

Formulation and evaluation of melatonin and curcumin sunscreen cream

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Abstract

Skin is affected by oxidative stress due to the formation of reactive oxygen species, which trigger the development of sunburn, erythema, edema, photoaging, skin cancer, etc. Protection of skin from photodamage is an urgent concern. The use of sunscreen is the most common practice nowadays that protects from the adverse effect of UV radiation. In the present study, sunscreen is formulated using melatonin and curcumin which was further evaluated by measuring organoleptic characters, moisture content, % drug release, spreadability, antioxidant potential, and FTIR study, etc. Results showed that formulations have shown good consistency with no degradation of herbal actives while formulating it and shown good spreadability. FTIR study showed that the same functional groups were present in both drug and formulation. So it was confirmed that curcumin and melatonin were incorporated in the cream formulation. From the study, It can be concluded that melatonin, curcumin, and zinc oxide containing formulation can be a new sunscreen therapy with anti-aging property.

Keywords: sunscreen, curcumin, melatonin, skin

Introduction

Skin is the outermost and largest organ of the body hence it is most prone to photodamage as it is directly exposed to sunlight. In recent years the incidences of UV-related diseases & disorders are continuously growing. When the mammalian skin is exposed to long term UV radiation, it induces oxidative stress by generating the reactive oxygen species (ROS). These substances further trigger the development of sunburn, erythema, edema, immunosuppressant, photoaging, skin cancer, etc. The various other biological responses occur in the skin due to UV exposure which includes the development of erythema, edema, sunburn, cell deformation, hyperplasia, and melanogenesis ^[1, 2].

Melanin pigmentation of the skin absorbs UV light & thus protects skin cells from the detrimental effects of UV exposure. So in certain subjects, the amount of melanin produced is not sufficient enough to protect the skin, Hence the protection of the skin from photodamage is by use of natural sunscreen to counteract the reactive oxygen species by blocking the UV radiation exposure on the epidermis. The electromagnetic radiation in sunlight called the UV region is the main factor causing skin damage. Three subdivisions of UV based on wavelength include UV C (270-290nm), UVB (290-320nm), UV A (320-400nm). UV C is absorbed by the ozone layer while UV B & UV A penetrate the earth's surface. Hence both UV A & UV B can injure the human skin resulting in sunburn, solar keratosis, photoaging and can cause severe harm such as skin cancers

^[3-7]. Therefore, sunlight exposure protection by applying effective sunscreen products is necessary. The chemical sun blockers are more used but it has many limitations. Chemical sunscreens absorb UV rays and undergo potentially harmful chemical reactions. It can be fast degraded when gets in contact with UV rays. It turns the Chemical reaction into harmful heat. It increases the chances of irritation & stinging. It increases the chances of redness of skin because it changes UV rays into heat which can affect the skin. So due to this, the formulation of melatonin, curcumin & zinc oxide sunscreen cream has been formulated which has a synergistic effect on the human skin. It protects the skin from UV radiation and also acts as anti-aging, anti-inflammatory, antioxidant, and protect against cell and skin carcinoma.

The study aimed to formulate and evaluate the melatonin, curcumin & zinc oxide sunscreen cream by (O/W) type of emulsion system which will protect the skin from UV radiation & provide a sunscreen therapy and give the synergistic effect by protecting the skin from skin cancer and Antiaging.

Material and Methods

Procurement of the ingredients

Melatonin was given as a gift sample from Sisco lab, Mumbai, curcumin is obtained from Himedia labs, Mumbai as a gift sample and zinc oxide is obtained from LOBA chem, Mumbai, and other excipients are taken from college laboratory.

Method of Preparation

Weigh cetyl alcohol, stearic acid and mix the content and heat the organic mixture in a water bath until all the ingredients have melted at 70-80°C. Then add the melatonin and curcumin in heated solution along with continuous stirring and mix uniformly. The aqueous phase is prepared by mixing the deionized water and the water-soluble compounds like disodium EDTA, methylparaben, propylparaben, propylene glycol, etc. Then zinc oxide was added and heated up to 70-80°C.

After preparation of both the phases, the oil phase was added to the water phase at 80°C with continuous stirring with a mechanical stirrer for 20-25 min until a uniform emulsion is formed. Total 9 batches were prepared.

Evaluation of Nanosuspension

Organoleptic evaluation

Organoleptic evaluation of sunscreen like colour, odour, taste, touch, etc. conducted at Pharmaceutical chemistry laboratory of the institute following standard operative procedures.

Physico-chemical Evaluation

The physicochemical parameters like moisture content⁸, pH, viscosity, spreadability, etc. for sunscreen were established by following standard procedures recommended. pH is measured by using pH meter with magnetic stirrer^[9] and viscosity by using Brookfield RVDV-II + Pro having spindle-small sample volume adaptor and speed of rotation of stirring element is 0.5-100 rpm^[10]. The spreadability of the cream was determined using a spreadability apparatus^[11]. 0.5 gm of cream was placed between the two glass slides. 100 gm weight was allowed to rest on the upper slide for 1 to 2 minutes to expel the entrapped air between the slides and to provide a uniform film of the cream. The weight was removed and the top slide was subjected to a pull of 5 gm. The time necessary for the top slide to travel premarked 6.5 cm distance was noted. This gave an idea of the relative spreadability of the different creams. Spreadability was calculated by the following formula;

$$S = \frac{M \times L}{T}$$

Where, S= Spreadability, M= Mass attached with the slide, T = Time required to travel a distance to slide.

% drug release

The % drug release studies are an important parameter to determine and access the amount of drug is released and diffused within the skin membrane. Before the experiment, the cellophane membrane was washed with phosphate buffer 7.4 and then soaked in the buffer for 24 hr to remove glycerin present on it. The % release of drug from cream is performed using the diffusion cell with a receptor compartment volume of 45 ml and an effective diffusion area of 3.14 cm². The cellophane membrane was soaked in the buffer. 1 gm of the formulation was placed on the donor side. The receptor medium is continuously stirred and maintained at 37± 0.5 C using a circulating jacket using 100 ml of 7.5 pH Phosphate buffer with a stirring speed of 200 rpm/ min. An aliquot of 5 ml was withdrawn at an interval of 15 minutes, suitable diluted, filtered, and analyzed for the content of melatonin & curcumin by UV Spectro photo

meter (Shimadzu 1700 japan) at melatonin (277nm) & curcumin (421nm). An aliquot was replaced with the equivalent volume of fresh dissolution medium to compensate for the loss due to sampling. Then evaluate the % drug release for cream sample formulation & calculated.

Determination of Sun Protection Factor by using Mansur equation

Accurately about 1.0g of the sample was weighed and transferred to 100mL volumetric flask; Ethanol was added up to 3/4th volume of the flask and contents were sonicated for about 10 minutes and volume made up to the mark using ethanol. The solution was filtered through Whatman No.1 filter paper and filtrate collected by rejecting the first few mL of the filtrate. 5mL of the aliquot was taken in a 50mL volumetric flask and volume made up to the mark using ethanol. Then 5mL of the diluted solution was taken into 25mL volumetric flask and volume made up to the mark using ethanol. The absorption spectra of the sample solution were obtained in the range of 250 to 400 nm using 1 cm quartz cell, and ethanol as blank. The absorption data were obtained in the range of 290 to 320, every 5 nm, and 3 determinations were made for each samples^[12]. The SPF of the samples were calculated using the below equation (a mathematical expression derived by Mansur)

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

Where, CF is correction factor (=10); EE (λ) - erythema effect of radiation with wavelength λ; I(λ) -solar intensity spectrum; Abs (λ) - Absorbance of a sunscreen product.

Determination of Antioxidant activity

DPPH Free radical scavenging activity (DPPH assay): Free radical scavenging activity of the methanolic extracts was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. The methanolic extracts at different concentrations (50.0, 100.0, 150.0, 200.0µg/ml and 250 µg/ml) were used in the analysis. The reaction mixture was prepared by mixing 2ml of methanolic solution of extract, 1 ml solution of DPPH, and further 2.0ml of methanol was added to all the test tubes and the mixture was allowed to stand in dark for 30.0 minutes. The same procedure was repeated for the standard solution of Ascorbic at the same concentrations and the calibration line was constructed. Blank was concomitantly prepared, containing 4ml methanol and 1ml solution of DPPH. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. All the readings were performed in a triplicate manner. Then, absorbance was measured at 517nm by using a UV-Vis spectrophotometer¹³. The percent DPPH scavenging effect was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} \text{ or Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100.$$

Where A0 was the absorbance of the control and A1 absorbance of the test or standard samples.

Determination of the infrared absorption spectrum of melatonin and curcumin and their physical mixture and cream formulation

Melatonin powder was triturated with Potassium bromide powder (1:100) and placed in the sample holder, and then

the IR spectrum was recorded using an FTIR spectro photo meter. For the IR spectrum of the physical mixture, melatonin and curcumin were mixed and then dissolved in chloroform and filtered to get the clear sample and placed in the sample holder. The infrared spectrum was recorded using an FTIR spectrophotometer. Sample solution of gel base and the gel was prepared by dissolving gel base and gel in chloroform and filtered to get the clear sample. IR absorption spectrum was recorded by using chloroform in which sample was placed in the sample holder and infrared spectrum was recorded using FTIR spectro photo meter [14].

Results

The prepared formulation batches are evaluated by evaluating the organoleptic characters, the concentration of

drug, viscosity, spreadability of the formulation, sun protection factor (SPF) of resultant sunscreen cream formulation, etc as shown in table no. 1 & 2. During preparation, it was observed that formulation variables significantly had an impact on % drug release, the viscosity of formulation spreadability of formulation, SPF of formulation, and physical stability of sunscreen cream.

Table 1: Results of organoleptic characters

Parameter	melatonin
Appearance	White powder
Colour	white
Odour	characteristic
Texture	Clear

Table 2: Results of evaluation parameters Moisture content, pH, Viscosity, Spreadability, % Drug release, and SPF

Batch	Moisture content	pH	Viscosity in cps	Spreadability	% Drug release	SPF
F1	9.20±0.001	6.71±0.5	7000±0.2	0.26±0.005	61 %	0.9±0.5
F2	9.10±0.004	6.80±0.04	9000±0.4	0.28±0.002	34%	10.15±0.02
F3	8.75±0.001	6.70±0.03	13000±0.1	0.19±0.001	68 %	12.56±0.1
F4	9.05±0.009	6.95±0.05	6600±0.08	0.21±0.003	71%	13.68±0.1
F5	11.29±0.002	6.66±0.06	7200±0.2	0.16±0.001	69%	12.51±0.05
F6	7.63±0.006	6.64±0.02	7900±0.6	0.32±0.002	80%	16.55±0.01
F7	7.70±0.003	7.11±0.05	5800±0.4	0.43±0.001	63%	18.64±0.5
F8	9.04±0.06	6.87±0.06	6600±0.4	0.33±0.006	71%	21.11±0.1
F9	5.42±0.001	6.90±0.002	6200±0.1	0.95±0.003	86%	22.56±0.05

Determination of Sun Protection Factor for drug and sunscreen formulation

SPF of drugs and cream formulation was checked by absorption spectroscopy using the Mansur equation method.

Absorbances obtained in the spectrum were considered for SPF calculations and results are shown in table no 3 and fig no. 1

Table 3: SPF Determination of Melatonin, Curcumin, and Sunscreen Cream by using Mansur equation

Wavelength	EF × I	Melatonin		curcumin		Cream Formulation	
		Abs	EF×I×A	Abs	EF×I×A	Abs	EF×I×A
		3.26	0.0489	0.087	0.0013	2.811	0.0121
295	0.081	3.23	0.2638	0.086	0.0070	2.793	0.0647
300	0.287	3.20	0.9196	0.085	0.0244	1.788	0.2264
305	0.327	3.14	1.0292	0.085	0.0278	1.785	0.2579
310	0.1864	3.08	0.5741	0.085	0.0158	1.781	0.1455
315	0.083	3.00	0.2511	0.084	0.0070	1.777	0.06503
320	0.018	2.93	0.0527	0.084	0.0015	2.773	0.0139
SPF		24.39		0.850		20.05	

Each value is expressed as the mean± SD of three determinations.

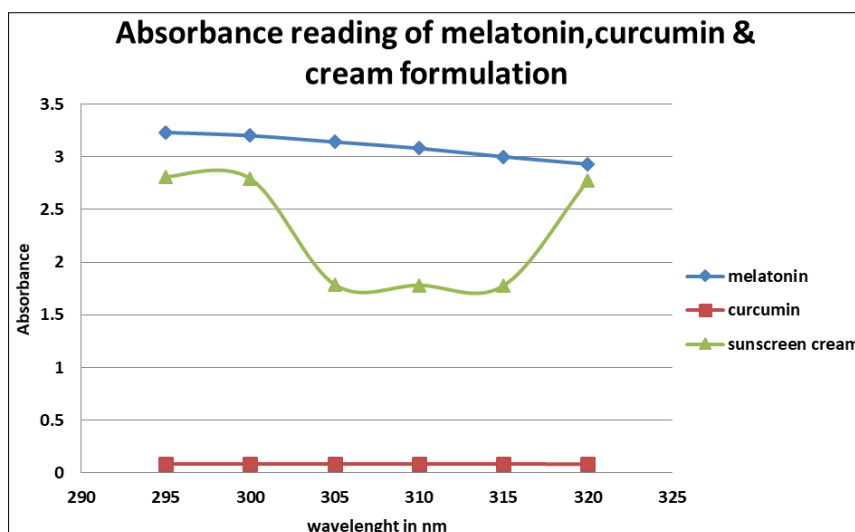


Fig 1: UV spectral absorbance readings of melatonin, curcumin, and cream formulation

SPF determination of melatonin, curcumin, and the cream formulation were performed by UV-Spectrophotometer in the range of 200-400 nm. The maximum absorption for the cream and drug were found between 220-300 nm. SPF of melatonin, curcumin, and Sunscreen cream formulation was

found to be 24.39, 0.850, and 20.05 respectively. Since the formulation has zinc oxide as a physical barrier, the SPF value will be much higher than 20.05.

Radical scavenging activity DPPH assay method

Table 4: Results of % Radical scavenging activity of melatonin

Concentrations (µg/ml)	Ascorbic acid (% scavenging activity) Mean ± S.D (n=3)	(% Scavenging activity) Mean ± S.D (n=3)	
		Melatonin	Curcumin
50	61.71±0.004	56.25±0.030	53.12±0.040
100	70.31±0.002	60.15±0.007	56.25±0.008
150	75.78±0.006	64.84±0.014	61.17±0.010
200	84.375±0.001	69.53±0.008	66.40±0.005
250	89.84±0.004	77.34±0.004	74.21±0.014

FTIR analysis

The use of the analytical technique Fourier Transform Infrared (FTIR) Spectroscopy is an approach used in compatibility tests based on the hypothesis that the same

functional group changes during drug-excipients interaction and if the functional groups remain unchanged means the drug excipients are compatible with each other. Results are shown in Fig no. 2-5

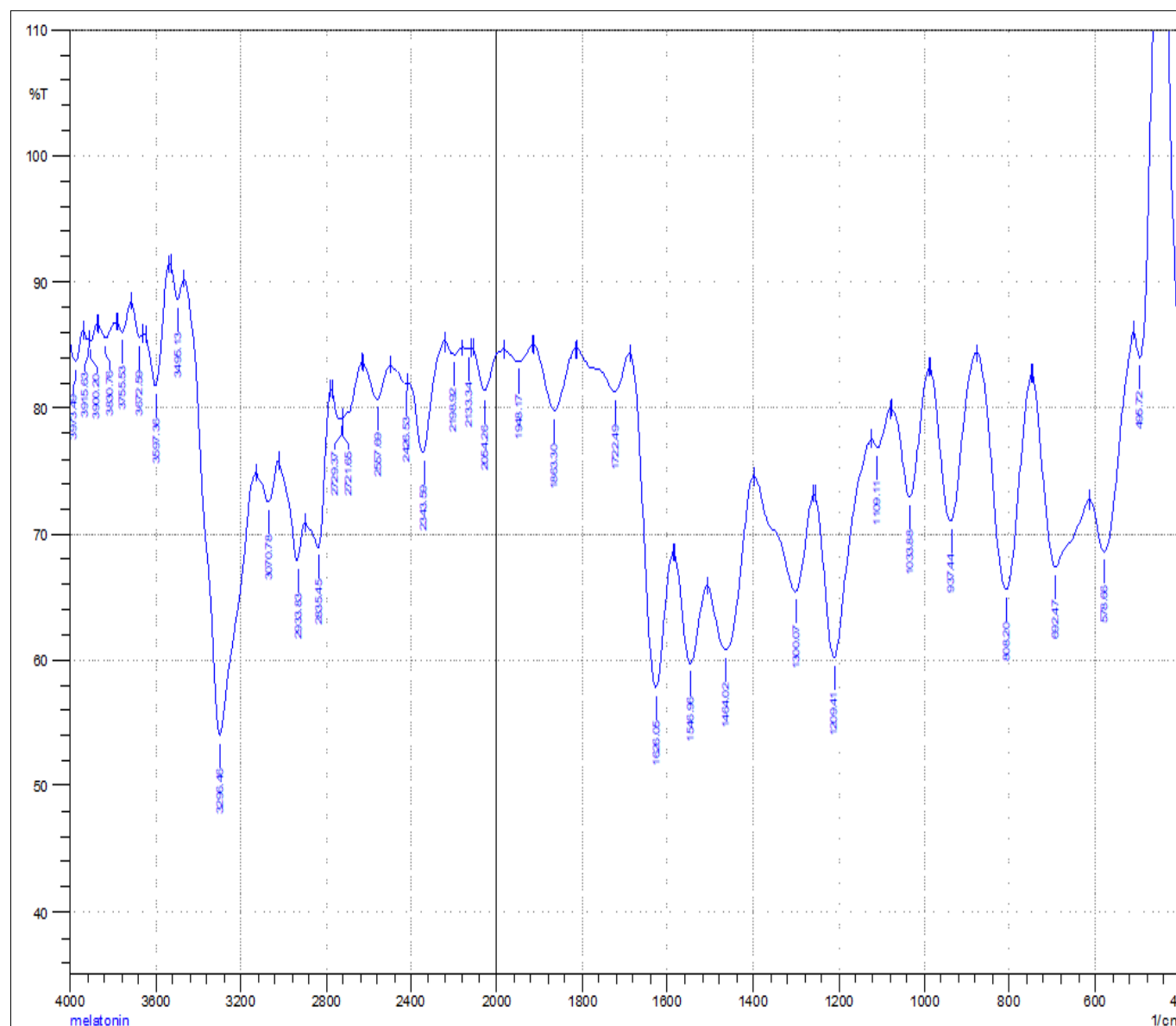


Fig 2: FTIR analysis of melatonin

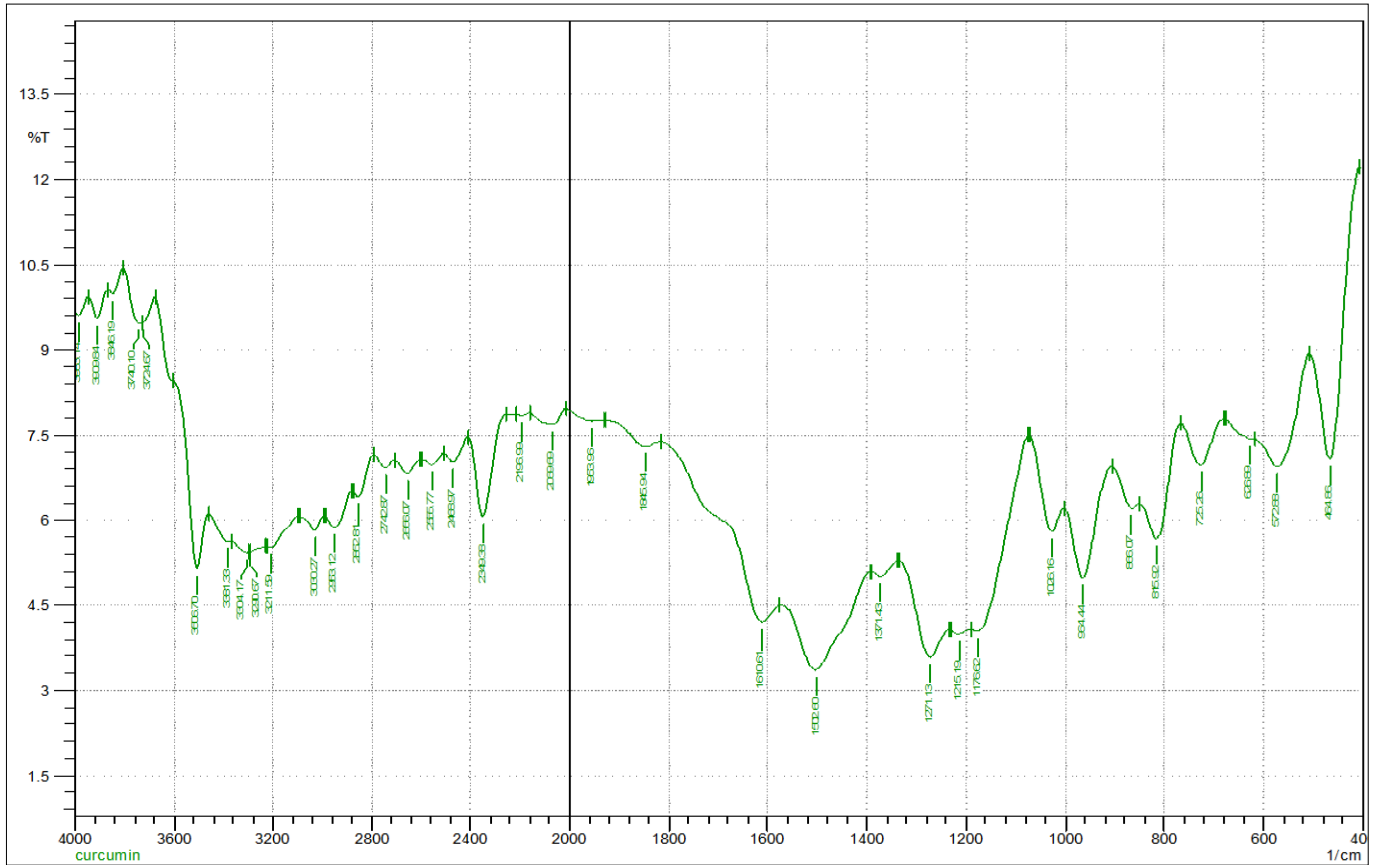


Fig 3: FTIR spectrum of curcumin

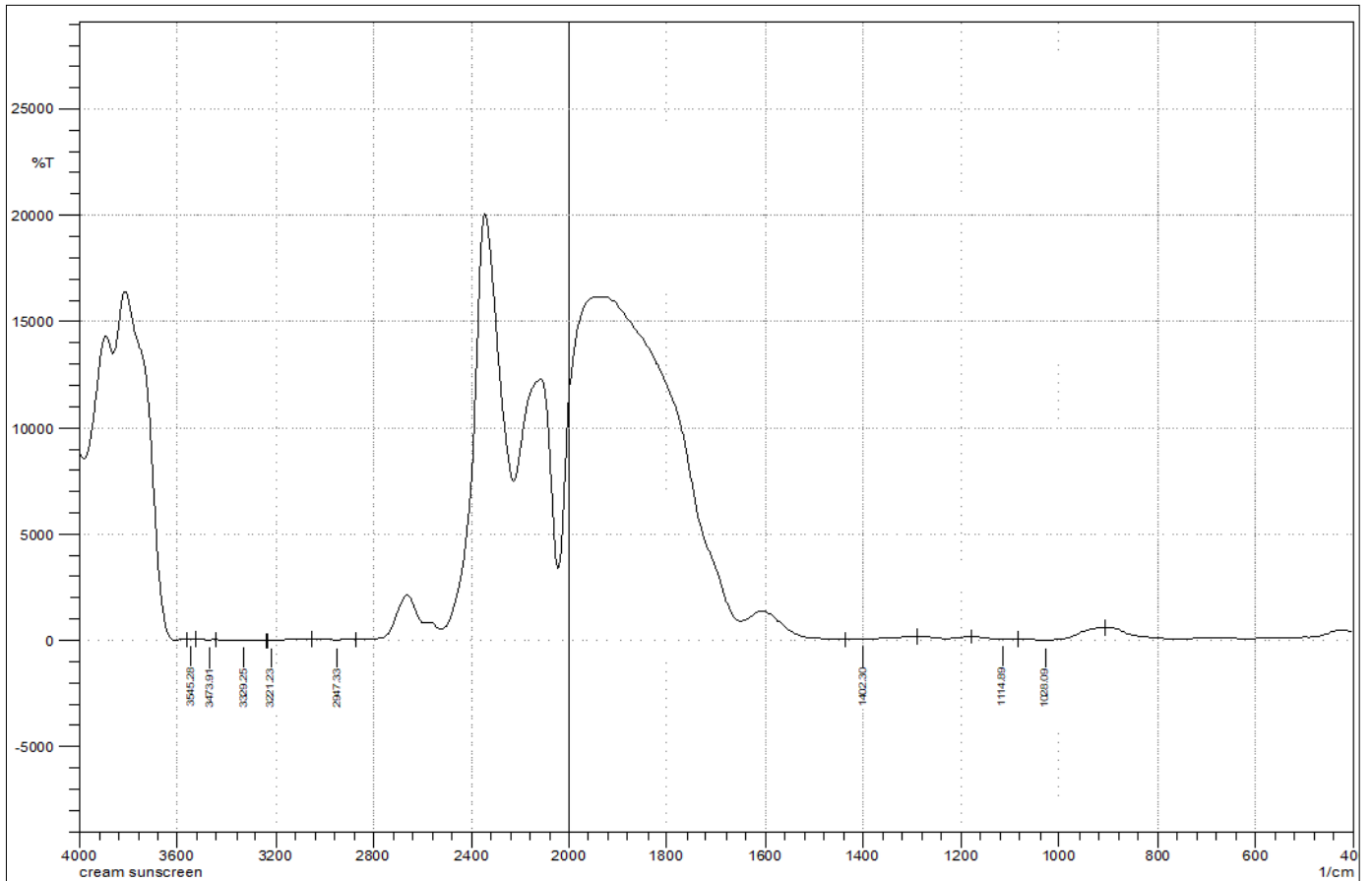


Fig 4: FTIR spectrum of mixture melatonin and curcumin

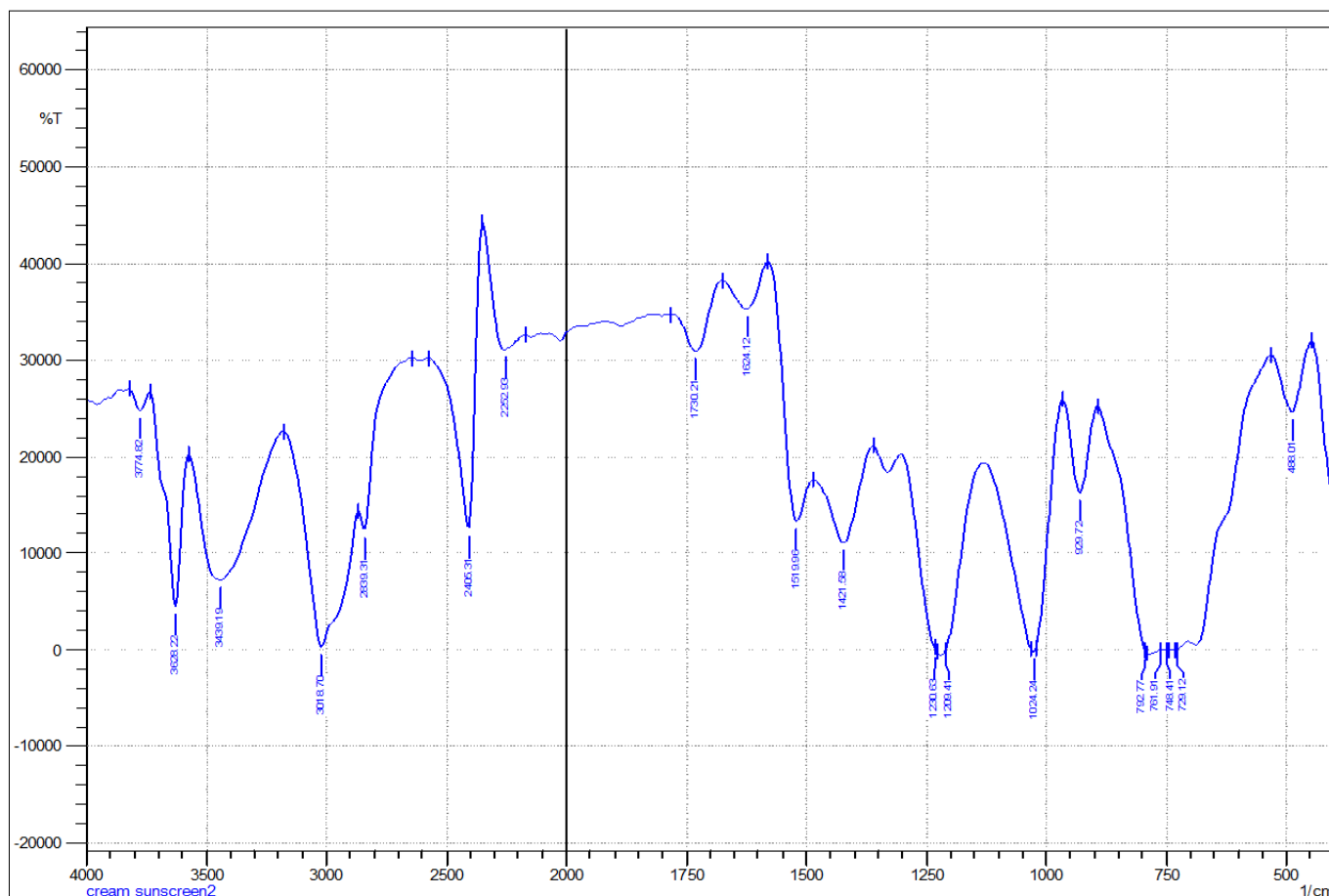


Fig 5: FTIR spectrum of sunscreen formulation

From FTIR of study of melatonin, curcumin, physical mixture of melatonin and curcumin, and sunscreen cream formulation was done and it is observed that same functional groups were present in both drug and formulation. So it was confirmed that curcumin and melatonin were incorporated in the cream formulation. Also, no additional peaks were observed in the FTIR spectrum of the physical mixture of drugs. Thus confirming no interaction between them i.e. the mixture was compatible.

Discussion

Sunscreen agents have been used to protect the human skin against the effects of UV radiations and also have a protective effect against the changes occurring due to exposure to UV radiations such as photoaging and skin cancer. Regular use of high SPF sunscreen can prevent photo carcinogenesis. Though the skin has an antioxidant defense mechanism to treat the UV-induced oxidative stress however when there is excessive exposure to UV radiations the body is unable to control the formation of free radicals. The use of antioxidants by oral and topical routes can help fight against the photo-oxidation.

The sunscreen gel formulation was developed by using curcumin and melatonin and further evaluated by determining the pH, viscosity, spreadability, % moisture loss. All the gel formulations have shown good consistency with no degradation of herbal actives while formulating it.

Conclusion

Melatonin, curcumin, and zinc oxide containing formulation can be a new sunscreen therapy with anti-aging property and

this formulation posses synergistic effect like anti-cancer activity and anti-aging therapy and produce protection from UV A & B induced skin damage. Finally it is concluded from present findings that melatonin and curcumin containing formulation may contribute as a cosmetic ingredient for protection from UVB induced skin damage.

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