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APPLICATION OF 2^2 FACTOIRAL DESIGN IN THE FORMULATION AND EVALUATION OF SUNSCREEN CONTAINING NON NANO ZINC OXIDE AND POLYPODIUMLEUCOTOMOS

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ABSTRACT

Sun exposure affects the skin in many ways. In the short term it can leads to reddening and tanning. In the long term effect of UV radiation which are irreversible and often cause sunburn, wrinkling, and skin cancer. For the reason, sun protection has become, a very important issue today. UV filter ingredients that can effectively protect the skin from UV radiation. To increases the Sun Protection Factor, value the incorporation of chemicaland physical UV filters this turns to toxicity. In this research our aim is to formulate and evaluate the sunscreen containing polypodium leucotomos and non-nano zinc oxide. Before the formulation, preformulation study of polypodium leucotomos and non-nano zinc oxide describes here includes macroscopic observation, particle size, hydroscopicity, flow property. Determine the main effect, interaction effect and surface plot. The formulated sunscreen was then evaluated for its colour, odour, PH, spreadability, invitro SPF etc.

KEYWORDS: Sun protection factor, non nano zinc oxide, polypodium leucotomos, UV filter, invitro SPF.

INTRODUCTION

The most source of UV ray comes from the sunlight .They are various kinds of UV beams arrive at the ground in various amount. Around 95% of the UV beams from thesun that arrive at the ground are UVA beams, with the leftover 5% being UVB beams.[1] UV likewise

helps human wellbeing by interceding regular amalgamation of vitamin d and endorphins in the skin; subsequently UV has intricate and blended impacts on humanwellbeing. In any case, inordinate exposure to UV conveys significant wellbeing chances, including sunburn, pigmentary changes, wrinkling and cancer.[2] UV rays react with compound in the skin called melanin. This is the principal protection against. The sun. Melanin prevents the perilous UV beams that can do genuine skin harm. However, melanin can't prevent all the UV beams, and certain individuals don't have a lot of melanin in their skin this prompts skin cancer, sunburn, photoageing.

The FDA defines sunscreen active agent as "an ingredient listed in sec.352.10 that absorbs, reflects, or scatter radiation in the ultraviolet range wavelength of 290 to 400 nanometer".[3] Sunscreen secure the skin they assume a significant part in hindering ultraviolet(UV) Radiation from being consumed by the skin. The standard utilization of uv filters might assist with decreasing the shot at the harmful impacts of UV. Hence sunscreen play an important role in cosmetic formulation with that it is necessary the sunscreen must be efficient[4] The efficacy of a sunscreen is usually expressed by the sun protection factor (SPF), which is defined as the UV energy required for producing as minimal erythema dose(MED) on protected skin a divided by the UV energy required producing a MED on unprotected skin.[5] The sunscreen is efficient in higher SPF. They are such countless definitions distinctive to builds the SPF esteems.

In that antioxidant assumes a model part by aiding the stability the sunscreen while additionally supporting its adequacy basically antioxidant super charges your sunscreen by guaranteeing greatest protection. Natural substances have been as of late considered as potential sunscreen assets as a result of their assimilation in the UV locale and their cancer prevention agent activity. Antioxidant from normal sources might give additional opportunities to the treatment and counteraction of UV intervened diseases.[6] Polypodium leucotomos tropical plant from local and South America. Equivalent word for the plant name Phlebodurn aureum. Both its slim, green leaves and underground stems (rhizomer) have been utilized for medicial purposes for centuries.[7] The greenery contains the compound P-Comaric acid, ferullic acid, caffeic acid, vanillic acid and chlorogenic acid all of which have incredible antioxdant properties. The family polypodiaceae degree research has shown that concentrates of Polypodium leucotomos have powerful cancer prevention agent, photoprotectant, antimutagenic and immunoreguulatory properties. The concentrate of

Polypodium leucotomos applied topically or taken orally has been demonstrated to be successful in serveral dermatores in research direct in the course of the most recent forty years.[8] A preformulation study for cosmetics is a study of physicochemical characteristic associated with the substance used in the formulation of cosmetic preparation. Preformulation study is necessary to produce quality cosmetic produce.

The Goals of this research are to formulate and evaluation of sunscreen containing the polypodium leucotomos and non nano zinc oxide. Selection of best formulation by 2 x 2 factorial design. Determine main effect, interaction effect and surface plot. We tested the creams physiochemical properties. The preformulation study described here includes macroscopic observation, particle size, hydroscopicity, flow property. Now a day's combination of chemical and physical sunscreen turn into toxicity. The scope of this research by the way combination of non nao zinc oxide and polypodium Leucotomos benefits are boost SPF value by using natural plant, it is possible to produce a high quantity at low price, Cosmetics containing natural herb all. Components are less certainly to the skin, especially skin that is hypoallergic point.

MATERIALS AND METHODS

Materials

Table 1: List of material used in experiment.

S.No	Ingredients	Trader name
1.	Non Nano zinc oxide	Sky Organics from amazon
2.	Polypodium Leucotomos Calaguala Extract	Herbodiet
3.	Sweet almond oil	Olvedic from amazon
4.	Cocoa butter	Minimal Confections from amazon
5.	Shea butter	Paiya Organics from amazon
6.	NIMIR industries	Stearic acid
7.	Glycerin	Hindustan drugs
8.	Sodium Benzoate	Qualigens
9.	Sorbitan Monostearate(SPAN60)	SRL
10.	Deionised water	Labpure
11.	Tween 80	Purenso

Equipment

Table 2: List of equipment used for experiment.

S.NO	EQUIPMENT	MANUFACTURE
1.	PH meter	Vision plus
2.	Centrifuge apparatus	Laboratory centrifuge
3.	UV spectrophotometer	Labman
4.	Homogenizer	Remi motor
5.	Brook field viscometer	DV PRO -II
6.	Weight balance machine	Health genie
7.	Incubator	B.o.d
8.	Laminar air flow	Fischer instrument

METHODS

I. DETERMINATION OF ABSORPTION MAXIMA (λMAX)

- The stock solution of 1000 µg /ml was prepared by dissolving approximately 100 mg of pure non nano zinc oxide in 100 ml of 0.1 N HCL. From the stock solution, 1 ml was taken and further diluted to 100 ml with 0.1 N HCL. The prepared solution was then scanned in a wave length range of 200-400nm, to find the maximum absorbance.
- The stock solution of 1000 µg /ml was prepared by dissolving approximately 100 mg of polypodium leucotomos in 100 ml of distilled water. From the stock solution, 1 ml was taken and further diluted to 100 ml with distilled water. The prepared solution was then scanned in a wave length range of 200-400nm, to find the maximum absorbance.[9]

II. DETERMINATION OF ABSORBANCE PROPERTY OF UV FILTERS

- Absorbance in the UV and visible spectra regions (200-400nm) of different concentration of polypodium leucotomos diluted in distilled water at different concentration (6.25, 12.5, 25, and 50 micrograms per ml).
- Absorbance in the UV and visible spectra regions (290-320nm) of different concentration of non-nano zinc oxide diluted in 0.1 N HCL at different concentration (5, 25, and 50 microgram per ml).[10]

III. PREFORMULATION CHARACTER DETERMINATION

1. PHYSICALCONSIDERATION

➢ **MACROSCOPIC OBSERVATION**

- **COLOUR**

To determine the colour of the compound, 0.2 g of the material was placed against a white background in diffuse day light, viewed by eye and its colour described accordingly.

- **ODOUR**

To determine the odour of the compound, 0.4 g of the material was placed in a 5 cm diameter watch glass, left for 15 minutes and there after the air above the sample was inhaled slowly and repeatedly. The strength of the odour was determined by classifying it as either non-existent, weak, distinct or strong and the odour sensation described as either aromatic, fruity, musky, mouldy or rancid.[11,12]

- **PARTICLE SIZE**

- In manual optical microscopy, the dispersed particle are viewed by transmission, and the area of the magnified image are compared with the area of know sizes inscribed on a graticule. The relative number of particles are determined in each of a series of size classes, these represent the size distribution by number from which it is possible to calculate the distribution. For reproducible result, at least 300-500 particles must be observed.[12,13]

- **SOLUBILITY**

Finally the solubility of the material was described using the common descriptive phrases of solubility and the corresponding quantitative solubility ranges given in the BP 2013 and expressed in terms of "parts", which represented the number of millilitres (ml) of thesolvent, in which 1g of solid was soluble.[12,13]

Table: 3 common descriptive phrase of solubility and the corresponding quantitative solubility ranges.

Descriptive phrase	Approximate quantities of solvent by volume for 1 part of solute by weight
Very soluble	Less than 1 part
Freely soluble	From 1to 10 parts Soluble
soluble	From 10 to 30 parts
Sparingly soluble	From 30 to 100 parts
Slightly soluble	From 100 to 1000 parts
Very slightly soluble	From 1000 to 10000 parts
Practically insoluble	More than 100000 parts

- **HYGROSCOPICITY**

First weight the empty china dish and weigh the china dish with the sample. Place sample in electric oven maintain temperature for 24 hours, again weigh the dry sample.[12,13]

$$\frac{Weight\ of\ sample\ (\ in\ atmosphere) -\ oven\ dry\ weight}{Oven\ dry\ weight}$$

Table: 4 classification of hygroscopicity.

Classification	% water uptake at 25 c % rh[w/w]
Non hydroscopic	0-0.12
Slightly hydroscopic	0.2-2
Moderately hydroscopic	2.0-15.0
Very hydroscopic	>15.0

- **FLOW PROPERTY**

Angle of repose

The angle of repose of blend was determined by funnel method. Accurately weighed powder blend was taken in funnel just touches the apex of the powder blend. The powder blend was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose is calculated using the following equation.[12]

$$\theta = tan\text{-}1h/r$$

Where, h and r are height and radius of the powder cone.

Table 5: Flow property depending upon angle of repose.

Angle of repose	Type of flow
25-30	Excellent
30-35	Good
35-40	Fair
40-45	Poor
45-50	Very poor

IV. CHARACTERISATION OF CREAM

1. PERCENTAGE YIELD

Percentage practical yield was calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production.[24] Cream prepared were collected and weighed to determine practical yield from the followingequation:

$$\text{Yield} = \frac{practical\ yield\ X\ 100}{\text{theorical yield}}$$

V. SELECTION OF BEST FORMULATION OF SUNSCREEN BY 2X2 FACTORIAL DESIGN AND DETERMINE THE MAIN EFFECT ANDINTERACTION EFFECT

2^2 factorial design is a experimental design that allow to acknowledge the effect of two independent variables on a single dependent variable

- **Independent variable #1:** non nano zinc oxide
  - **Levels:** Low, High

- **Independent variable #2:** polypodium leucotomos
  - **Levels:** Low, High

And there is one dependent variable: sun protection factor

A 2×2 factorial design allows you to analyze the following effects:

Main Effects: It is the reaction of one independent variable on the dependent variable. For example, in our previous scenario we could analyze the following main effects:

- Main effect of sun protection factor.
  - We can find the mean SPF that received low non nano zinc oxide
  - We can find the mean SPF that received high non nano zinc oxide
- Main effect of sun protection factor.
  - We can find the mean SPF that received low polypodium leucotomos .
  - We can find the mean SPF that received high polypodium leucotomos

Interaction Effects: the reaction of one independent variable has on the dependent variable on the level of the other independent variable

For example, in our previous scenario we could analyze the following interaction effects:

- Does the effect of non nano zinc oxide on SPF depend on polypodium leucotomos ?
- Does the effect of polypodium leucotomos on SPF depend on the non nano zinc oxide?[13,14]

Surface plot: Surface plot displays the three-dimensional relationship in two dimensions, with the variables on the x- and y-axes, and the response variable (z)represented by a smooth surface.

VI. PREPARATION OF SUNSCREEN CREAM

Phase A: polypodium leucotomos (5%W/W), glycerine (2%W/W) were dissolved indeionsed water (12%W/W) and heated up to 70 c

Phase B: almond oil (5%W/W), shea butter (7%W/W), coco butter (10%W/W), sorbitan monostearate (1.25%W/W), tween 80 (0.75%W/W),stearic acid (1%W/W), non nano zinc oxide (5%W/W)was added and heated upto 70 c

Mixing phase: oil phase was added to water phase to 70 c with continuous stirrind for 20-25 min and then its was homogenized (8000 rpm) till a homogenous.them sodium benzoate (1%W/W)and lavender flavor added.[10]

Table: 6 formulation of sunscreen.

S.no	Ingredients	Quantity in grams F_1	Quantity in grams F_2	Quantity in grams F3	Quantity in grams F_4	Use
1.	Almond oil	5%	5%	5%	5%	Emollient
2.	Shea butter	7%	7%	7%	7%	Emollient
3.	Coco butter	10%	10%	10%	10%	Emollient
4.	Sorbitan monostearate	1.25%	1.25%	1.25%	1.25%	Emulsifier
5.	Tween 80	0.75%	0.75%	0.75%	0.75%	Emulsifier
6.	Stearic acid	1%	1%	1%	1%	NIMIR industries
7.	Non nano zinc oxide	3%	5%	3%	5%	UV filter
8.	Polypodium leucotomos	3%	5%	5%	3%	UV filter
9.	Distilled water	12%	12%	12%	12%	Vehicle
10.	Sodium benzoate	1%	1%	1%	1%	Preservative
11.	Lavender oil	0.5%	0.5%	0.5%	0.5%	Flavor agent

VII. EVALUATION OF SUNSCREEN

1. COLOUR

Appearance and texture of the creams was optically observed.[16,17]

2. ISCOSITY

Viscosity of cream was measured by the Brookfield viscometer at 12 rpm using spindle.[17,18]

3. PH

1g of creams dispersed in 9 ml of distilled water to determine the pH at 25 c using the ph meter.[18,19]

4. STABILITY

The sample characteristic were evaluated after preparation or after storage in freeze andthaw condition 24 hours for each storage temperature of 6 cycle.[20]

5. CENTRIFUGATION TEST

Centrifugal test were performed for emulsion directly after preparation. Those test were repeated after 6 cycle. They were performed at 5000 rpm and 25 c for 10 minutes by place10 g of each sample in centrifugal test.[18,19]

6. WATER RESISTANCE TEST

Apply cream on the dorsal surface of the hand and sprinkle some water.[20]

7. TESTOF SPREADABILITY

The parallel plate method is most widely used method for determining the spreadability of semisolid preparation. A modified laboratory apparatus was used to evaluate spreadability. The setup consist of two glass slides placed on a tripod stand on which excess of cream (3g) was applied in between two glass slides. The upper slides is movable and the lower slide was firmlyfixed to the stand. 100g weight placed on them for 5 minutes to compress the cream to uniform thickness and the excess of cream was scrapped off from the edges. then 50 g weight was added to one side of the slide and the slide is pulled till it cover a distance of 10 cm. the time in second required to separate two glass slides by 10 cm taken as measure of spreadability. The spreadability was calculated by using the formula

$$S= M \times I/l$$

Where s= spreadability, m= weight tied to upper glass slide, i= length of glass slide, t=time taken to separate them.[16]

8. MICROBIAL LIMIT TEST

Take 10ml Or 10gm sample add in 90ml buffered sodium chloride peptone solution it is solution.

TOTAL AEROBIC MICROBIAL COUNT

Add 1ml sample of solution A in two sterile Petri plate and add about 15_20 ml soyabean casein digest agar cool at 45 degree centigrade in both Petridish and allow the medium to solidify. Along with sample preparation make two sterile petriplate one plate for negative control (only media) and one plate for positive control (media with Bacillussubtillis) Incubate all the plates at 30_35 degree centigrade for 3_5 days in inverted.

TOTAL YEAST AND MOLD COUNT

Add 1ml sample of solution A in two sterile petriplate and add about 15_20ml sabouranol chloramphenical agar cool at 45 degree centigrade in both Petridish and allow the medium to solidify. Along with sample preparation make two petriplate one plate for negative control (only media) and one plate for positive control (media with C. Albicans) Incubate all the plates at 20_25 degree centrigeate for 5_7 days in an invertedposition.[21,22]

9. EMULSION TEST

1 gram of cream placed in beaker and water is poured .the cream and water dispersed with glass rod .we added a drop of food colour.[23].

10. IRRITANCY TEST

The cream was applied on left hand dorsal side surface of 1 sq.cm and observed in equal interval up to 24 hours for irritancy, redness, and edema.[23]

11. DIFFUSION STUDY

The diffusion study was carried out for the prepared formulation bt preparing agar nutrient medium of any concentration. It was poured into petridish a hole bored at the centre and cream was placed in it. The time taken for the cream to get diffused was note.[23]

12. DETERMINATION OF INVITRO SPF OF SUNSCREEN CREAM

This method which involve the measurement of absorption or the transmission of UV radiation through sunscreen formulation. In which the absorption characteristics of the sunscreens agents are determined on spectrophotometric analysis of dilute solutions of sunscreen Mansur et al. (1986), developed a very simple mathematical expression which substitutes the in vitro method which is proposed by Sayre et al., (1979), Sun protection factor calculated by using the following equation and utilizing UV spectrophotometry.

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

Where: EE (l) – erythemal effect spectrum; I (l) – solar intensity spectrum; Abs (l) - absorbance of sunscreen product CF – correction factor (= 10). The values of EE x I are constants. This were determined by Sayre et al. (1979), and are showed below in Table.

Table 7: Normalized product function which are used in the calculation of SPF (Sayre et al., 1979).[16]

WAVELENGTH (NM)	EE X I (NORMALIZED)
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.018
Total	1

RESULT

1. ABSORPTION MAXIMA

Figure 1: Image of volumetric flask containing 10 microgram per ml of non nano zinc oxide and polypodium leucotomos.

The maximum absorbance (λmax) of the non nano zinc oxide was estimated by scanningthe non nano zinc oxide solution (10µ/ml) between 200nm-400 nm regions in the UV spectrophotometer. The obtained spectrum that the absorption maximum (λmax) at 256nm in 0.1N Hydrochloric Acid.

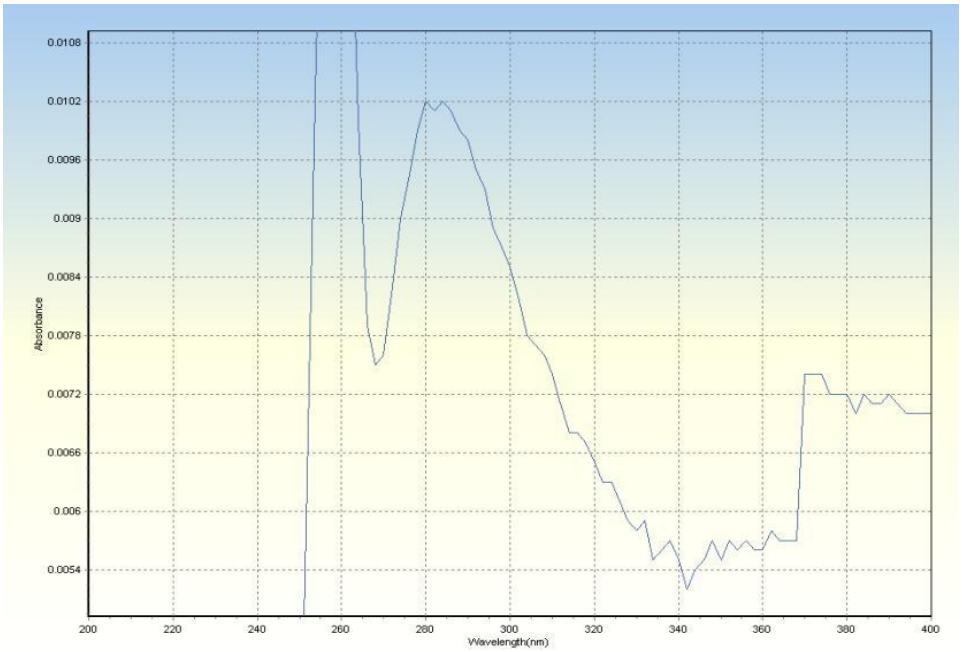

Figure 2: Absorption maxima of non nano zinc oxide in 0.1N Hydrochloric Acid at 256 nm.

The maximum absorbance (λmax) of the polypodium leucotomos was estimated by scanning the polypodium leucotomos solution (10µ/ml) between 200nm-400 nm regions in the UV

spectrophotometer. The obtained spectrum that the absorption maximum (λmax) at 276 and 340 nm in deionsed water.

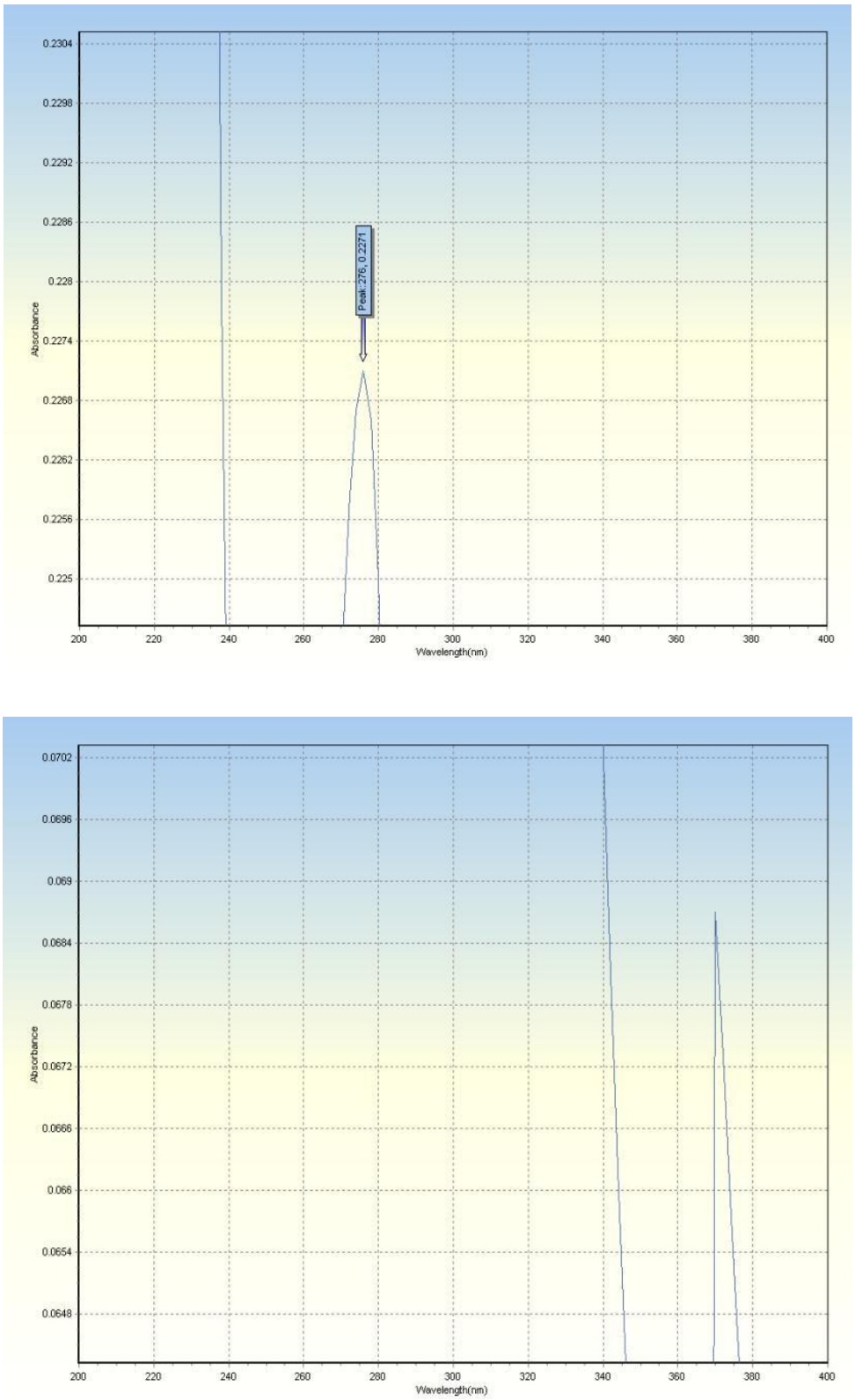

Figure 3: Absorption maxima of polypodium leucotomos in water at 276 nm and 340 nm.

2. ABSORBANCE PROPERTY OF UV FILTER

Figure: 4 image of volumetric flask containing different concentration of non nano zinc oxide.

Absorbance property of non nano zinc oxide: The different concentration of non nano zinc oxide diluted in 0.1 N hydrochloric acid .increased absorbance in the UV spectrumfrom 290-320 nm was observed.

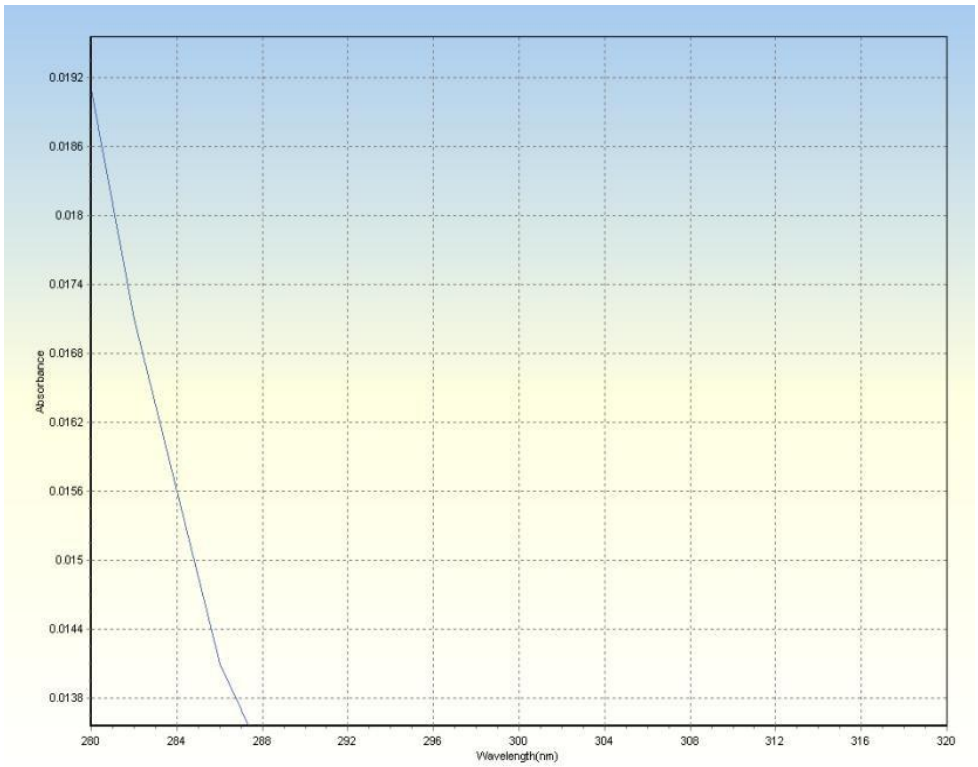

Figure 5: Absorption of nano zinx oxide in 0.1 N hydrochloric acid at 290-320nm andits concentration 5 microgram per ml.

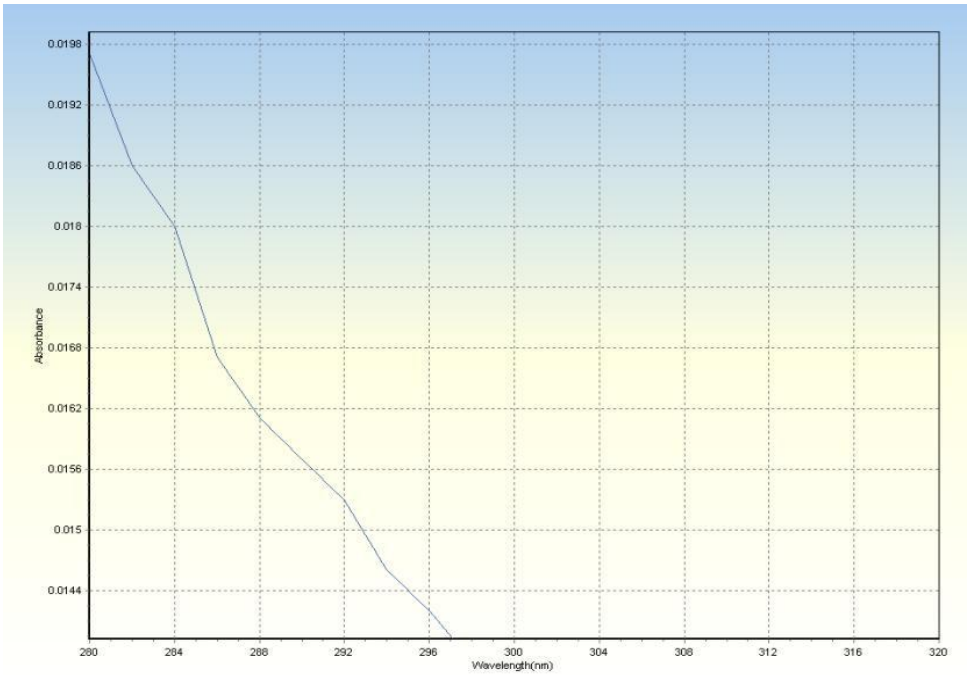

Figure 6: Absorption ofnon nano zinx oxide in 0.1 N hydrochloric acid at 290-320nm and its concentration 10 microgram per ml.

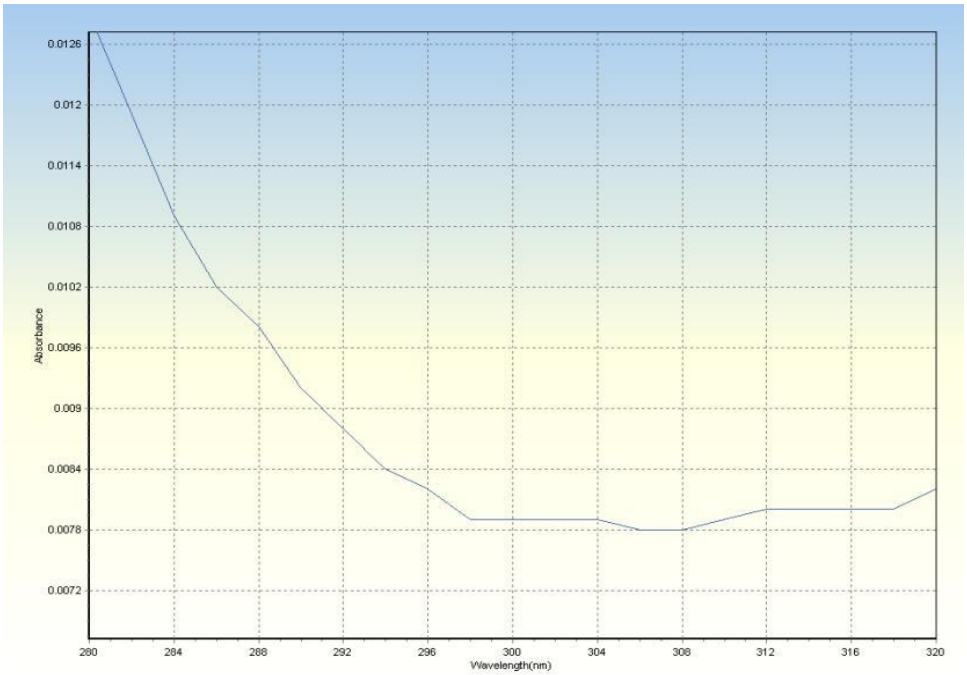

Figure 7: Absorption of non nano zinx oxide in 0.1 N hydrochloric acid at 290-320nm and its concentration 25 microgram per ml.

Absorbance property of polypodium leucotomos: The different concentration of polypodium leucotomos diluted in deionsed water .increased absorbance in the UVspectrum from 200-400 nm was observed.

Figure 8: Image of volumetric flask containing different concentration of polypodium leucotomos.

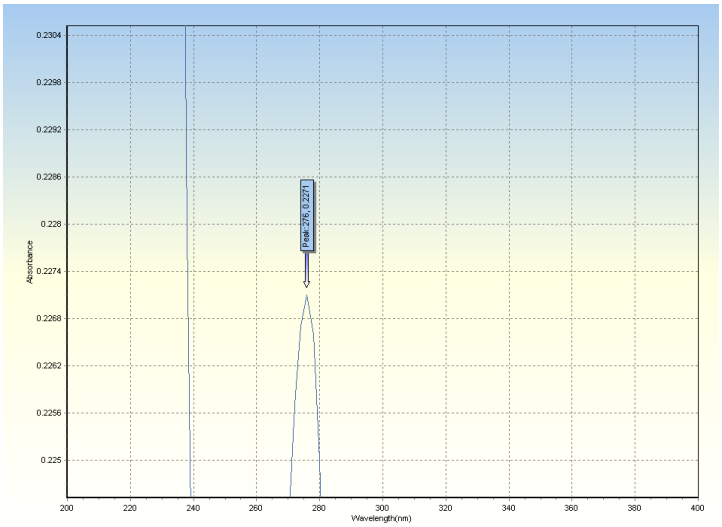

Figure 9: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 6 microgram per ml.

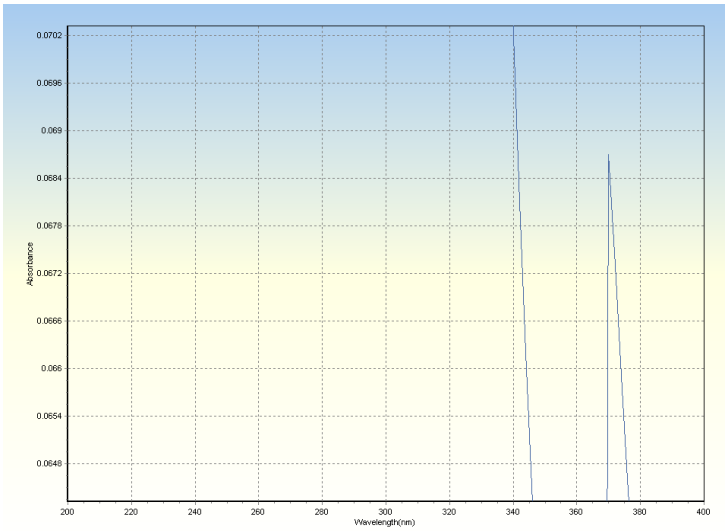

Figure 10: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 6 microgram per ml.

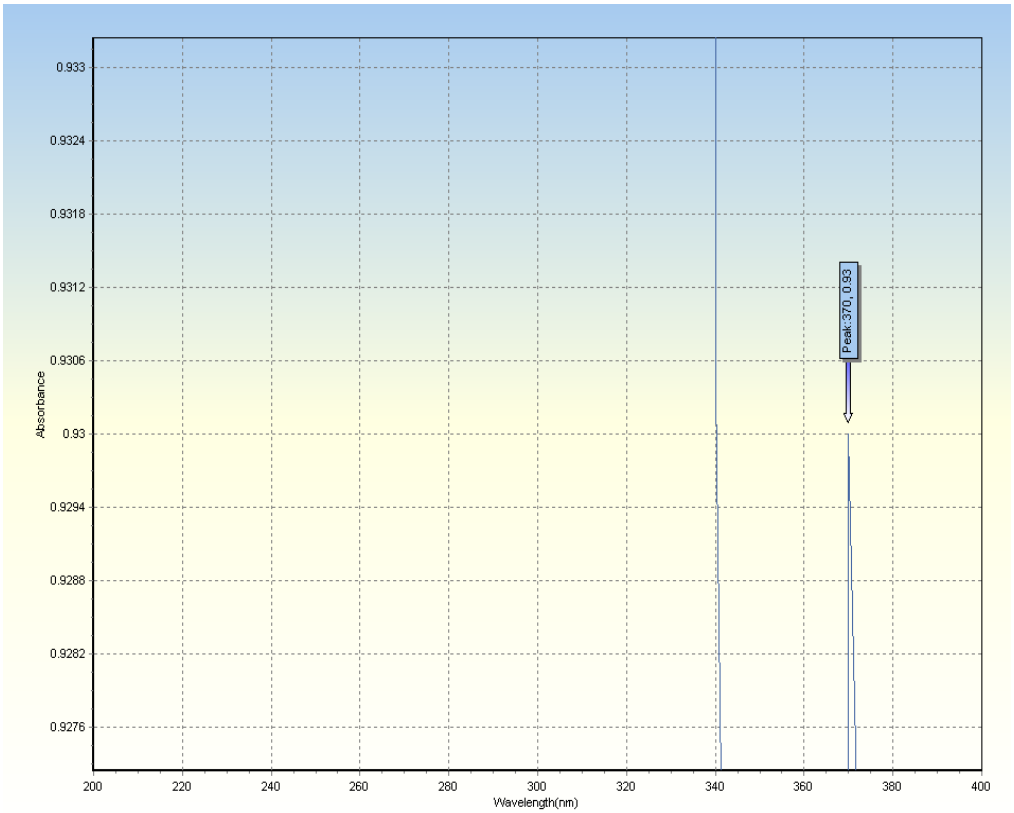

Figure 11: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 12.5 microgram per ml.

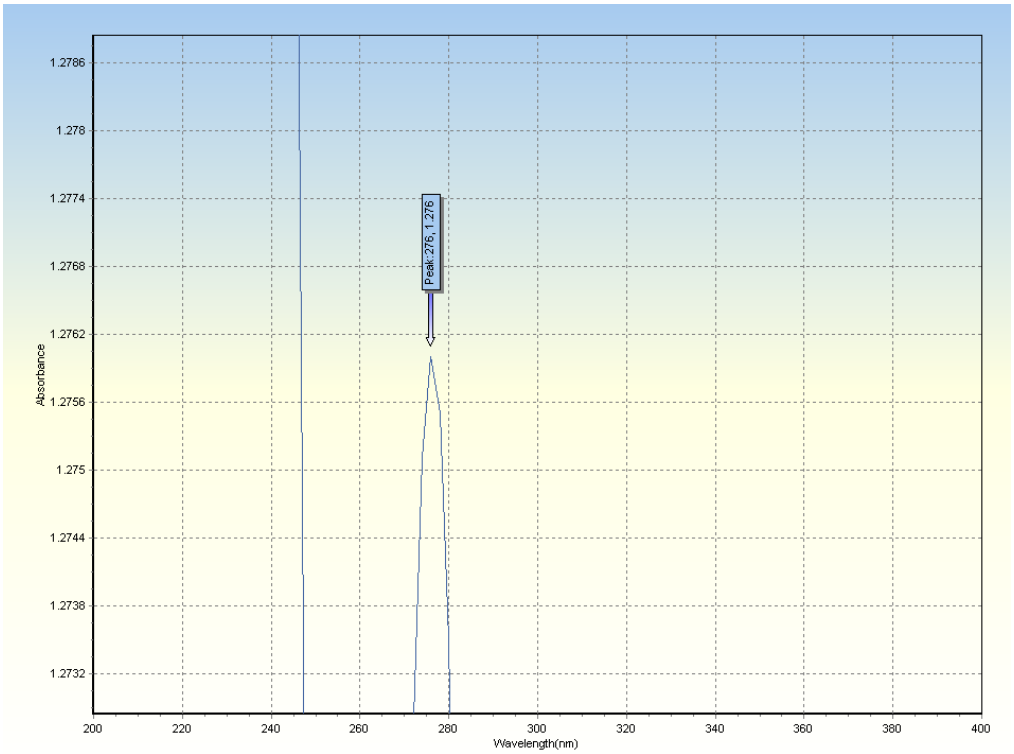

Figure 12: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 12.5 microgram per ml.

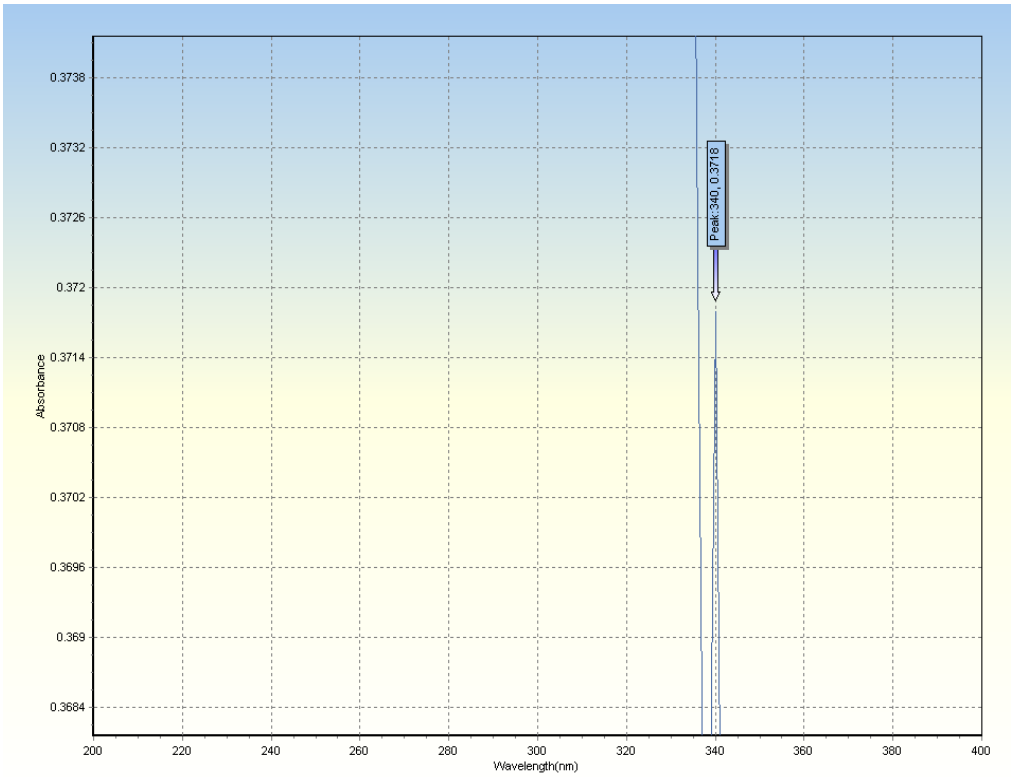

Figure 13: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 25 microgram per ml.

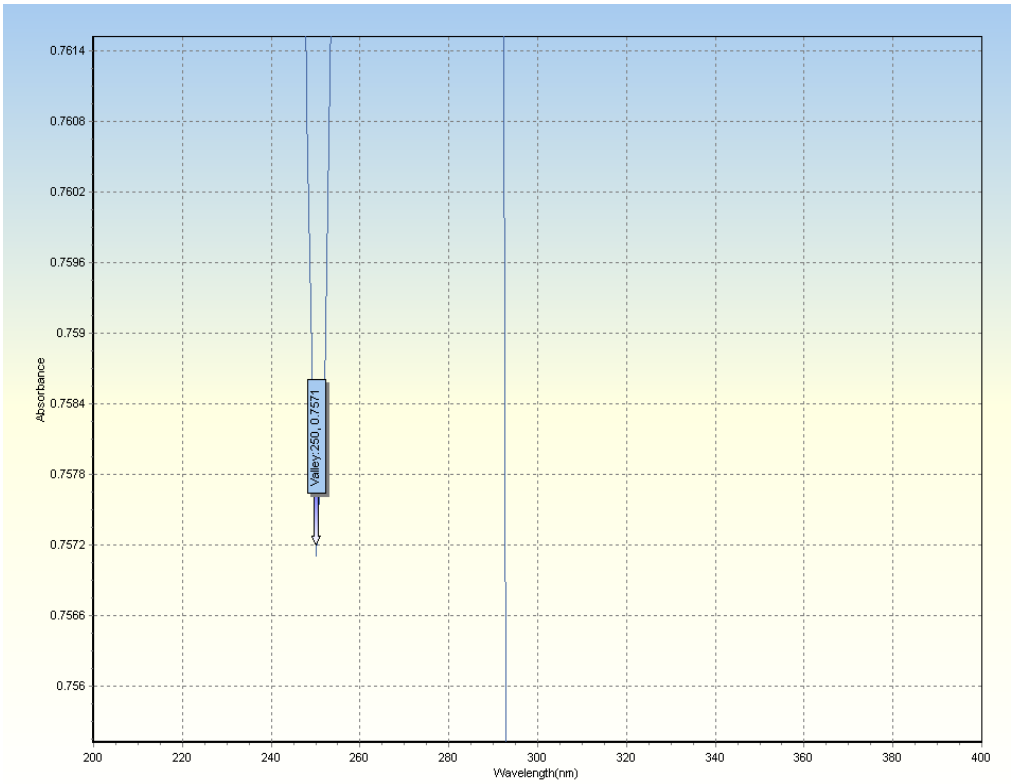

Figure 14: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 25 microgram per ml.

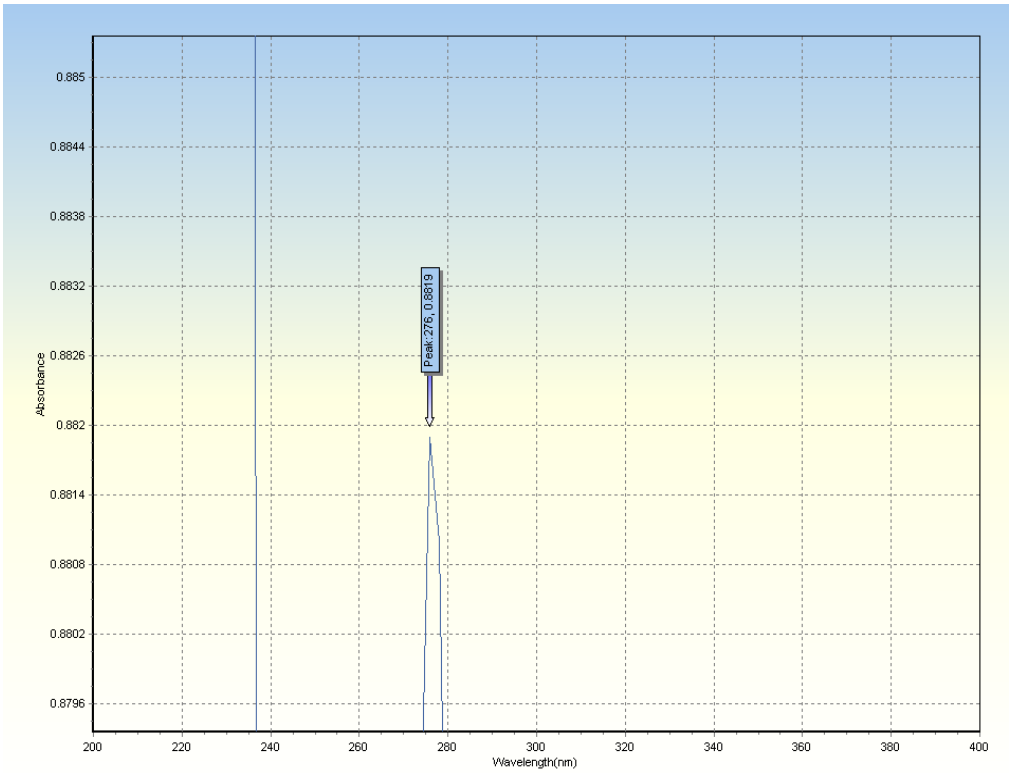

Figure 15: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 25 microgram per ml.

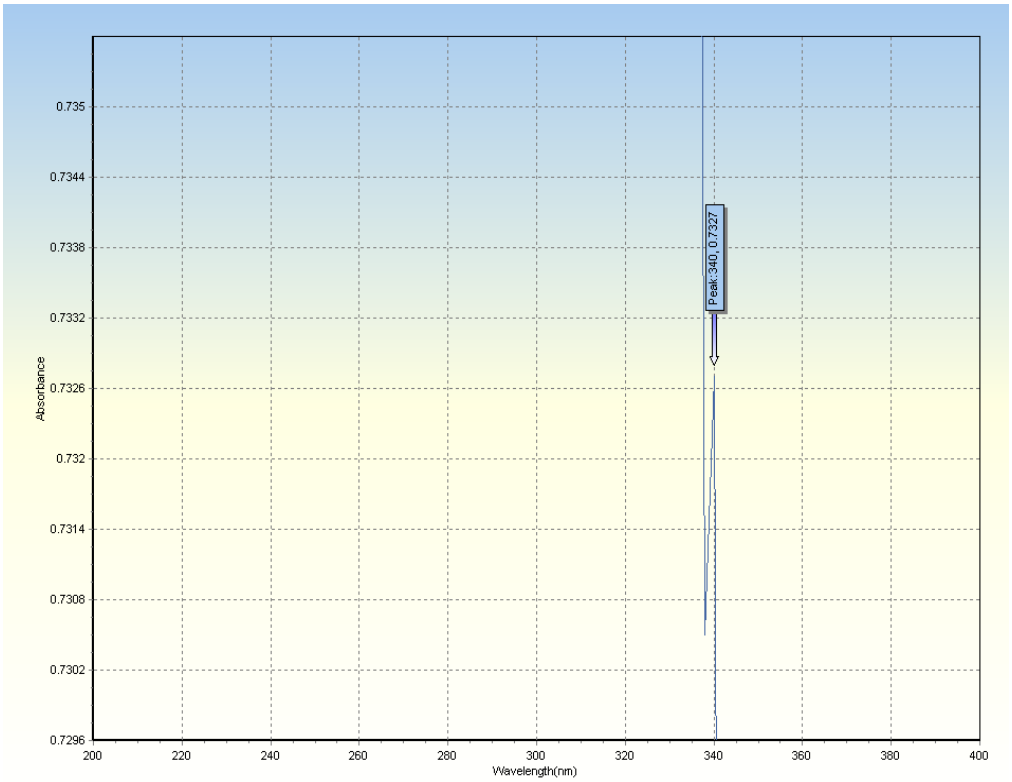

Figure 16: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 50 microgram per ml.

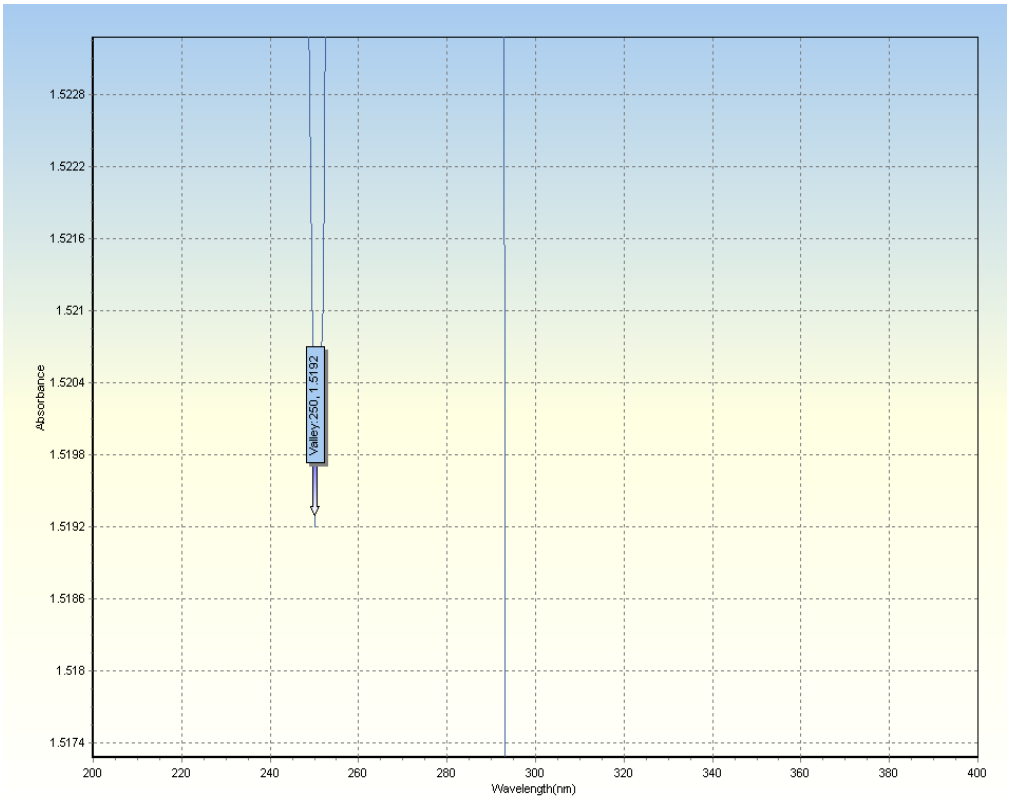

Figure 17: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 50 microgram per ml.

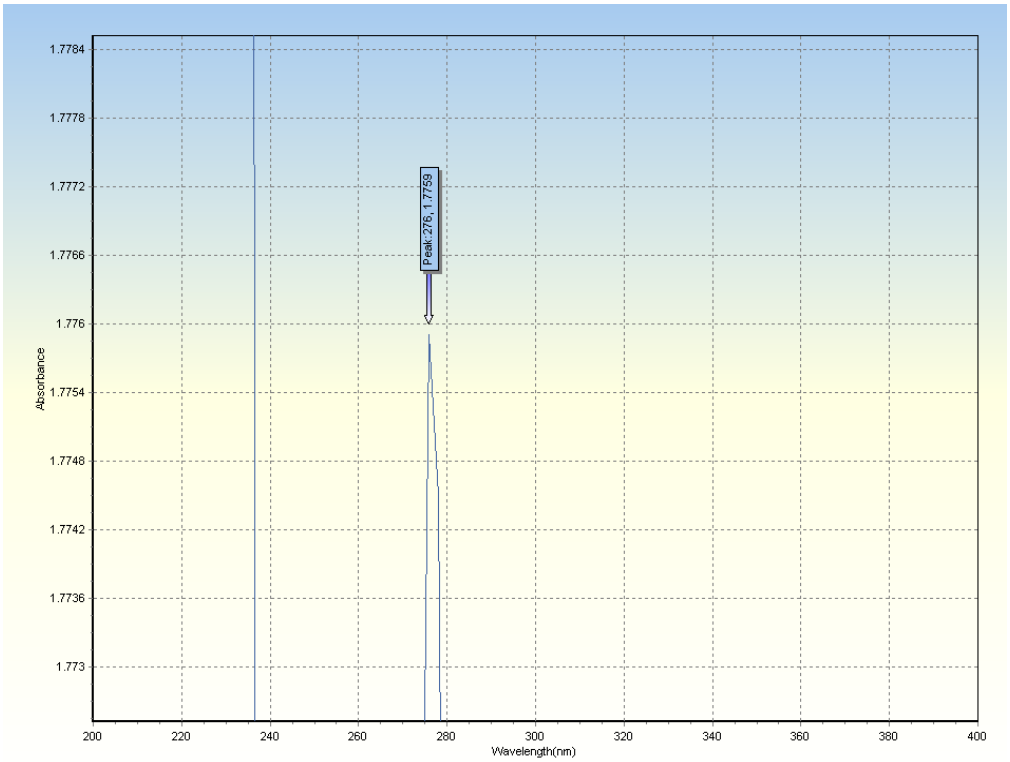

Figure 18: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 50 microgram per ml.

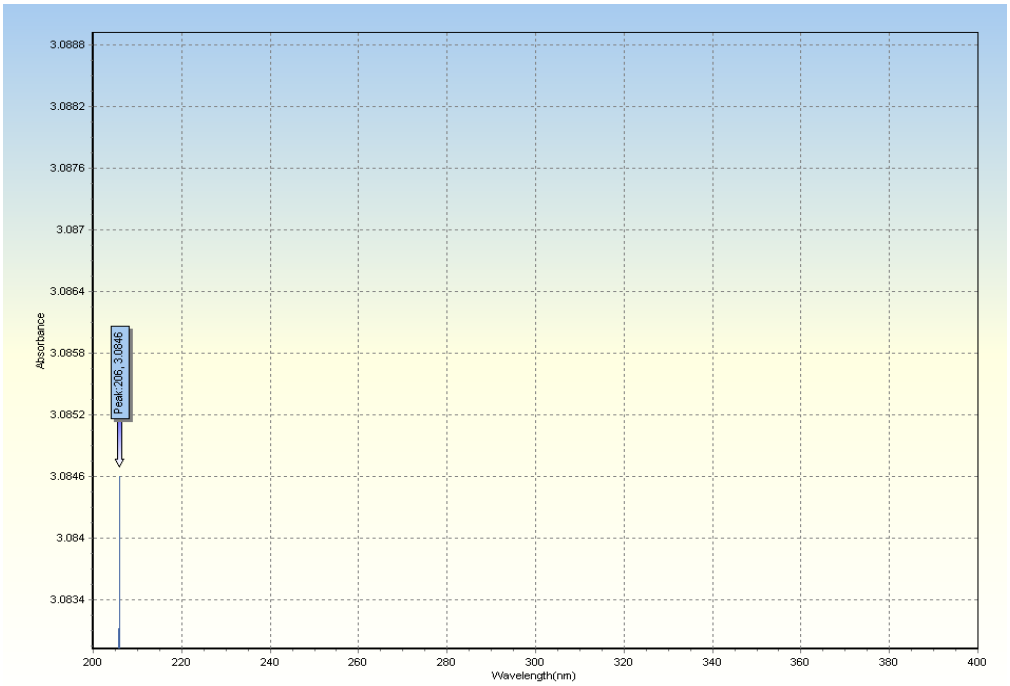

Figure 19: Absorption of polypodium leucotomos in water at 200-400 nm and its concentration 50 microgram per ml.

3. PREFORMULATION CHARACTER

1. PHYSICALCONSIDERATION

➢ MACROSCOPIC OBSERVATION

Table 8: Macroscopic observations.

UV filter	Colour	Odour
Non nano zinc oxide	White	-
Polypodium leucotomos	Brown	Musky,aromatic

➢ PARTICLE SIZE

- **Particle size of non nano zinc oxide**

Table 9: Observation of Particle Size of Non Nano Zinc Oxide.

Size range (micrometer)	Mean of sizerange micrometer (d)	Number of particle (n)	% particles	nd
00-50	25	14	4.67	350
50-100	75	23	7.67	1725
100-150	125	38	12.67	4750
150-200	175	18	6.00	3150
200-250	225	62	20.67	13950
250-300	275	44	14.67	12100
300-350	325	73	24.33	23725
350-400	375	28	9.33	10500
		Sum of n =300		Sum of nd =70250

ARITHMETIC MEAN DIAMETER

$$\text{ARITHETIC MEAN DIAMETER} = \frac{sum\ of\ nd}{sum\ of\ n}$$

$$\frac{70250}{300}$$

Arithmetic mean diameter= 234.1 micrometer

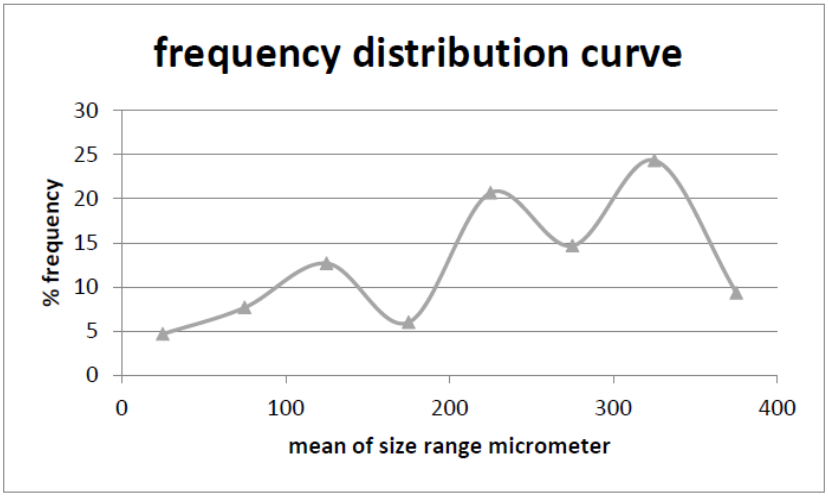

Figure: 20 frequency distribution curve for non nano zinc oxide.

- **Particle size of polypodium leucotomos**

Table 10: Observation of Particle Size of Polypodium Leucotmos.

Size range (micrometer)	Mean of sizerange micrometer (d)	Number of particle (n)	% particles	nd
00-50	25	28	.9.33	700
50-100	75	73	24.33	5475
100-150	125	44	14.67	5500
150-200	175	62	20.67	10850
200-250	225	18	6.00	4050
250-300	275	38	12.67	10450
300-350	325	23	7.67	7475
350-400	375	14	4.67	5250
		Sum of n =300		Sum of nd = 49750

ARITHMETIC MEAN DIAMETER

$$\text{ARITHETIC MEAN DIAMETER} = \frac{sum\ of\ nd}{sum\ of\ n}$$

$$\frac{49750}{300}$$

Arithmetic mean diameter= 165.8 micrometer

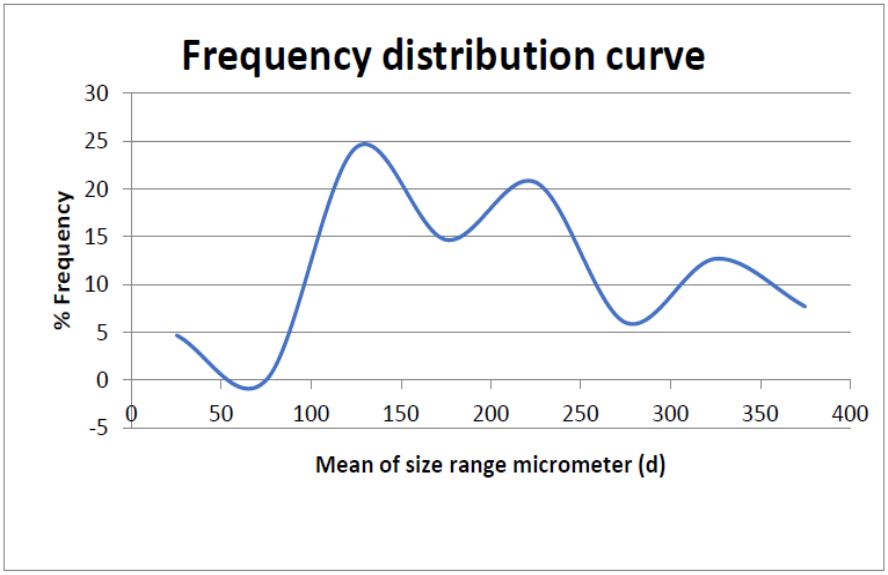

Figure: 21 frequency distribution curve for polypodium leucotomos.

➢ SOLUBILITY ANALYSIS

- Solubility analysis of non nano zinc oxide

Figure 22: Image of solubility analysis of non nano zinc oxide.

Table 11: Solubility analysis of non nano zinc oxide.

SOLVENT	SOLUBILITY
Water	Very slightly soluble
0.1 N Hcl	Freely soluble
Propanol	Very slightly soluble
Chloroform	Practically insoluble
Diethyl ether	Practically insoluble

- Solubility analysis of polypodium leucotomos

Figure 23: Image of Solubility Analysis of Non Nano Zinc Oxide.

Table 12: Solubility analysis of polypodium leucotomos.

Formulation	Appearance	Colour	Homogeneity
F_2	Cream like	Brown colour	Uniform and homogenous

➢ **HYGROSCOPICITY**

Figure: 24 image of hygroscopicity of non nano zinc oxide and polypodium leucotomos.

Table 13: Hydroscopicity of Non Nano Zinc Oxide And Polypodium Leucotomos.

Compound	Classification	% water uptake at 25 ◦c/80 %RH (w/w)
Non nano zinc oxide	Non hygroscopic	0
Polypodium leucotomos	Slightly hygroscopic	0.2

- **FLOW PROPERTY**

Figure 25: Image of Flow Property of Non Nano Zinc Oxide And Polypodium Leucotomos.

Angle of repose

Table 14: Flow Property Of Non Nano Zinc Oxide And Polypodium Leucotomos.

compound	Angle of repose	Type of flow
Non nano zinc oxide	35-40	Fair
Polypodium leucotomos	35-40	Fair

4. CHARACTERISATION OF CREAM

- **PERCENTAGE YIELD**

% yield of sunscreen product was found to be 90%

5. SELECTION OF BEST FORMULATION OF SUNSCREEN BY 2X2 FACTORIAL DESIGN AND DETERMINE THE MAIN EFFECT ANDINTERACTION EFFECT

- **MAIN EFFECT**

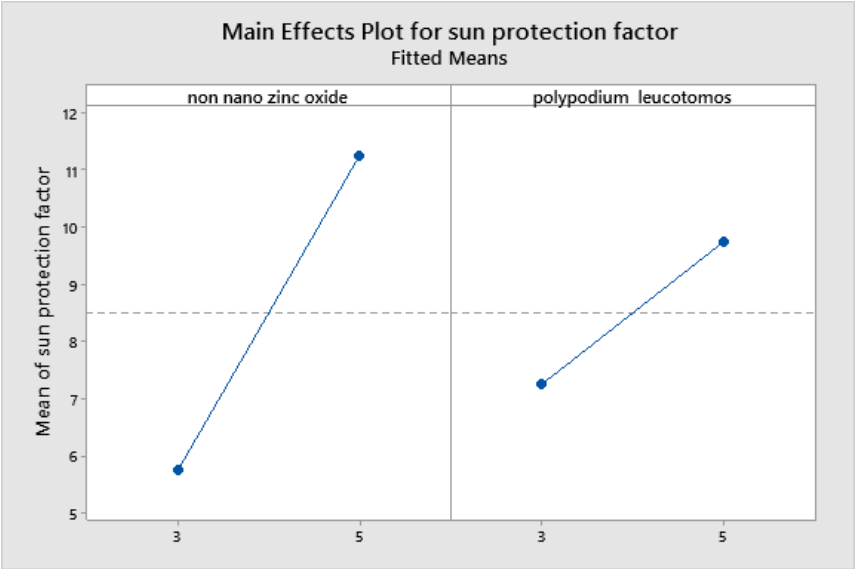

Figure 26: Main Effect of Non Nano Zinc Oxide and Polypodium Leucotomos.

Non nano zinc oxide – seems to affect the sun protection factor. Because the line is not horizontal. Non nano zinc oxide concentration 5 has a higher sun protection. Factor meanthan non nano zinc oxide concentration 3. Polypodium leucotomes seems to affect the sunprotection factor Because the line is not horizontal. Polypodium leucotomes concentration 5 has a higher sun protection factor mean than poly podium leucotomes concentration 3.

INTERACTION EFFECT

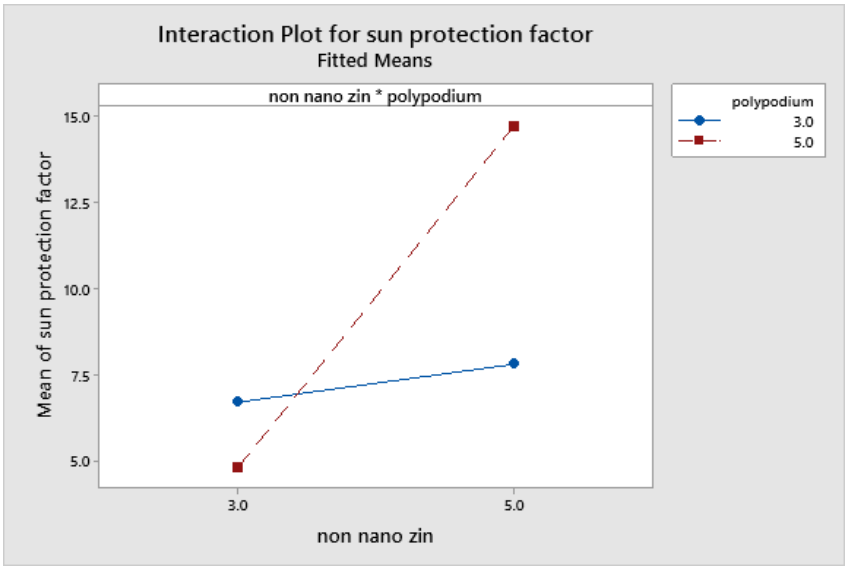

Figure 27: Interaction Effct of Non Nano Zinc Oxide And Polypodium Leucotomos

The two lines are not parallel at all, which indicates that there is likely an Interaction effect between polypodium leucotomes and non nano zinc Oxide is effect of non nano zinc oxide has an SPF depend on the Polypodium leucotomes. Hence this plot indicates an interaction between polypodium leucotomes and Non nano zinc oxide. The SPF has high percentage when polypodium leucotomes high concentration& Non nano zinc oxideconcentration high.

SURFACE FACTOR

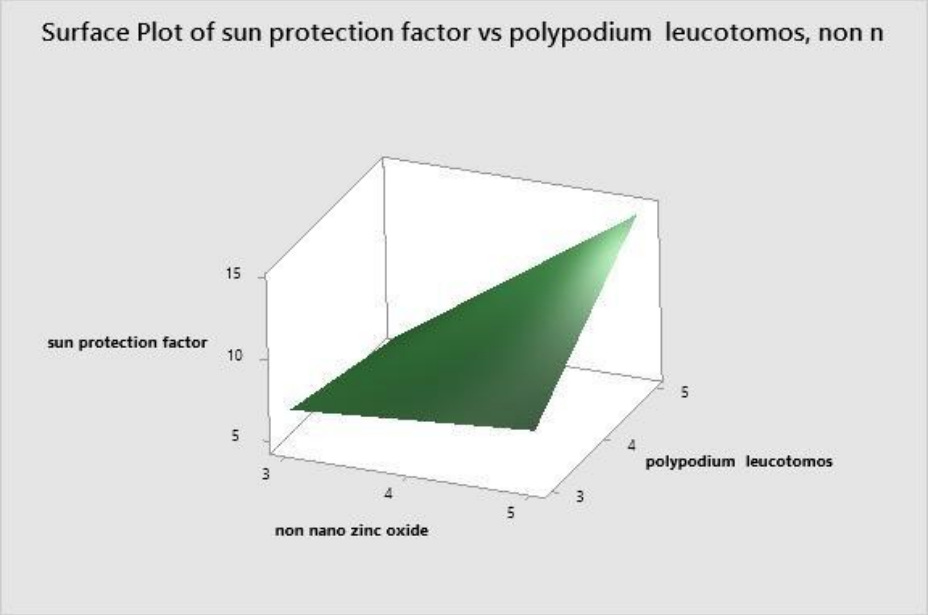

Figure 28: Surface Plot of Sunscreen.

The higher values of SPF are in the upper light corner of the plot, which Corresponds with high values of non nano zinc oxide & polypodium Leucotomos. The lowest values of SPF are in the lower left corner of the plot, which Corresponds with low values of non nano zinc oxide & polypodium Leucotomos.

6. EVALUATION OF SUNSCREEN

1. PHYSICAL PARAMETERS

Table 15: Physical Parameter of Cream.

Forumaltion	Appearance	Colour	Homogeneity
F_2	Cream Like	Brown Colour	Uniform and Homogenous

2. VISCOSITY

Figure 28: Image of Viscometer.

Table 16: Viscosity of Cream.

Formulation	viscosity
F_2	10,000cps

3. PH

Figure 29: Photo of Ph of The Cream.

Table 17: PH of the cream.

Formulation	F2
PH	**7.03**

4. STABILITY

7. Table: 18 freeze thaw test.

PARAMETER	CONDITION	DAYS											
		1	2	3	4	5	6	7	8	9	10	11	12
Colour	**Freeze**	BC		BC		BC		BC		BC		BC	
	Thaw		BC		BC		BC		BC		BC		BC
Odour	**Freeze**	No		No		No		No		No		No	
	Thaw		No		No		No		No		No		No
Ph	**Freeze**	7.03		7.0		7.0		7.0		7.02		7.0	
	Thaw		7.0		7.02		6.9		7.0		7.0		7.02
Homogeneity	**Freeze**	No		No		No		No		No		No	
	Thaw		No		No		No		No		No		No
Phase separa`tion	**Freeze**	No		No		No		No		No		No	
	Thaw		No		No		No		No		No		No

BC-BROWN COLOUR, NO-NO CHANGES

1. CENTRIFUGATION TEST

Figure 30: Photo of centrifugation of cream.

Table 19: Centrifugation Test.

Formulation	Centrifugation Test
F_2	No phase separation

2. TEST FOR SPREADABILITY

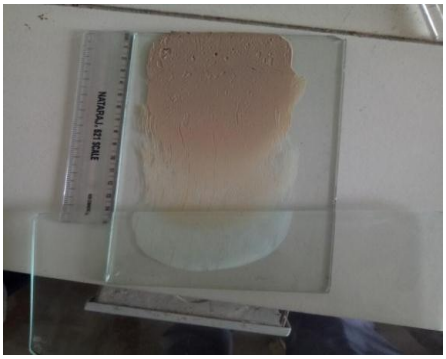

Figure 31: Photo of Spreadability of Cream.

Table 20: Spreadability Of Cream.

Formulation	Weight tiedto upper slide (m)	Length of glass slide moved(i)	Time taken(t)	Spreadability
F_2	50	10	4	125g.cm/s

3. MICROBIAL LIMIT TEST

Figure 32: Photo of Microbial Limit Test.

Table 21: Microbial Limit Test.

Microbial Limit Test	Colony –Forming Unit
Total Aerobic Microbial	NO CFU
Total Yeast and Mold Count	NO CFU

b. EMULSION TEST

Figure 33: Photo of Emulsion Test.

The cream settle down after stirred cream repels water and does noty blent with water.after addinf drop of food colour emulsion remains brown in a surrounding yellowsolution.

13. IRRITANCY TEST

Table 22: Irritation Test.

Formulation	Irritancy test
F_2	No irritation

14. DIFFUSION STUDY

Figure 34: Photo of Diffusion Study.

Table 23: Irritation Test.

Formulation	Diffusion study
F_2	Cream not diffused into agar

11. WATER RESISTANCE TEST

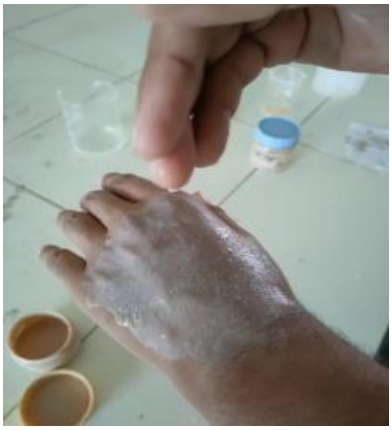

Figure 35: Photo of water resistance test.

Table: 24 water resistance test.

Formulation	Water resistance test
F_2	Water proof

11. INVITRO SPF OF SUNSCREEN CREAM

Figure 36: Image F_1, F_2, F_3,F_4 Cream Dilution Solution for SPF Test.

I. SPF DERMINATION OF F1 FORMULATION CONCENTRATION (LOW-LOW)

Table: 25 SPF F_1 FORMULATION CONCENTRATION (LOW-LOW).

Wavelength (NM)	EE X I (Normalized)	Absorbance	EE X I X Abs
290	0.015	0.7371	0.011057
295	0.0817	0.714	0.058334
300	0.2874	0.6952	0.1998
305	0.3278	0.6727	0.220511
310	0.1864	0.6563	0.122334
315	0.0839	0.6509	0.054611
320	0.018	0.6464	0.011635
			0.678282

SUN PROTECTION FACTOR=6.7

II. Spf Determination Of F2 Formulation Concentration (High-High).

Table 26: SPF F_2 formulation concentration (high-high).

WAVELENGTH (NM)	EE X I (NORMALIZED)	ABSORBANCE	EE X I X Abs
290	0.015	1.5629	0.023444
295	0.0817	1.5208	0.124249
300	0.2874	1.4954	0.429778
305	0.3278	1.4738	0.483112
310	0.1864	1.4551	0.271231
315	0.0839	1.4452	0.121252
320	0.018	1.4346	0.025823
			1.478888

SUN PROTECTION FACTOR FACTOR=14.7

III. Spf Determination of F3 Formulation Concentration (High-Low)

Table: 27 SPF F_3 formulation concentration (high-low)

WAVELENGTH (NM)	EE X I (NORMALIZED)	ABSORBANCE	EE X I X Abs
290	0.015	0.8351	0.012527
295	0.0817	0.8128	0.066406
300	0.2874	0.7964	0.228885
305	0.3278	0.7801	0.255717
310	0.1864	0.7671	0.142987
315	0.0839	0.7629	0.064007
320	0.018	0.7613	0.013703
			0.784233

SUN PROTECTION FACTOR=7.8.

IV. Spf Determination Of F4 Formulation Concentration (Low-High)

Table: 28 SPF F_4 formulation concentration (low-high).

WAVELENGTH (NM)	EE X I (NORMALIZED)	ABSORBANCE	EE X I X Abs
290	0.015	0.5423	0.008135
295	0.0817	0.5174	0.042272
300	0.2874	0.4978	0.143068
305	0.3278	0.4805	0.157508
310	0.1864	0.4677	0.087179
315	0.0839	0.464	0.03893
320	0.018	0.4625	0.008325
			0.485416

SUN PROTECTION FACTOR=4.8

DISCUSSION

The finding presented in current study indicated that λ max of non nano zinc oxide was found to be 256 nm in 0.1N HCL (fig:2) and λ max of polypodium leucotomos was found to be 276 and 340 nm in deionised water(fig:3). The different concentration of polypodium leucotomos was prepared and observed in 200 to 400 nm. It describes that increases in the concentration increases in the absorbance property(fig: 9,10,11,12,13,4,15,16,17,19). likewise the different concentration of non nano zinc oxidewas prepared and observed in 290 to 320 nm . It is also describes that increase in the concentration increase in the absorbance property (fig:5,6,7). To produce quality cosmetic product performulation study is very necessary. In this study the preformulationof non zinc oxide was found to be white in colour and no odour. The particles size of non zinc oxide 234.1 micrometer. The non nano zinc oxide freely soluble in 0.1 N HCL (fig: 22). Non hygroscopic was found in the non nano zinc oxide. The flow property of non nano zinc oxide was fair(fig:25). Likewise the preformulation study of polypodium

leucotomos was found to be brown in colour of musky,aromatic odour. The particle size of polypodium leucotomas 165.8 micrometer. The polypodium leucotomas freely soluble in deionized water(fig:23). Polypodium leucotomos slightly hygroscopic in nature. The flow property of polypodium leucotomos was fair(fig: 25). The formulated sunscreen have the percentage yield of 90%. The factorial design methodology was applied in the study. The main effect and interaction effect was determined. The main effect of non nano zinc oxide seems to effect SPF, non nano zinc oxide concentration 5 has higher SPF (fig:26). Similarly the main effect of polypodium leucotomos seems to affect the SPF polypodium leucotomos concencentration 5 has higher SPF(fig: 26). In interaction effectthe two lines are not parallel at all which indicates they was likely interaction effect between polypodium leucotomos and non nano zinc oxide(fig: 27). Hence the one independent variable depended variable depend on the another inindepended variable for the response. According to (jose agular al 2021) apparently synergistic effect of polypodium containing formulation was proved in this study by interaction effect.

In surface factor the higher value was found to be in the upper right corner of the plot by the surface factor F2 formulation was high SPF hence it is selected has best formulation (fig:28). F2 formulation produce a good SPF value ie 14.7 it offer 93 % of protection. F2 formulation have good physical characteristic, skin tolerated PH ie 7.03. Stable in freeze thaw test. No colony formation in total aerobic count, total yeast and mold count. In centrifugation test no phase separation. The prepared formulation was w/o emulsion. The formulated sunscreen do not cause any skin irritation. The main property of sunscreen its have waterproof and do not enter into skin. In this study we observed that it has waterproof and do not enter into the agar medium. SPF was determine by in vitro method SPF of F1 formulation was found to be 6.7 (table: 25)ie it offer 75% protection. SPF of F2 formulation was found to be 1 4.7(table:26) it offers 93.3% . SPF of F3 formulation was found to be 7.8 (table:27) it offers 87.5% SPF of F4 formulation was found to be 4.8(table: 28) it offer below 75% protection. The limitation of this study UVAprotection is not determined.

CONCLUSION

Thus the result of the present study conclude that the formulated f2 cream has 93.3% potency to protect against UVB ray as per SPF value.

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