ml.

From the above stock solution (SS I) 10 ml of the sample was withdrawn and the volume was made up to 100 ml with distilled water in a volumetric flask (SS II) to obtain a concentration of 100 µg/ml. This sample (SS II) was scanned in the range of 200-800 nm using a UV Spectrophotometer (Shimadzu 1900i) [14].

Standard calibration curve of pure drug phycocyanin

100 mg of pure phycocyanin was accurately weighed and dissolved in 100 ml of distilled water in a volumetric flask, which is given as the stock solution I (SSI).

From SSI, different volumes (1 ml, 2 ml, 3 ml, 4 ml, and 5 ml) were withdrawn and brought up to a volume of 10 ml with distilled water, resulting in concentrations of 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml respectively. These solutions' absorbance was measured at 615.5 nm using a UV Spectrophotometer. The Beer's range was found to be 100-500 µg/ml. The readings were taken in triplicate, and the average absorbance values were used to plot the calibration curve [14].

Fourier transform infrared spectroscopy

The pure drug was stored in an amber-colored bottle and placed in a stability chamber set to 40 °C with a tolerance of ±2 °C and relative humidity of 75 % with a tolerance of ±5 °C and relative humidity of 75 % with a tolerance of ±5 °C for a month. Dried samples were grounded in an agitate mortar (Shimadzu, Japan). Using a Fourier Transform Infrared Spectroscopy (FT-IR) was used to identify the active functional groups of the drug. The spectrum was scanned over a range of 4,000–400 cm^{-1} [15, 16].

Differential scanning calorimetry (DSC)

The DSC was performed to check for the thermal behavior of the pure drug (Phycocyanin). It was carried out by using the instrument Shimadzu DSC-60 calorimeter. The pure drug was (4-6 mg) crimped in an aluminum pan and the melting point was determined [17].

In vitro screening of sun protection factor determination of drug

Phycocyanin was screened for its absorbance spectra in the range of 290 to 400 nm, using a UV Spectrophotometer. The 50 mg/10 ml, 75 mg/10 ml, and 100 mg/10 ml stock solution of the drug were scanned from 290-320 nm to check absorbance [6].

$$SPF = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times abs(\lambda) \text{ (1)}$$

Where CF = 10 (Correction factor),

EE (λ) = Erythemogenic effect of radiation at wavelength λ,

I (λ) = Intensity of solar light at wavelength λ and

abs (λ) = Absorbance of wavelength λ by a solution of the preparation.

The values for the term "EE x I" are constants.

In vitro antioxidant activity

DPPH method: 2, 2-diphenyl picrylhydrazyl (DPPH) free radical scavenging assay was used to measure the antioxidant activity of a drug.

Preparation of DPPH solution: DPPH (4 mg) was dissolved in 100 ml phosphate buffer in a conical flask. The conical flask containing the DPPH solution was covered with aluminum foil and kept for 30 min at room temperature. Dissolved 10 mg of ascorbic acid and phycocyanin (API) in 100 ml phosphate buffer to obtain 100 µg/ml concentration. This standard solution was diluted serially to make various concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml. Pipetted out 1 ml from each concentration in a 10 ml volumetric flask. To this solution, 3 ml of DPPH solution was added and the volume was made up to 10 ml using phosphate buffer and the absorbance was measured using UV Spectrophotometer at 517 nm [18]. The expression of *in vitro* antioxidant activity is shown in Equation No. 2.

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \text{ .. (2)}$$

Optimization of phycocyanin-loaded ethosomal formulation using a central composite design

The two levels, two factorial central composite designs, were selected using design expert software, and the experimental design was developed by considering dependent and independent variables. The selected independent variables include X1=Concentration phosphatidylcholine (%) and X2=Concentration of ethanol (%) with one center point as shown in table 1.

A total of 9 formulations were obtained from the experimental design. Particle size (Y1) and zeta potential values (Y2) were chosen as dependent variables [19].

Formulation of drug-loaded ethosomal dispersion

The preparation of phycocyanin-containing ethosomes was achieved through the cold method. Soyaphosphotidylcholine (1-4 % w/v) was dissolved in ethanol (20-50 % v/v) and heated in a closed vessel over a water bath to 30±1 °C. Then, propylene glycol (20 %) was added to the above solution. After heating the distilled water (1 %

w/v solution) to 30±1 °C, the drug was dissolved in distilled water and gradually added in a fine stream to the ethanolic lipid solution. The mixture was continuously mixed using a magnetic stirrer operating at 900 rpm. The resulting vesicular dispersions were then allowed to cool for 45 min at room temperature (25±1 °C) after another five minutes of mixing [20]. As shown in table 2, nine batches (F1–F9) of ethosomes were prepared by varying the concentrations of phospholipid and ethanol.

Table 1: List of the independent and dependent variables that were used in central composite design to formulate phycocyanin ethosomes

Factor	Levels used	
Independent variables	Low (-1)	High (+1)
X1=Phosphatidylcholine	1	4
X2=Ethanol	20	50
Dependent variables		
Y1 = Particle size		In range
Y2 = Zeta potential		In range

Table 2: Composition of ethosomes formulation

Formulation code	Drug (%)	SPC (%)	Ethanol (%)	Propylene glycol (%)	Water (%)
PE1	0.2	1	50	20	28
PE2	0.2	4	50	20	25
PE3	0.2	4	20	20	55
PE4	0.2	4.62	35	20	40
PE5	0.2	2.5	35	20	42
PE6	0.2	2.5	56.21	20	21
PE7	0.2	1	20	20	58
PE8	0.2	0.3	35	20	44
PE9	0.2	2.5	13.78	20	63

Characterization of ethosomes

Microscopic examination of particle size and shape

Through microscopic inspection, the average size of ethosomes was calculated. To examine individual vesicles, distilled water was used to dilute a sample of ethosomes adequately. After applying a coverslip to a glass slide containing a drop of the diluted solution, the sample was examined under a microscope with a magnification of 15 x 45. All measurements were taken in triplicate, and the final vesicles had a spherical shape [21].

Determination of vesicle size and size distribution measurements

Vesicle size and size distribution of drug-loaded ethosomal vesicular dispersion were determined by using a zeta sizer (Malvern instrument) [18]. A clear, disposable zeta cell was used to hold the sample after a few drops of the formulation were diluted in distilled water, and measurements were made [22].

Determination of zeta potential measurements

Zeta potential (ζ) measurements were performed by using a zeta sizer and the drug-loaded vesicular ethosomes were analyzed [18]. After diluting ethosomes with distilled water, the samples were put in an electrophoretic cell with a potential of 150 mV, and analysis was carried out [22].

Determination of entrapment efficiency (EE)

The ultracentrifuge method was utilized to determine the ethosome's entrapment efficiency. The prepared ethosomes were centrifuged in an ultracentrifuge for 30 min at 10,000 rpm after being stored overnight at 4 °C. After that, the liquid supernatant was collected, diluted with water, and its EE was measured at 615.5 nm using a UV Spectrophotometer [14]. In the end, the entrapment efficiency was determined using Equation No. 3.

$$\% \text{ Entrapment efficiency} = \frac{(\text{Total drug} - \text{Amount of drug in supernatant layer})}{\text{Total drug}} \times 100 \text{ .. (3)}$$

Scanning electron microscopy (SEM)

After the ethosomal dispersion was suitably diluted, it was sonicated. A few drops of the dispersion were applied to the grid, and then they were left to dry. The samples were fully dried before the images were captured using a scanning electron microscope at a temperature of 25±2 °C, an accelerating voltage of 12.0 kV, and a magnification of 60x [21, 23].

Formulation of ethosomal topical gel

Xanthum gum was mixed with glycerine (2 ml and 1 ml) and soaked in distilled water (7 ml and 3 ml) for overnight. Then, the optimal formulation of 10 g and 15 g of ethosomes were added to the gel base, respectively (EG1 and EG2), and the mixture was continuously agitated in a closed jar at 700 rpm and 30 °C until a homogenous gel was formed. Finally, citric acid was added gradually to the gel to correct its pH [15].

Evaluation of topical ethosomal gel

a) Homogeneity

The formulations were tested for homogeneity by visual appearance and by touching [24].

b) Viscosity

Using a Brookfield viscometer (DV 2PL), the viscosity for different gel designs were measured at a temperature of 25.0±0.5 °C. In the 10-100 % range, the spindle speeds have been changed to yield the maximum torque values. Spindle TL6 was used to measure viscosity at 10 rpm [5].

c) pH

The pH of the gel was measured using a calibrated digital pH meter that was standardized using standard buffers with a pH of 7.4 before using 1 g of gel, precisely weighed and dispersed in 100 ml distilled water and its pH was measured [5].

d) Spreadability

A 20 by 20 cm glass plate was covered with about 1 g of gel in the shape of a circle with a diameter of 1 cm, and another glass plate was placed on top of it. After five minutes of resting a 500 g weight on the upper glass plate, it was discovered that the cream had spread, increasing in diameter [5].

$$\text{Spreadability} = M \times \frac{L}{T} \text{ (4)}$$

Where, S-Spread ability

M-Weight coupled to the upper slide

L-Length of the glass slide

T-Time is taken to separate the slides in seconds.

Table 3: Composition of optimized formulation

Formulation	Drug (%)	SPC (%)	Ethanol (%)	Propylene glycol (%)	Water (%)
OF1	0.2	1.85	39.32	20	38

SPC: Soya phosphatidylcholine

Table 4: Formulation of ethosomal gel (20g)

Formulation code	Xanthum gum	Phycocyanin ethosomes
EG1	1 g	10 g
EG2	0.5	15 g

e) Drug content

Using spectrophotometry, the drug content was determined. 100 mg of the formulation was diluted with distilled water and filtered. Distilled water was added to make the volume up to 10 ml. After properly diluting the resulting solution with water, the absorbance was measured at 615.5 nm using Shimadzu1900i UV Spectrophotometer [25].

f) SPF activity of the formulation

1g of gel was placed in a 25 ml volumetric flask along with 25 ml of water. A Whatman filter paper was used to filter the contents after the flask was placed on the sonicator for fifteen minutes. A 1 ml aliquot was put into a 10 ml volumetric flask, and the volume was then brought down to 10 ml by adding distilled water. Samples in solution yielded absorption spectra in the 290–320 nm wavelength region. Using a UV Spectrophotometer, the produced aliquots were scanned between 290 and 320 nm. The absorbance readings were acquired and multiplied by the corresponding EE (λ) and I (λ) values. They then took their aggregate and increased it by the Correction Factor (CF) [26]. The expression to determine sun protection activity is shown in Equation No. 1.

g) Anti-oxidant activity

1 ml of various quantities of formulations was taken in 10 ml volumetric flasks. To this 3 ml of DPPH phosphate buffer solution was added and the volume made up to 10 ml and incubated for 20 min at 37 °. The absorbance was measured at 517 nm using phosphate buffer as a blank. As a control, the DPPH absorbance was employed [9]. Using Equation No.2, the percentage of anti-radical action was determined.

h) Stability studies

The ethosomal gel formulations were subjected to accelerated stability testing at 40 ºC±2 º/75%±5 % RH for 3 mo following ICH recommendations. The gels were stored in accelerated stability chambers with controlled relative humidity and temperature, and they were packaged in amber-colored jars. The formulations underwent rapid stability testing at room temperature and 40 °C. The parameter readings were taken on 1, 30, and 90 days. The physical characteristics, color shift, pH, viscosity, and sun protection factors of the formulations were evaluated [11].

RESULTS

Absorption maxima for phycocianin

The absorption maxima for phycocyanin was determined by using a UV Spectrophotometer in the colorimetric measurement range of 200-800 nm. The absorption maximum for phycocyanin was found to be 615.5 nm. The linear equation of standard calibration for phycocyanin was estimated.

Compatibility study using FTIR spectroscopy

The data obtained from FTIR studies is shown in table 5. It indicates that the drug and the excipients present in the formulations were compatible with each other no signs of any interactions. The FTIR spectra of phycocyanin and formulation are shown in fig. 1 and 2.
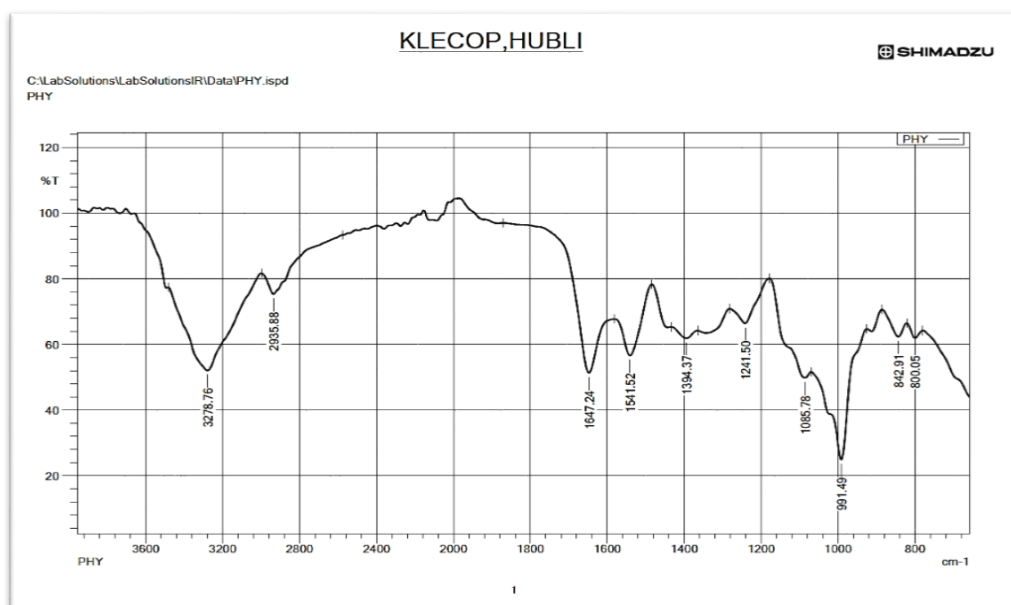
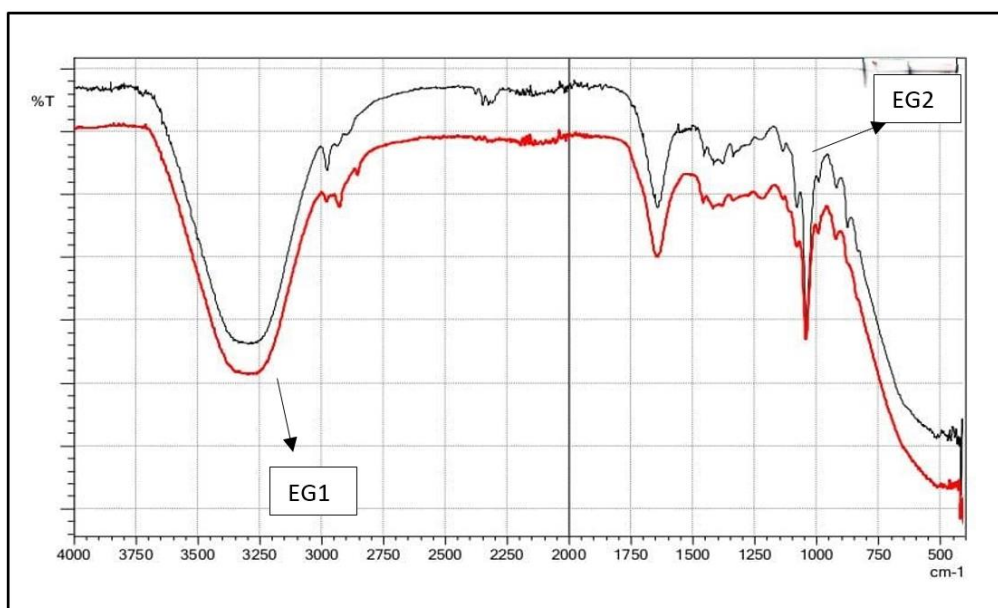**Fig. 1: FTIR spectra of phycocyanin (API) (Caption, Centered)**

Fig. 2: FTIR spectra of formulations (EG1and EG2)

Table 5: Data obtained from FTIR spectral peaks

Functional groups	Vibrational mode	Wave number (cm^{-1}) literature value	Frequency range cm^{-1}		
			Drug	EG1	EG2
Phenol	O-H stretch	3400-3200	3278.76	3307.92	3298.28
Ketone	Amide I (C=O stretch)	1650-1550	1647.24	1643.35	1643.35
Aromatic	Amide II (N-H bend, C-N stretch)	1550-1450	1541.52	1456.26	1456.26
	Fingerprints region	1250-1000	1241.50	1043.49	1041.56

The compatibility between the phycocyanin and the added excipients was evaluated by Fourier-transform infrared spectroscopy (FTIR) analysis, both in its pure form and inside a gel formulation. Key functional groups were detected in the FTIR spectrum: a broad O-H stretching vibration in the 3400–3200 cm^{-1} range suggested the presence of hydroxyl groups; the amide I band, which reflects peptide bonds and is characterized by C=O stretching, within 1650–1550 cm^{-1}; and the amide II band, which appears between 1550–1450 cm^{-1} and is linked to C–N stretching and N–H bending. These functional groups attest to phycocyanin's proteinaceous state and the stability of its structural framework within the gel. Because hydroxyl groups and amide bonds can donate hydrogen atoms to eliminate free radicals, they are essential to phycocyanin's antioxidant activity. Thus, the appearance of these functional groups indicates that phycocyanin may be effective in gel formulations for antioxidant activity.

Differential scanning calorimetry (DSC)

According to the literature search, the melting point of phycocyanin ranges from 60 to 80 °C. The phycocyanin sample demonstrated an endothermic peak at 83.23 °C, indicating that the substance is amorphous. The DSC thermogram of phycocyanin is depicted in fig. 3.
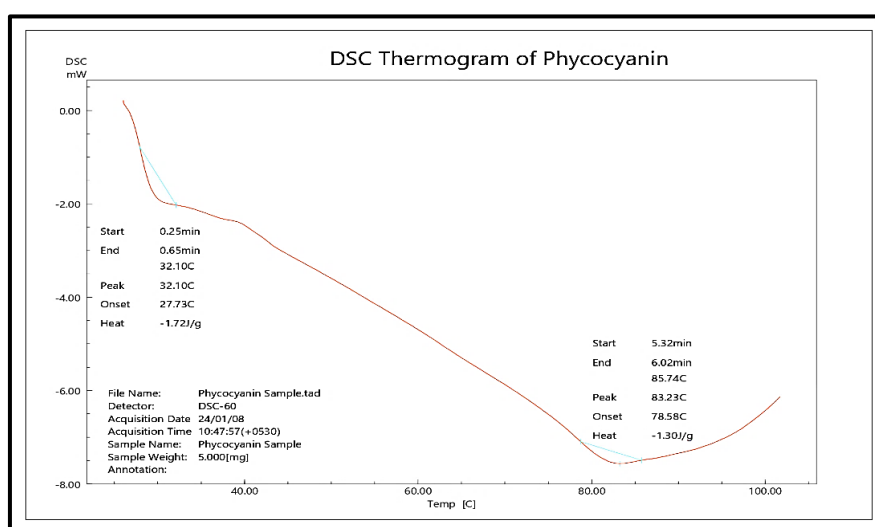

Fig. 3: DSC thermogram of phycocyanin (API)

In vitro antioxidant activity of the drug

At a dosage of 100 mg/ml, the phycocyanin exhibited a 78.94 % radical scavenging activity. Phycocyanin's capacity to scavenge DPPH radicals was demonstrated and presented in table 6.

All formulations exhibited a higher percentage of DPPH radical inhibition than the phycocyanin (API) when taken alone, indicating a verified scavenging effect of DPPH radicals.

The sun protection factor values for different drug sample concentrations are displayed in table 7 and the graphical representation of sun protection factor activity is illustrated in fig. 4. The maximum sun protection factor value in this study was achieved by diluting 100 mg of phycocyanin with 10 ml of water. As the wavenumber increased the UV transmittance value also increased. As the concentration of the medication (Phycocyanin) increased, the sun protection factor value demonstrated a significant enhancement in UV protection.

Therefore, 100 mg/10 ml exhibited a sun protection factor of more than 30. Therefore, 100 mg of the drug was considered an optimized concentration, which was further loaded into ethosomal vesicles.

**Table 6: *In vitro* antioxidant activity for standard and drug**

S. No.	Concentration(µg/ml)	Standard ascorbic acid (% inhibition)	Drug (%)
1	20	56.89±0.027	38.44±0.01
2	40	68.39±0.035	46.31±0.04
3	60	77.87±0.03	55.86±0.03
4	80	89.63±0.012	66.35±0.01
5	100	96.33±0.024	78.94±0.02

All data are given in mean±SD, n=3; n is the number of observations.

Table 7: *In vitro* SPF values of various concentrations of drug

WL	EE*I	50 mg/10 ml		75 mg/10 ml		100 mg/10 ml	
		Abs	SPF	Abs	SPF	Abs	SPF
290	0.015	3.135	0.047025	3.297	0.049455	3.731	0.055965
295	0.0817	2.43	0.198531	3.456	0.2823552	3.624	0.2960808
300	0.2874	2.165	0.622221	2.319	0.6664806	3.324	0.9553176
305	0.3278	1.991	0.6526498	2.395	0.785081	3.189	1.0453542
310	0.1864	1.934	0.3604976	2.700	0.50328	2.915	0.543356
315	0.0839	1.953	0.1638567	2.458	0.2062262	2.984	0.2503576
320	0.0180	2.026	0.036468	2.240	0.04023	2.235	0.04023
Total SPF			20.81		25.33		31.86

WL: Wavelength, EE: Erythemogenic effect, I: Intensity, SPF: Sun protection factor, Abs: Absorbance
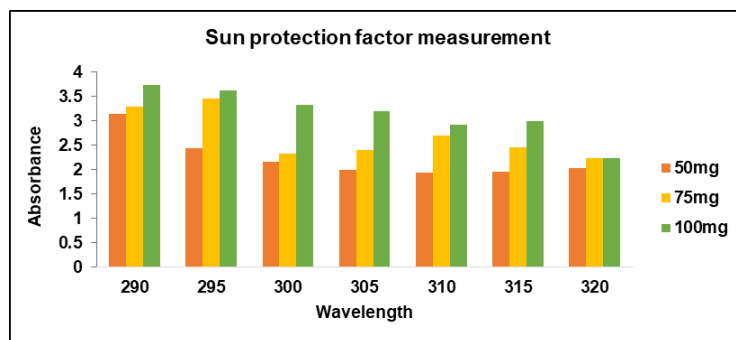
Fig. 4: *In vitro* sun protection factor activity of drug phycocyanin

Central composite design's optimization of ethosomes

The Central composite design yielded a total of nine experimental runs to generate an optimized ethosomal formulation. The data values obtained from the experimental study design are shown in table 8. The response variables particle size (Y1) and zeta potential (Y2) exhibited data values that ranged between 161.6 to 492.3 nm and-34.6 to-75.43 mV, respectively. The results from all 9 formulations indicated that the quadratic polynomial equations were found to be the best fit for the data obtained. The contour and 3D surface plots (fig. 5 and 6) illustrated the relationship between the independent variables and the selected response variables particle size and zeta potential.

Table 11 indicates R^2, standard deviation, and percentage coefficient variation values for both the response variables.

The statistical analysis for particle size and zeta potential is shown in table no 9 and 10.

Table 8: Observed responses for the phycocyanin-loaded ethosomal formulations during the development

Formulation code	SPC (X1) (%)	Ethanol (X2) (%)	Particle size (Y1) (nm)	Zeta potential (Y2) (mV)
PE1	1	50	161.6	-34.6
PE2	4	50	472.3	-51.09
PE3	4	20	339.5	-59.14
PE4	4.62	35	492.3	-75.43
PE5	2.5	35	304	-46.98
PE6	2.5	56.21	283.7	-38.51
PE7	1	20	188.3	-36.38
PE8	0.37	35	79.03	-28.08
PE9	2.5	13.78	223	-44.26

Table 9: Statistical analysis for particle size (ANOVA)

Source	Sum of squares	df	mean Square	F – value	*p*-value	
Model	1.478E+05	3	49273.96	56.49	0.0003	significant
A-Phospholipid	1.369E+05	1	1.369E+05	156.89	<0.0001	
B-Ethanol	4605.25	1	4605.25	5.28	0.0700	
AB	6360.06	1	6360.06	7.29	0.0428	
Residual	4361.45	5	872.29			
Cor Total	1.522E+05	8				

Table 10: Statistical analysis for zeta potential (ANOVA)

Source	Sum of squares	df	Mean square	F – value	*p*-value	
Model	1450.41	2	725.21	19.96	0.0022	significant
A-Phospholipid	1410.15	1	1410.15	38.81	0.0008	
B-Ethanol	40.26	1	40.26	1.11	0.3330	
Residual	218.01	6	36.33			
Cor Total	1668.42	8				

Response 1 (Y1): The effect of independent variables on particle size,

Particle size (Y1) =+282.76 130.69 *X1+23.99 *X2+39.88*X1X2

By the above quadratic polynomial equation, soya phosphatidylcholine (X1) and ethanol (X2) have exhibited synergistic effects on response variable (Y1) particle size. It was observed that as the concentration of soya phosphatidylcholine was increased, the particle size of ethosomes was also increased. Furthermore, when the concentration of soya phosphatidylcholine was increased from 1% to 4%, it was observed that the particle size of the ethosomes increased from 79.03 nm to 492.3 nm. Similarly, it was noticed that an increased concentration of ethanol from (20 % to 50%) has shown a positive effect on particle size.

Zeta potential (Y2) =-46.06-13.26*X1+2.24 X2

According to the above quadratic polynomial equation, soya phosphatidylcholine (X1) has shown a negative effect on the response variable (Y2) zeta potential. It was observed that, with an increased concentration of soyaphosphotidylcholine, the zeta potential value was found to be decreased. Similarly, it was proved that ethanol exhibited a reciprocal impact on the zeta potential of the ethosomes. As the concentration of ethanol in ethosomal formulation increases, the zeta potential value of the ethosomes increased from -28.08 mV to-75.43 mV.

Negative zeta potential results in improved permeability. The ethosomes vesicle is stable, as indicated by the negative zeta potential values of all formulations.

Table 11: Projected values produced by design expert

Quadratic model	R²	Adjusted R²	Predicted R²	SD	% CV
Y1	0.9714	0.9543	0.9012	29.49	10.43
Y2	0.8692	0.8255	0.6803	6.03	13.10

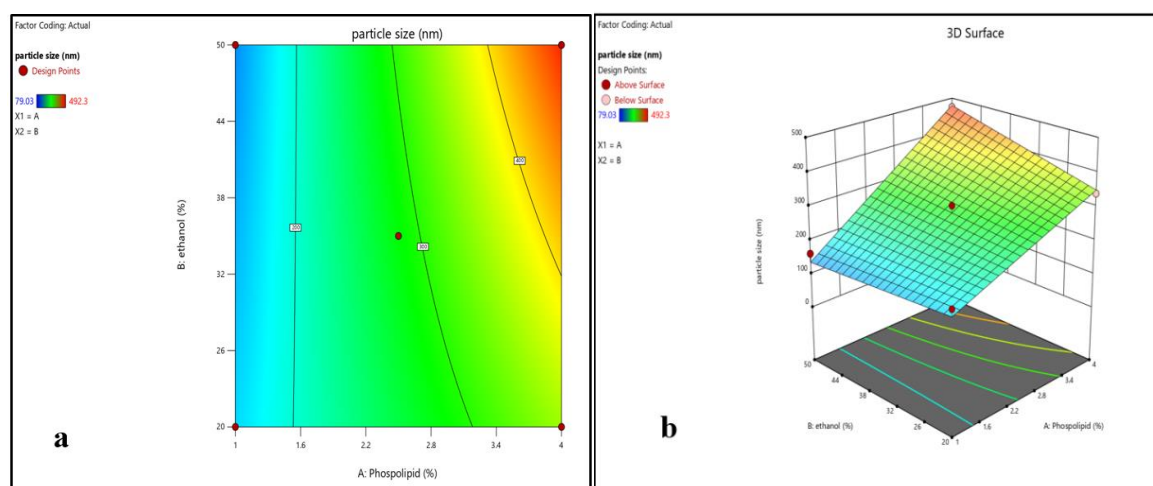SD: Standard deviation, %CV: Percentage co-efficient variance

Fig. 5: An illustration of a) Contour plot and b) 3D surface plots showing the correlation between the particle size and independent variables

Optimization of ethosomal formulation

The phycocyanin-loaded ethosomal formulation was optimized from central composite design using design expert software, in consideration with soya phosphatidylcholine (1.85 %) and ethanol

(39.32 %). The optimized formulation was further characterized for different parameters particle size, zeta potential, polydispersity index, and entrapment efficiency. The data obtained for the optimized formulation is shown in table 13. The overlay plot for optimized ethosomal formulation is shown in fig. 7.
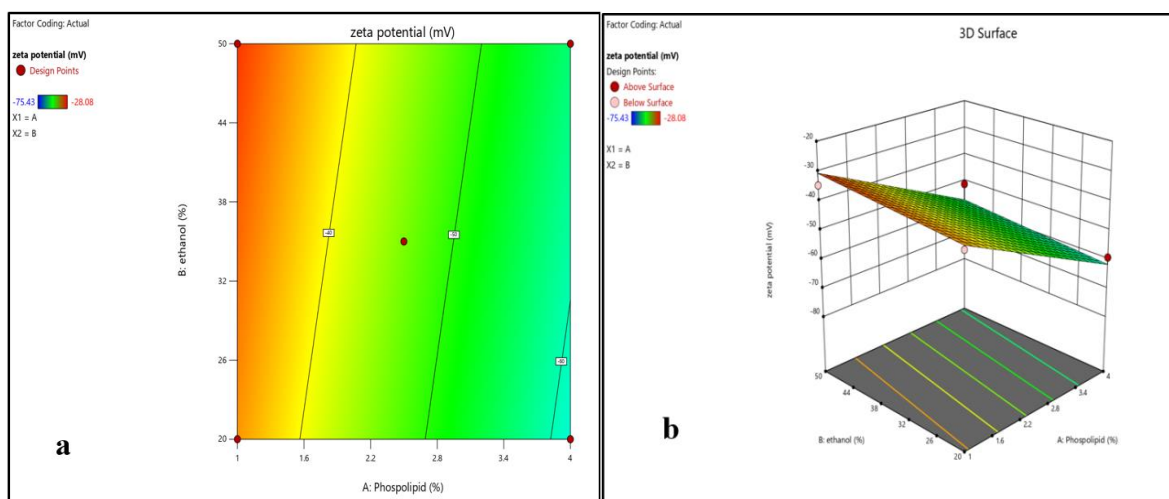
Fig. 6: An illustration of a) Contour plot and b) 3D surface plots showing the correlation between the zeta potential and independent variables

Table 12: Physicochemical characterization of different batches of ethosomes

S. No.	Formulation code	Particle size (nm)	Zeta potential (mV)	PDI	Entrapment efficiency (%)*
1	PE1	161.6	-34.6	0.518	98.39±0.015
2	PE2	472.3	-51.09	0.402	99.82±0.021
3	PE3	339.5	-59.14	0.179	99.64±0.026
4	PE4	492.3	-75.43	0.519	99.84±0.012
5	PE5	304	-46.98	0.015	99.56±0.017
6	PE6	283.7	-38.51	0.191	99.43±0.032
7	PE7	188.3	-36.38	0.342	98.57±0.045
8	PE8	79.03	-28.08	0.470	98.05±0.023
9	PE9	223	-44.26	0.283	99.29±0.011

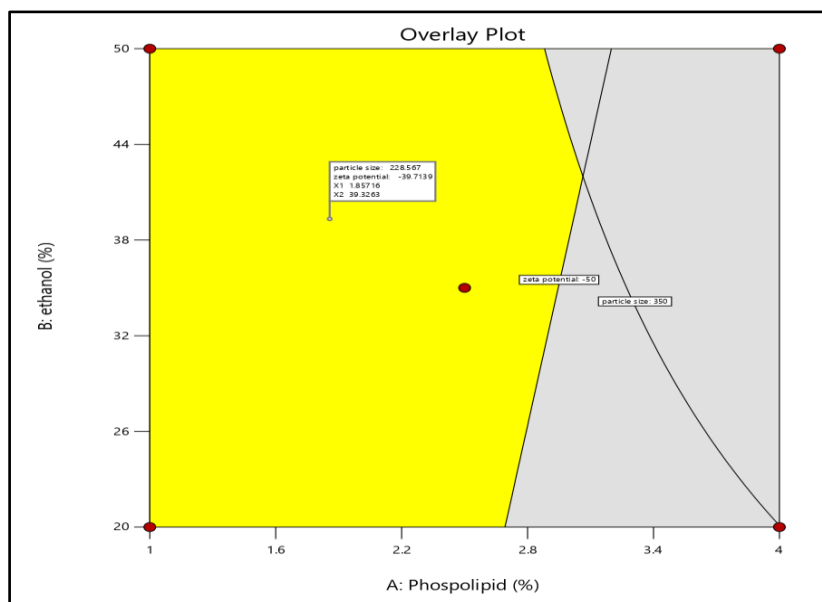*The data is expressed in terms of mean±SD, n=3; n is the number of observations, PDI: Polydispersity index

Fig. 7: Overlay plot for optimized ethosomal formulation

Table 13: Optimized formulation of ethosomes

S. No.	Formulation code	Particle size (nm)	Zeta potential (mV)	Polydispersity index	Entrapment efficiency (%)*
1	OF1	221.8	-28.21	0.747	99.36±0.027

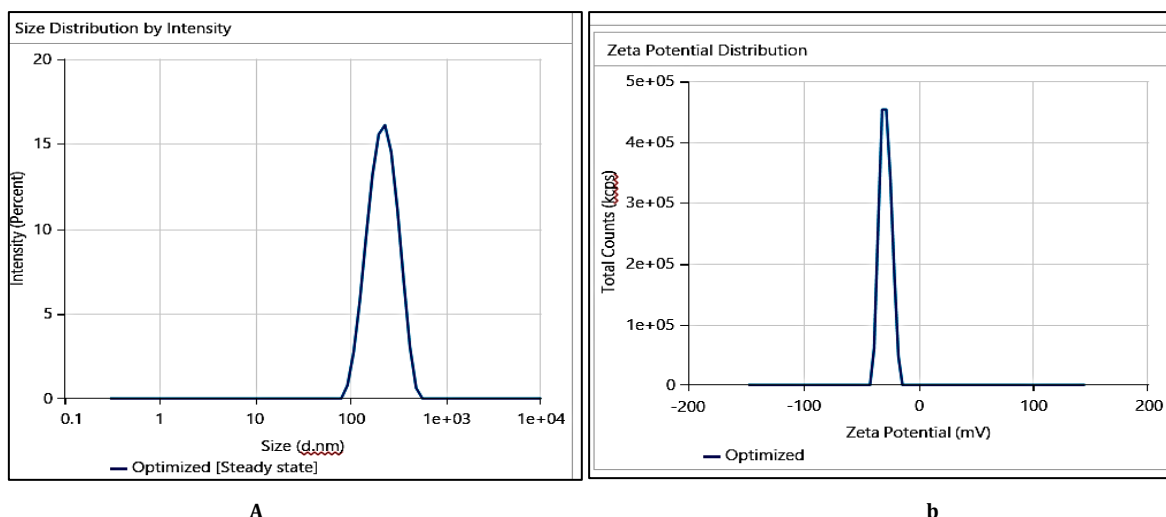*The data is expressed in terms of mean±SD, n=3; n is the number of observations.

Fig. 8: a) Particle size b) Zeta potential for optimized ethosomal formulation

Concerning the values presented by central composite design, phycocyanin-loaded ethosomes optimized formulations were designed and developed by considering the criteria of reaching the minimum particle size and zeta potential. Particle size distribution was 221.8 nm with a zeta potential value of -28.21 mV. The particle size distribution and zeta potential curve for optimized ethosome formulation is depicted in fig. 8.

Scanning electron microscopy (SEM)

The spheres in the phycocyanin ethosomal dispersion were clustered together and displayed (fig. 9) an irregularly spherical structure. The smooth, spherical surface of ethosomes was uniform. Scanning electron microscopy test results also show that ethosomal

dispersions with smooth surfaces are less likely to undergo oxidation reactions, but because of their larger surface area, those with rough surfaces are more susceptible to these processes. Therefore, it is assumed that the aggregated spheres may also increase stability due to the reduction of surface area.

Evaluation of topical dosage form

Appearance

The color, homogeneity, and consistency of the prepared formulations were visually inspected. The topical formulation is pale blue with good consistency. The physicochemical characterization of ethosomal gel is shown in table 14.
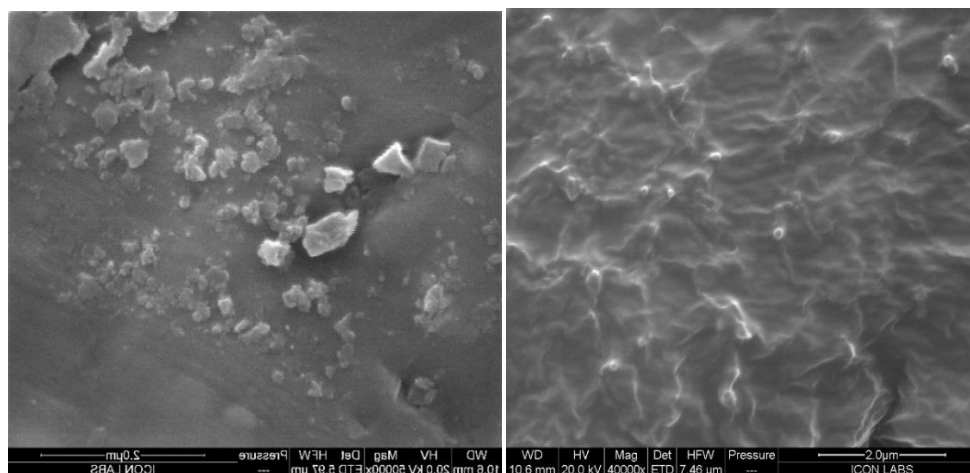

Fig. 9: SEM study of optimized ethosome formulation

Table 14: Physicochemical characterization of ethosomal gel

Formulation code	Appearance	Consistency	Homogeneity
EG1	Pale blue	Good	Homogenous
EG2	Pale blue	Good	Homogenous

EG: Ethosomal gel

Determination of pH

A digital pH meter was used to measure the pH of the gel. The pH of the formulation ranged from 6.0-7.0. Hence, it resulted in being non-irritant to the skin. The data obtained for pH values were tabulated in table 15.

Readings were taken in triplicate and data was presented as mean±SD.

Spreadability

Spreadability values contribute to the efficacy of a formulation. The values of spreadability were found to be 7.8±0.1 cm and 8.3±0.05 cm

as shown in table 15. Spreadability was found to be decreased with an increased concentration of the xanthum gum.

Viscosity

The viscosity of the gel was found to be in the range of 4500 cps to 5000 cps and the viscosity was increased with the increased in concentration of the xanthum gum as expressed in formulation table 4. The data obtained for viscosity values are tabulated in table 15.

Drug content

The percent drug content of gel formulations EG1 and EG2 was found to be 96.18 % ±0.35 and 98.75 % ± 0.12, respectively. Where EG2 formulation has exhibited greater drug content as compared to EG1 ethosomal formulation. The drug content analysis is tabulated in table 15.

In vitro antioxidant activity of gel

Using the DPPH technique, the anti-radical activity of ethosomal formulations (EG1 and EG2) was determined. It was shown that the antioxidant activity of EG1 and EG2 was 85.48% and 88.49 % respectively. However, the EG2 formulation has exhibited the highest antioxidant activity as compared to the EG1 formulation and standard ascorbic acid. The data values for the antioxidant activity of the gels are shown in fig. 10.

Table 15: Evaluation of ethosomal gel formulation

Formulation code	pH	Spreadability (cm)	Viscosity (cps)	Drug content (%)
EG1	6.45±0.05	7.8±0.1	4910±5.50	96.18±0.35
EG2	6.76±0.04	8.3±0.05	4570±4.04	98.75±0.12

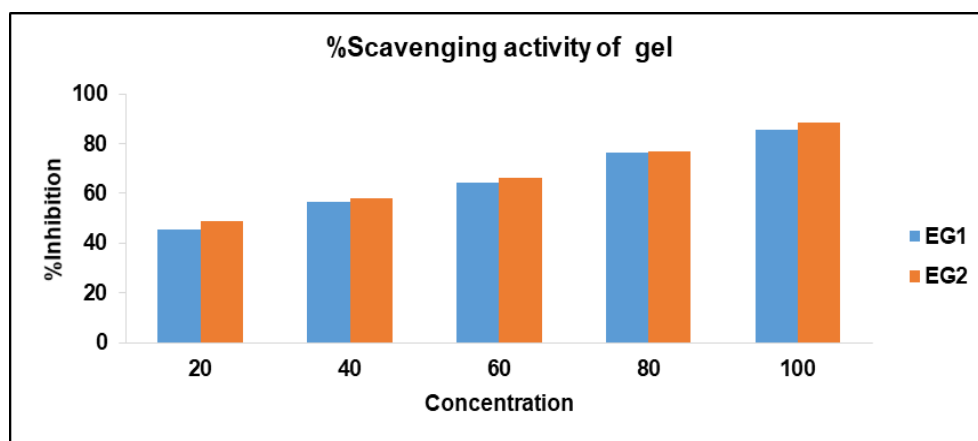All data are given in mean±SD, n=3; n is the number of observations.

Fig. 10: Antioxidant activity of ethosomal gel

EG1: Ethosomal Gel 1

Ethosomal Gel 2

Determination of sun protection factor values of formulations

In comparison to ethosomal formulations, the EG2 formulation has a higher sun protection factor rating than the EG1 formulation. The data obtained for sun protection factor values for both formulations are tabulated in table 16. The graphical representation of sun protection factor values for both the ethosomal formulations are depicted in fig. 11.

The stability analysis of the formulated gel was conducted as per the ICH guidelines for 3 mo. The results shown in table 17 indicate no visible changes in appearance with good consistency. However, there were slight changes in pH and viscosity, and the sun protection factor of the formulations was increased with the increase in days. Overall, the formulations demonstrated stability throughout the study period, with only minor variations in some parameters. Notably, formulation EG2 exhibited the highest sun protection factor activity, maintaining its effectiveness over time.

Table 16: Sun protection factor values of ethosomal gel formulations

Wavelength (nm)	EE*I	EG1		EG2	
		Absorbance	SPF	Absorbance	SPF
290	0.015	4.000	0.06	4.023	0.060345
295	0.0817	3.792	0.3098064	3.867	0.3159339
300	0.2874	3.566	1.0248684	3.754	1.0788996
305	0.3278	3.304	1.0830512	3.736	1.2246608
310	0.1864	3.297	0.6145608	3.625	0.6757
315	0.0839	3.112	0.2610968	3.677	0.3085003
320	0.0180	2.959	0.053262	3.554	0.063972
SPF			34.06		37.28

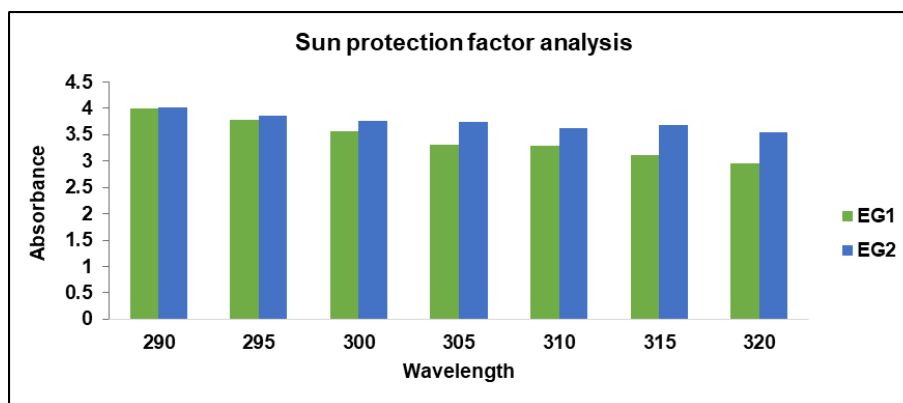SPF: Sun protection factor

Fig. 11: *In vitro* SPF analysis of ethosomal gel

Table 17: Accelerated stability for ethosomal gel

Formulation code	No of days	Evaluation parameters at 30 °C and 65.5 % RH				
		Color	Homogeneity	pH	Viscosity	SPF
EG1	1	Pale blue	Homogenous	6.5±0.025	4908±7.0	35.11±0.02
	15	Pale blue	Homogenous	6.6±0.037	4881±4.16	36.18±0.03
	30	Pale blue	Homogenous	6.7±0.045	4805±5.50	37.10±0.04
	45	Pale blue	Homogenous	6.8±0.051	4783±5.03	38.04±0.04
	60	Pale blue	Homogenous	6.6±0.032	4694±5.56	39.01±0.05
	75	Pale blue	Homogenous	6.7±0.047	4609±6.0	40.03±0.06
	90	Pale blue	Homogenous	6.8±0.045	4615±5.50	41.03±0.05
EG2	1	Pale blue	Homogenous	6.7±0.035	4565±5.50	38.27±0.03
	15	Pale blue	Homogenous	6.8±0.045	4505±5.68	39.20±0.04
	30	Pale blue	Homogenous	6.9±0.055	4474±4.50	40.03±0.05
	45	Pale blue	Homogenous	7.0±0.03	4406±7.63	41.02±0.05
	60	Pale blue	Homogenous	6.8±0.025	4386±4.58	42.01±0.04
	75	Pale blue	Homogenous	6.9±0.02	4304±4.50	43.02±0.06
	90	Pale blue	Homogenous	7.0±0.03	4314±4.00	44.02±0.05

All data are given in mean±SD, n=3; n is the number of observations, SPF: Sun protection factor.

DISCUSSION

Using the Central Composite Design statistical model, the current study intended to develop and optimize a phycocyanin-loaded ethosomes formulation [18]. In the present study, phycocyanin algae was scanned for its UV protectant activity. Phycocyanin algae exhibited an SPF of 30 and this concentration was used to develop ethosomal transdermal delivery [6].

The analysis of FTIR demonstrated no chemical interactions [14, 15]. The detection of the melting point of the drug by using DSC was found to be amorphous [16].

The optimized ethosome formulation (OF1) has a particle size of 221.8 nm, which is comparable to previous reports of Phycocyanin loaded ethosome formulations sizes ranging from 79.03 nm to 492.3 nm. Zeta potential of optimized ethosome formulation (OF1) of -28.21 mV, which is comparable to previous reports of phycocyanin-loaded ethosome formulations ranging from-28.08 mV to-75.43 mV [18].

The optimized formulation gel EG2 exhibited good antioxidant activity with UV protection which can be used as a remedy for digital ageing.

The study investigated the potential of phycocyanin ethosomes for sunscreen formulations.

Using the Central Composite Design statistical model, the current study intended to develop and optimize a phycocyanin-loaded ethosomes formulation [18]. In the present study, phycocyanin algae was scanned for its UV protectant activity. Phycocyanin algae exhibited an SPF of 30 and this concentration was used to develop ethosomal transdermal delivery [6].

FTIR studies confirmed the compatibility of phycocyanin with other formulation components [14, 15]. The DSC study showed a lack of a distinct melting peak, suggesting an amorphous structure [16]. Phycocyanin demonstrated 78.94% radical scavenging activity at 100 µg/ml, indicating strong antioxidant properties. *In vitro* SPF screening revealed that 100 mg of phycocyanin in 10 ml of water yielded the highest SPF value.

Ethosomes were prepared using a cold method, with increased phospholipid and ethanol concentrations resulting in larger particle sizes and decreased zeta potential. Entrapment efficiency increased with higher phospholipid concentrations. SEM analysis showed uniform, smooth spherical ethosomes. The optimized ethosomes were incorporated into xanthan gum gel bases.

The optimized ethosome formulation (OF1) has a particle size of 221.8 nm, which is comparable to previous reports of Phycocyanin loaded ethosome formulations sizes ranging from 79.03 nm to 492.3 nm. Zeta potential of optimized ethosome formulation (OF1) of -28.21 mV, which is comparable to previous reports of phycocyanin-loaded ethosome formulations ranging from-28.08 mV to-75.43 mV [18].

The gel formulations were pale blue with good consistency and acceptable pH. Spreadability values were 7.8±0.1 cm and 8.3±0.05 cm, and viscosity was 4910 and 4570 cps, respectively. Drug content in gel formulations EG1 and EG2 was 96.57±0.35% and 98.71±0.12%, respectively. Antioxidant activity was 85.48% for EG1 and 88.49% for EG2, with EG2 showing better results. Both formulations exhibited good SPF activity, with EG2 having a higher SPF rating. Stability studies over three months showed minor changes in pH and viscosity, with an increase in SPF over time, confirming the stability and effectiveness of the formulations, particularly EG2.

The optimized formulation gel EG2 exhibited good antioxidant activity with UV protection, which can be used as a remedy for digital ageing.

CONCLUSION

The study aimed to evaluate ethosomes containing phycocyanin for their potential in sunscreen formulations. Fourier-transform infrared spectroscopy confirmed the proteinaceous nature and structural stability of phycocyanin in gel formulations. The substance demonstrated significant antioxidant potential, which, when incorporated into gel formulations, enhanced antioxidant and skin-protective properties, reflected in higher SPF values at increased drug concentrations. The ethosomal dispersion's structure suggested sensitivity to oxidation, highlighting the importance of surface morphology for stability and performance. The ethosomal gel with a pH compatible with skin physiology showed good spreadability and viscosity, with better drug content and antioxidant activity in one formulation compared to another. An ethosome formulation containing phycocyanin displayed especially high stability and SPF against UV radiation. Conclusion is written in short and specific

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Nil

AUTHORS CONTRIBUTIONS

All the authors have equal contribution and participation in the proposed research work. Fatima Sanjeri Dasankoppa: Conceptualization, Methodology, Writing-Reviewing and Editing, Supervision. Revati Dharampal Sagare: Methodology and Data analysis. Megha N Sureban: Conceptualization, Data curation, Investigation, Writing – Original draft preparation. Hasanpasha N Sholapur: Software, Methodology and Visualization. Arunkumar Gundaiah Ramesh: Reviewing and Data Analysis. Vijayakumar Murugesan: Software and Reviewing.

CONFLICT OF INTERESTS

Declared none

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