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EVALUATION OF VITAMIN E IN MILLIGLOBULES

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Abstract: Skin is the largest organ of the human body which is greatly affected by the external as well as internal factors. Drying of skin is a very common problem that can result from various factors such as sunlight, dry air, low humidity, age and medical conditions etc. A wide variety of actives are used to prevent the drying of skin as well as to nourish and moisturize the skin. Milliglobules of Vitamin E have emerged as a promising innovation for enhancing the delivery and efficacy of Vitamin E in dermatological and pharmaceutical applications. However, despite their potential, critical gaps persist in the understanding of their optimal formulation, stability and bioavailability. So, in order to investigate the optimal formulation parameters, long-term stability and also to explore the interaction with other formulation components the current research work is done. This study will focus on the development of effective and stable gel containing Vitamin E milliglobules, maximizing its therapeutic and commercial potential.

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1. INTRODUCTION[1]

Skin acts as a frontier that mediates between body and environment. Skin covers the entire body and protects it from various types of external stimuli, damage as well as from moisture loss.
The skin is composed of three layers:

1) The outer epidermis
2) The inner dermis
3) The sub cutaneous tissue deep the inner skin.

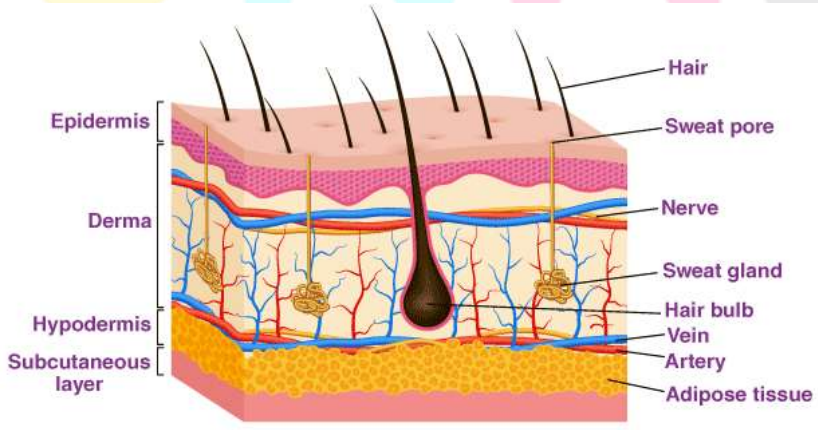


Fig.1 - Structure of Skin[2]

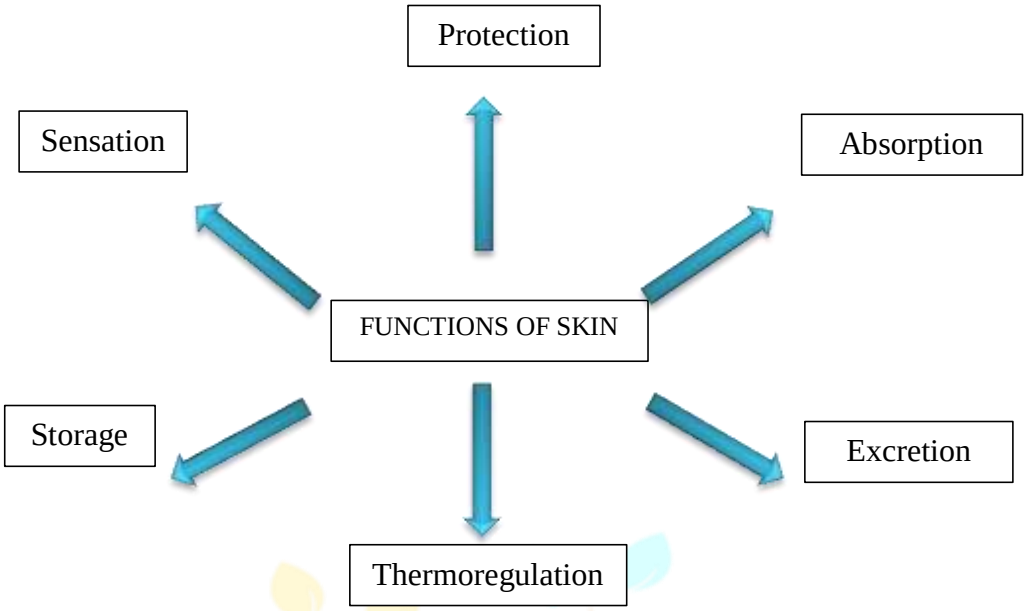


Fig.2 - Functions of Skin

INTRODUCTION TO VITAMIN E MILLIGLOBULES

Milliglobule is a soft, spherical, structure made from natural plant polymer material in which vitamins, oils, waxes, oil soluble herbal extracts, natural essential oils and fragrances, aroma therapy oils, micronised powders are homogeneously dispersed in the particle. Colourants and glitters can also be encapsulated. The globule breaks easily on gentle application of pressure, blending into the skin, leaving no debris [3].

Fig.3 - Milliglobules of Vitamin E

IMPORTANCE OF MILLIGLOBULES

In context of skincare milliglobules have significant influence on the effectiveness as well as the stability of the product. Milliglobules are important for the following reasons:

Stability: Milliglobules offer an advantage that hydrophobic actives can also be incorporated in the hydrophilic products and vice-versa. This helps in the improved stability of the product.

Enhanced Absorption: As compared to their volume milliglobules offer a large surface area which in turn helps in improving the absorption of active ingredients into the skin or body.

Improved Product Performance: In skincare formulations, milliglobules can help deliver active ingredients more effectively. They can encapsulate vitamins, antioxidants and other beneficial compounds, protecting them from degradation and ensuring a controlled release.

Enhanced Sunscreen: In sunscreen formulations, milliglobules can enhance the effectiveness of UV filters by ensuring even distribution on the skin. They can also reduce the greasy feel often associated with sunscreen.

2. MATERIALS AND METHODS

2.1 PRELIMINARY TESTING OF MILLIGLOBULES: Vitamin E milliglobules were used as an active ingredient in the formulation of Gel which was procured by Natural Odours and Polymers Pvt. Ltd., Mumbai. A procurement data of the silicone was provided based on that the following examination was done.

1. **Organoleptic Evaluation:**
   The physical evaluation of the milliglobules was done by its appearance, color, odour, taste and size as shown in Table no.1

Table No.1 - Organoleptic Properties of Milliglobules:

1.	Appearance	Beads
2.	Color	Mustard Yellow
3.	Odour	Characteristic
4.	Taste	Tasteless
5.	Size	10-16

2. **Solubility Test:** The milliglobules were mixed with different solvents to check its solubilty as shown in Table no.2

Table No.2 - Solubility of Milliglobules:

1.	Hot water	Miscible
2.	Water	Insoluble
3.	Ethanol	Insoluble
4.	Methanol	Insoluble
5.	Chloroform	Insoluble
6.	Di-ethyl ether	Insoluble

3. **Melting Point [4]:** The melting point was determined and it was found to be between 80 - 85˚ C.

4. **Limit Test [5]:**
   A. **Limit Test for Chloride:** The opalescence produced by the test sample was less than the opalescence produced by the control. Hence the samples passed the test for the absence of chloride.
   B. **Limit Test for Sulphate:** The opalescence produced by the test sample was less than the opalescence produced by the control. Hence the samples passed the test for the absence of sulphate.

5. **Drug Entrapment Capacity of Milliglobules [6]:**

Spectrophotometric method was used to determine the quantity of active present in milliglobules. Methanol was used as blank solution in the study. The *lamda max* was also determined for the sample. Vitamin E present in milliglobules in methanol showed maximum absorbance at 284nm. The calculated concentration of the test solution was 152.62 µg/ml. The calculated concentration present in the 1g gram of the milliglobules in gel after one month was found to be 0.152 gm.

2.2 FORMULATION AND DEVELOPMENT OF GEL

In today's increasingly competitive personal care market, skin care product manufacturing requires technologies that can help provide consumers with innovative and aesthetically pleasing product forms, as well as easy and cost efficiency methods that can meet satisfactory manufacturing requirements. Consumers prefer clear gel product forms for its clarity, perception of purity, mildness, consistency, spreadability, non-sticky and cool feeling after application. Also the clear gel offers an excellent appearance to the milliglobules.

Hence it was decided to prepare clear gel base formula for the incorporation of Vitamin E milliglobules as an active.
A gel base was formulated using carbopol as gelling agent. The carbopol gel has the advantage of being relatively easy to formulate and have a very few stability problems and all other necessary ingredients were used.

Table No.3 - Formulation table showing incorporation of Vitamin E milliglobules in Gel base

S.NO	INGREDIENTS	QUANTITY (100%)
1.	Carbopol 940	1-2
2.	Glycerine	8-9
3.	Sodium benzoate	0.15
4.	Triethanolamine (TEA)	0.5-1
5.	Vitamin E milliglobules	1.5
6.	Water	Upto 100 ml

2.3 EVALUATION OF GEL

A. **Organoleptic Characteristics:** The parameters of the gel such as its color, spreadability, pH and odour were observed as shown in Table no.4

Table No.4 - Organoleptic Characteristics of Gel

S.no	Parameter	Observation
1.	Color	Transparent
2.	Spreadability	Good
3.	pH	6
4.	Odour	Odorless

B. **Homogeneity of Gel:** The formulated gel was tested for homogeneity by visual inspection after the gel was set in the container. It was tested for appearance, presence of any aggregates and flocculates [7].

Observation:

Fig.4 - Homogeneity of Gel

C. Spreadability of Gel: The weighed quantity of gel (2g) was sandwiched between two glass slides. 500g of weight was placed on the slides. The weight was placed for specific period of time for 5 minutes. Then weight was removed and diameter of the spread circle was measured. Spreadability was calculated by using formula [7] –

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

where,

S = Spreadability

M = weight placed on the slide

L = diameter of circle

T = time in seconds

Observation:

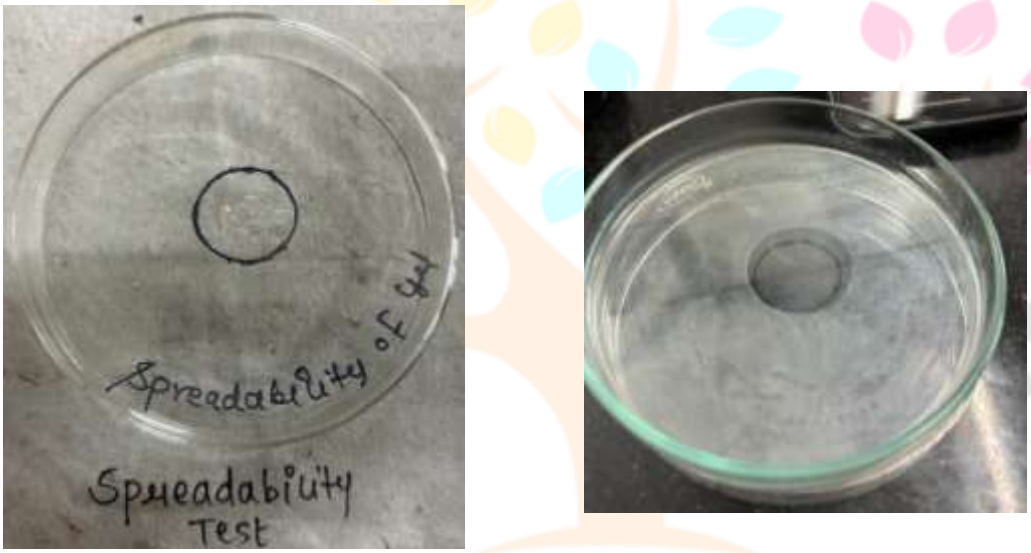


Fig.5 - Spreadability of Gel

Calculation:

$$S = \frac{500 \times 3}{300}$$

= 5 cm/sec

D. Accelerated Stability Studies: For gel, the accelerated stability studies were carried out for 1 month by keeping the samples under the following temperature and humidity conditions in a stability chamber as shown in Table no. 5

Table No.5 - Test conditions for gel stability testing

S.NO	STORAGE CONDITION	STORAGE TEMPERATURE
1.	Ambient	Room temperature (28 – 30^{O} C)
2.	Elevated	45^{O} C
3.	Refrigerator	4^{O} C

Table No.6 - Gel properties to be checked under stability testing

S.NO	PROPERTY	METHOD
1.	Colour	Visual
2.	Odour	Organoleptic
3.	Transparency	Visual
4.	pH	pH meter
5.	Viscocity	Visual

Observation:

Table No.7 - Colour, Odour and Transparency changes in product kept at different conditions

DAYS	ROOM TEMPERATURE (28 – 30^{O} C)			ELEVATED (45^{O} C)			REFRIGERATOR (4^{O} C)		
	Colour	Odour	Transparency	Colour	Odour	Transparency	Colour	Odour	Transparency
0	SC	OO	T	SC	OO	T	SC	OO	T
2	SC	OO	T	SC	OO	T	SC	OO	T
5	SC	OO	T	SC	OO	T	SC	OO	T
7	SC	OO	T	SC	OO	T	SC	OO	T
9	SC	OO	T	SC	OO	T	SC	OO	T
11	SC	OO	T	SC	OO	T	SC	OO	T
13	SC	OO	T	SC	OO	T	SC	OO	T
15	SC	OO	T	SC	OO	T	SC	OO	T
17	SC	OO	T	SC	OO	T	SC	OO	T
19	SC	OO	T	SC	OO	T	SC	OO	T
21	SC	OO	T	SC	SCO	T	SC	OO	T
23	SC	OO	T	SC	SCO	T	SC	OO	T
25	SC	SCO	T	SC	SCO	T	SC	OO	T
27	SC	SCO	T	SC	SCO	T	SC	SCO	T
29	SC	SCO	T	SC	SCO	T	SC	SCO	T
31	SC	SCO	T	SC	SCO	T	SC	SCO	T

SC= Same Color, OO = Original Odour, T = Transparent, SCO = Slight Change of Odour

Table No.8 - pH changes of product kept at different conditions

DAYS	ROOM TEMPERATURE (28 – 30^{O} C)	ELEVATED (45^{O} C)	REFRIGERATOR (4^{O} C)
0	6.0	6.0	6.0
2	6.12	5.9	5.9
5	6.15	5.95	5.88
7	6.20	6.0	6.05
9	6.20	6.11	6.14
11	6.24	6.13	6.08
13	6.30	6.05	6.21
15	6.22	6.07	6.18
17	6.18	6.10	6.22
19	6.32	6.17	6.25
21	6.36	6.25	6.30
23	6.24	6.28	6.22
25	6.32	6.26	6.34
27	6.30	6.32	6.28
29	6.34	6.30	6.27
31	6.30	6.34	6.25

Table No.9 - Viscosity of product kept at different conditions

DAYS	ROOM TEMPERATURE (28 – 30^{O} C)	ELEVATED (45^{O} C)	REFRIGERATOR (4^{O} C)
0	OV	OV	OV
2	OV	OV	OV
5	OV	OV	OV
7	OV	OV	OV
9	OV	OV	OV
11	OV	OV	OV
13	OV	OV	OV
15	OV	OV	OV
17	OV	OV	OV
19	OV	OV	OV
21	OV	OV	OV
23	OV	OV	OV
25	OV	OV	OV
27	OV	OV	OV
29	OV	OV	OV
31	OV	OV	OV

OV = Original Viscosity

E. Quantity of Active in Gel After One Month:

Spectrophotometric method was used to determine the drug entrapment capacity of the milliglobules after one month. Methanol was used as blank solution in the study. The concentration of the test solution was calculated.

F. Subjective Evaluation by Corneometer [8]:

A Corneometer is a device specifically designed to measure the hydration level of the stratum corneum, the outermost layer of the skin. It is a valuable tool for assessing the moisture content of the skin, which indirectly reflects the condition of the skin barrier.

Working Principle of Corneometer: The measurement of the skin moisture is based on the internationally recognized Corneometer-method, a capacitance method. This measurement is based on the completely different dielectric constant of water (81) and other substances (mostly < 7). The measuring capacitor shows changes of capacitance according to the moisture content of the samples. A glass lamina separates the metallic tracks (gold) in the probe head from the skin in order to prevent current conduction in the sample. An electric field between the tracks with alternating attraction develops. One track builds up a surplus of electrons (minus charge) the other a lack of electrons (plus charge). The scatter field penetrates the very first layer of the skin during the measurement and the capacitance is determined.

Unlike the impedance measurement no galvanic relation between the device and the measuring object or polarization effects exist.

Preparation of The Measurement:

The Corneometer CM 825 is a highly sensitive measurement instrument. For exact and reproducible measurement values it is important to follow the following instructions:

- **Preparation of the room:**

For all measurements of skin parameters, it is important to keep constant ambient conditions. Temperature and relative humidity should be constant. This is vital for the comparison of long-term measurements. The optimum room conditions are 20 C° and 40-60% relative humidity. Do not measure under direct lamp light or direct sunlight. Heat radiation might cause measurement inaccuracies. In the case of a series tests, always measure at the same time of the day and with the same light conditions.

- **Preparation of the volunteer:**

Allow your test persons to rest for at least 10-20 min., so that their blood circulation can regain a normal level after possible physical exercise. The skin area to be measured should not be covered with clothes during the acclimatization time. Emotional stress may also lead to increased transpiration. Make sure the acclimatization room is calm.

If possible measure on hairless skin areas. Should you wish to measure on hairy skin areas, it is recommended to shave the respective area some time (1-2 days) before the measurement or cut it very short with a pair of scissors.

The Moisture Measurement

1. Select Corneometer" by clicking on the button at the right margin of the screen. In addition, choose a display mode for the measurement (bar, digital display, curve or numeric table).
2. Place the probe head vertically on the skin area to be measured according to the pressure of the spring in the probe. Please make sure that the skin area is not hairy. The Corneometer-probe starts the measurement when in contact with the skin. A beep signals that the measurement has been carried out successfully. The screen will show the measuring value.
3. For the next measurement proceed in the same way. The average over all measurements is always shown and can be stored with the data in addition to temperature, air humidity, skin site, key and comment.
4. If you want to take continuous measurements instead of single measurements (measurement values are not taken one by one but taken as long as the probe is put onto the skin or until a certain stop event is reached), change the setting in the menu "Options".

Interpretation of the Results:

The following values are valid for healthy skin and normal room conditions (20°C and 40-60% air humidity) and will help you to determine the skin type as shown in Table No.11 below.

Table No.10 - Interpretation of Corneometer results

Skin condition	Inner forarm
Very dry	< 30
Dry	30 – 45
Sufficient moisturized	> 45

Obervation :

Fig.6 - Moisture measurement of Skin by Corneometer

G. Microbiological Analysis of Gel [9]:

The total microbial count, also known as total viable count or total plate count, is a method used in microbiology to estimate the number of viable microorganisms present in a sample. The principle is based on the fact that viable microorganisms will grow and form visible colonies when provided with appropriate nutrients and environmental conditions. TMC. provides a quantitative estimate of the microbial load in a sample and helps assess the microbiological quality of products or environments. It is a valuable tool for ensuring product safety & quality control.

Procedure:

Sterilization of Glassware

1. All the apparatus were washed properly with detergent & then cleaned with tap water & left out to dry then cleaned with alcohol. All apparatus were wrapped with paper, media was kept closed with cotton plugged & paper was placed over it & tied with rubber. All the apparatus were kept in autoclave at 121°C for 20 minutes.

Preparation & Sterilization of media

1. SCDA (Soybean Casein Digest Agar): - As per instructed on the media label, 40 gms was suspended in 1000 ml distilled water. Heated to boiling to dissolve the medium completely.
2. SDA (Sabouraud Dextrose Agar): - As per instructed on the media label, 65 gms was suspended in 1000ml of distilled water. Heated to boiling to dissolve the medium completely.
3. The prepare SDA & SCDA media was sterilized by autoclaving at 15 lbs pressure (121°C) for 20 minutes. Allowed to cool to 40-45°C. The sterilized media was mixed well & the poured into sterile petri plates.

Preparation of Sample

1. 1 gm of gel was accurately weighed in sterile test tube.
2. 9 ml of distilled water was added & stirred properly using sterile glass rod.

Preparation of Dilution

1. For dilution $10^{-1:}$ 1 ml of sample was transferred in sterile test tube. Then 9 ml of distilled water was added & mixed properly.
2. For dilution $10^{-2:}$ Aseptically 1 ml sample from dilution 10^{-1} was transferred using sterile pipette & then 9 ml of water was added & mixed properly.
3. For dilution $10^{-3:}$ Aseptically 1 ml of sample from dilution 10^{-2} was transferred using sterile pipette & 9 ml of water was added & mixed properly.

Procedure:

1. The petri plates were labelled & agar tubes were labelled as 10^{-1}, 10^{-2}, 10^{-3} for dilutions.
2. 1 ml sample of dilution 10^{-1} was aseptically transferred into the petri plate labelled as 10^{-1} using a sterile pipette.
3. In the same manner one plate each for dilution 10^{-2} & 10^{-3} were also prepared.
4. After the sample, 20-25ml of media was poured in the petri plates. This step was carried out quickly to avoid solidification of the medium in test tube or formation of lumps because of rapid drop in temperature.
5. The media in the petri plates was allowed to solidity & then the petri plates were incubated in an inverted position at 37°C for 24 hrs for bacterial growth & 25-30°C for fungal growth.

Observation :

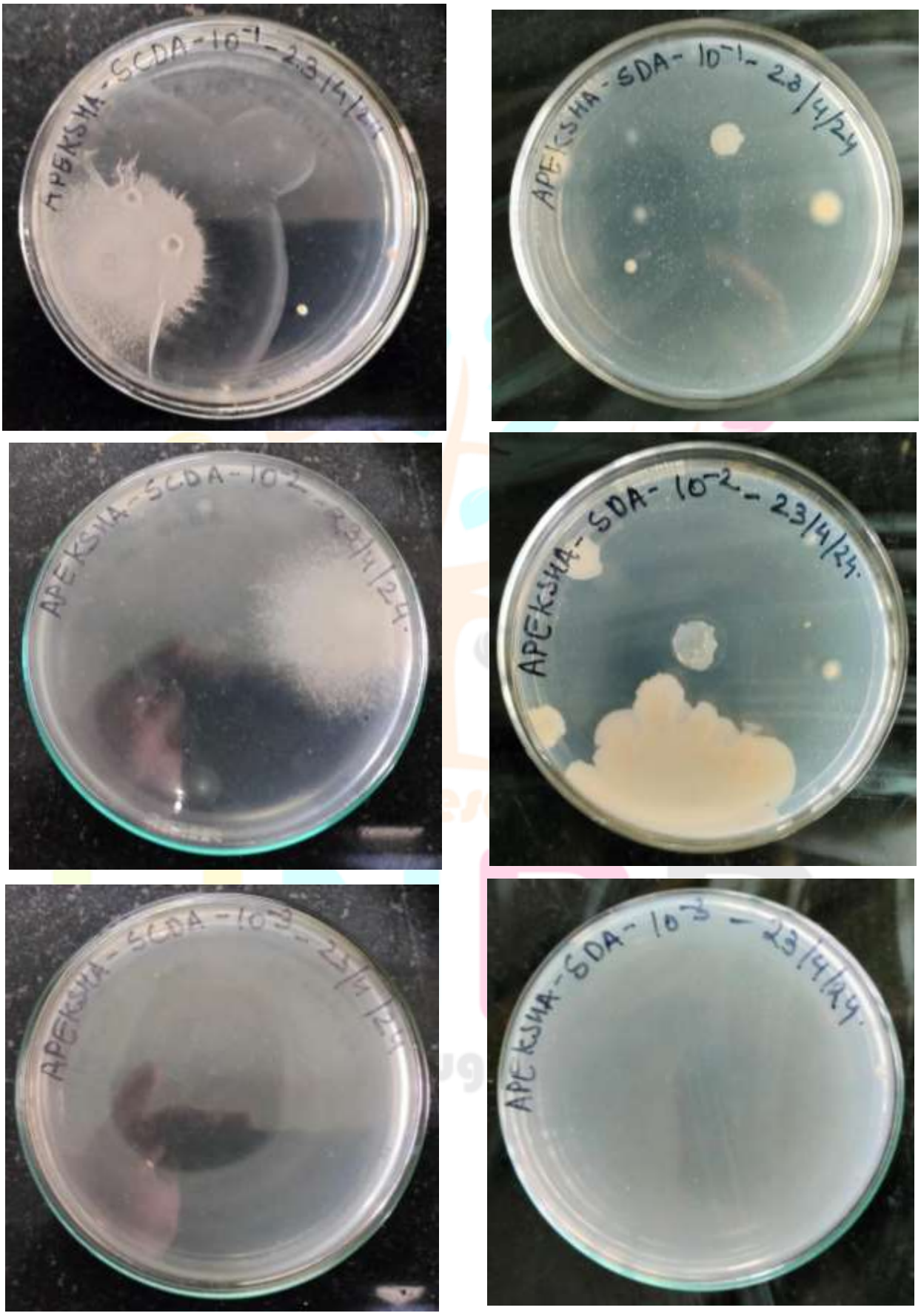


Fig.7 - Bacterial and Fungal growth in SCDA and SDA media

3. RESULT

Evaluation of Gel

A. Organoleptic Characteristics:

When formulated gel was evaluated for its organoleptic properties, it was found that it had the desired transparency. The spreadability of the gel was found to be excellent and feels smooth and soft. pH of the gel was also found to be 6. The gel was also found to be odorless.

B. Homogeneity of Gel:

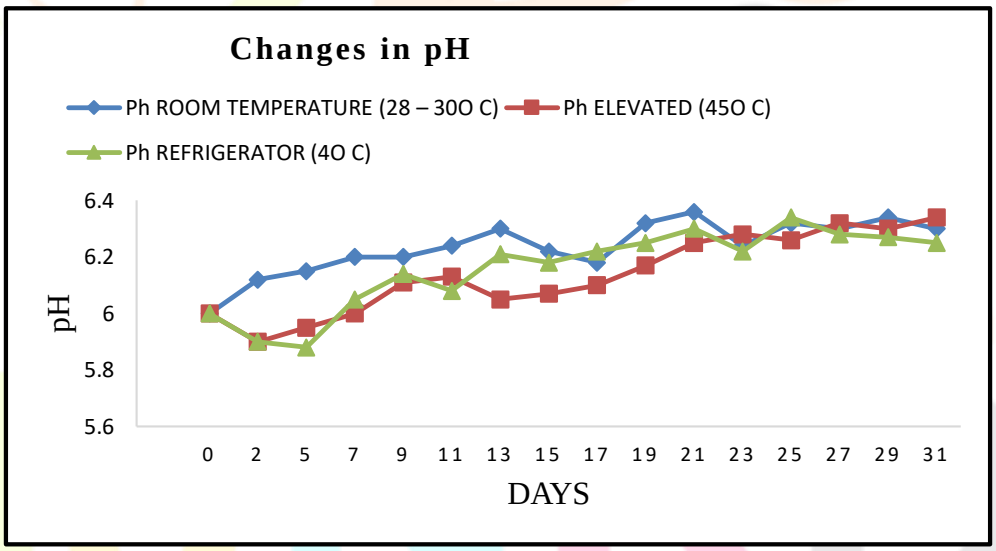
The formulated gel was smooth as well as homogenous and no agglomerates were found.

C. Spreadability of Gel:

Spreadability is an important factor which affects the consumer acceptability and also helps in the uniform application of the product. The spreadability of the formulated gel was found to be 5cm/sec.

D. Accelerated Stability Studies:

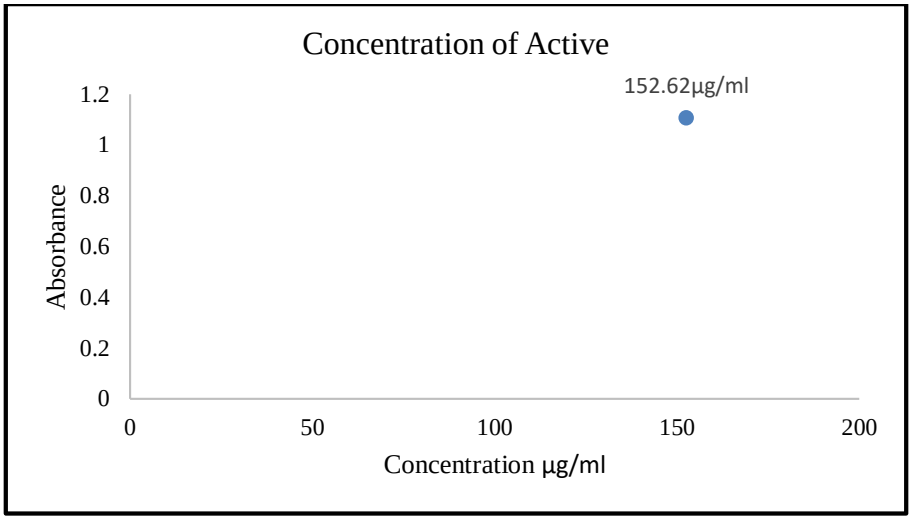
The accelerated stability study was carried out for the formulated gel at room temperature (28 - 30°C), elevated temperature (45 °C) and at refrigerator (4°C). Several parameters such as colour, odour, transparency, pH and viscocity of the formulated gel was noticed. Significant change in colour and transparency was not observed in 30 days. But slight change in pH, odour and viscocity of the gel was observed in 30 days.



Graph No.1 - Concentration of Vitamin E in fresh milliglobules

E. Quantiy of Active in Gel After One Month :

Spectrophotometric method was used to determine the quantity of active in gel after one month. Methanol was used as blank solution in the study. The lamda max was also determined for the sample. Vitamin E present in milliglobules in methanol showed maximum absorbance at 284nm. The calculated concentration of the test solution was 152.62 µg/ml. The calculated concentration present in the 1g gram of the milliglobules in gel after one month was found to be 0.152 gm.



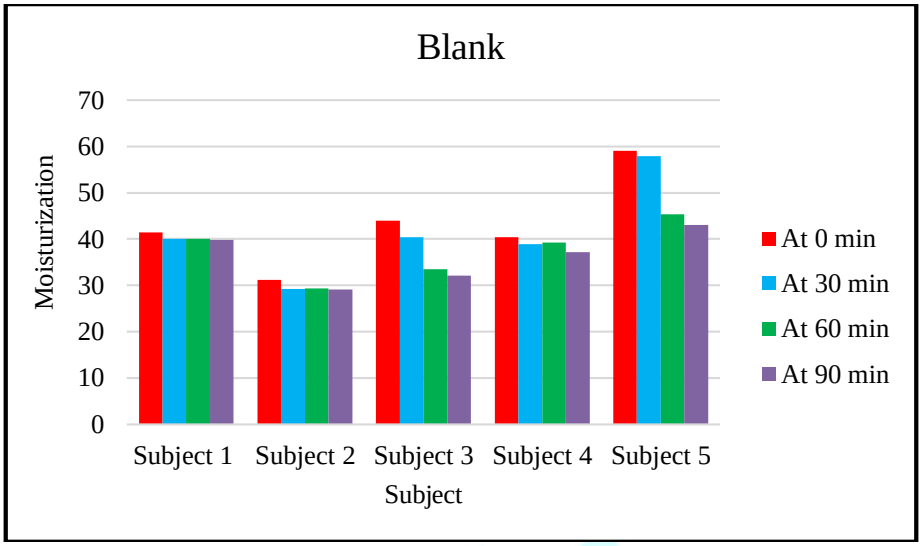
Graph No.2 - Concentration of Vitamin E in milliglobules after one month

F. Subjective Evaluation by Corneometer:

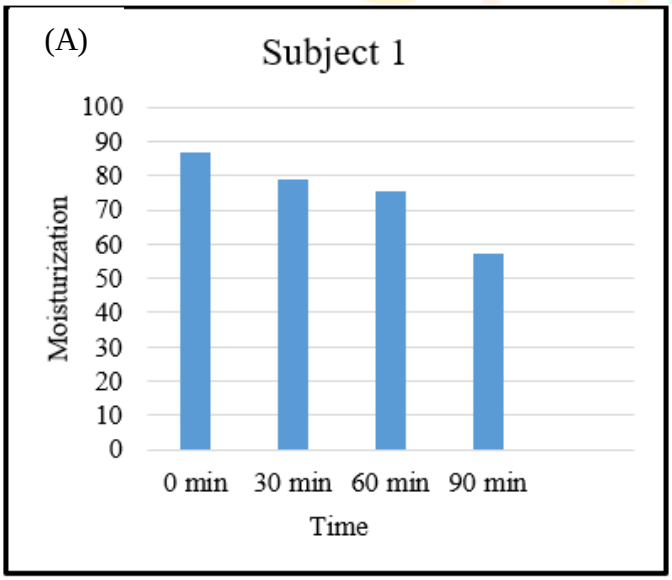
The moisture content values of the 5 test subjects were mentioned in the following table : Room Condition : 18-20˚C.

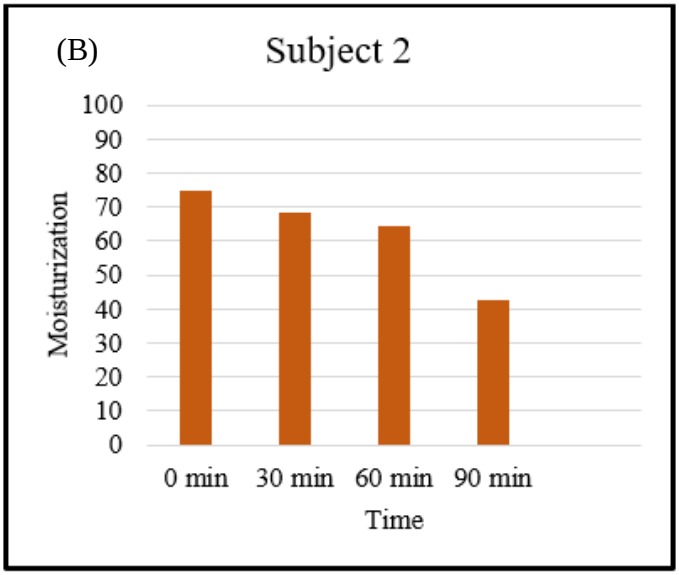
Table No.11 - Moisture Content Determined by Corneometer

Subjects	Product	Time (minutes)			
		O Min	30 Min	60 Min	90 Min
Subject 1	Blank	41.4	40	40.1	39.8
	Gel containing 10% Vitamin E Milliglobules	86.8	78.8	75.5	57.1
Subject 2	Blank	31.2	29.2	29.3	29.1
	Gel containing 10% Vitamin E Milliglobules	74.7	68.4	64.2	42.5
Subject 3	Blank	44.0	40.4	33.5	32.1
	Gel containing 10% Vitamin E Milliglobules	87.6	79.2	77.6	53.7
Subject 4	Blank	40.4	38.9	39.3	37.2
	Gel containing 10% Vitamin E Milliglobules	87.5	78.1	75.6	55.5
Subject 5	Blank	59.1	57.9	45.4	43.1
	Gel containing 10% Vitamin E Milliglobules	91.9	87.5	83.5	57.6



Graph No.3 - Determination of Moisture Content of Subject without product





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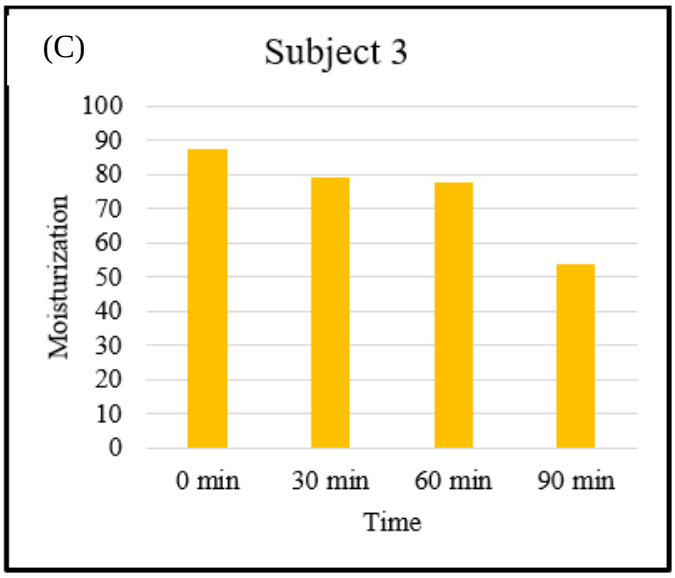

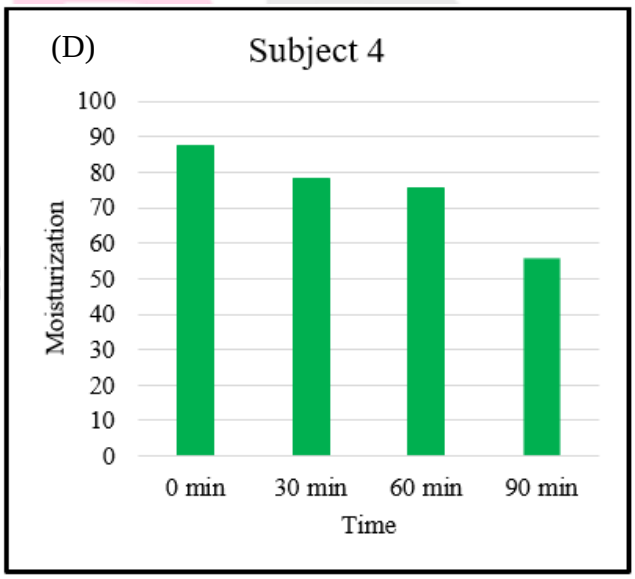

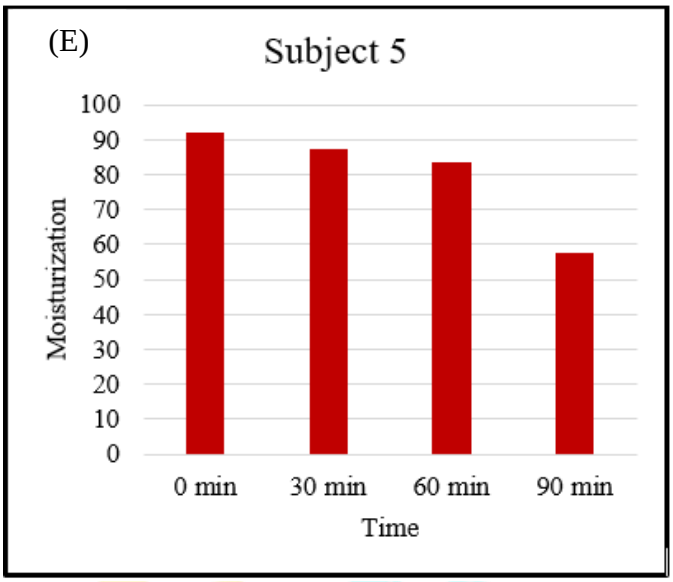

Graph No.4 - Determination of Moisture Content of Subjects with product at different time interval. (A) Subject 1, (B) Subject 2, (C) Subject 3, (D) Subject 4, (E) Subject 5

G. Microbiological Analysis of Gel :

The total microbial count of the gel was found to be 53 *cfu*. The microbial count was within the limits.

4. CONCLUSION

The current study involves the formulation of skin barrier gel containing Vitamin E milliglobules. The formulated gel was evaluated for its rheological characteristics. The stability and compatibility of milliglobuloes with gel was carried out for 1 month. From the results of organoleptic characteristics, it was concluded that it had the desired transparency. The spreadability of the gel was found to be excellent and feels smooth and soft. From the evaluation of the homogeneity of gel it was concluded that the formulated gel was smooth as well as homogenous and no agglomerates were found. From the spreadability test of the gel it was concluded that the formulated gel has good spreadability. From the accelerated stability studies which was carried out for 1 month and parameters such as colour, odour, transparency, pH and viscocity of the formulated gel was noticed. Significant changes in colour and transparency was not observed in 30 days. But slight changes in pH, odour and viscocity of the gel was observed in 30 days. The spectrophotometric method was used to determine the quantity of active in gel after one month. The calculated concentration present in the 1 g gram of the milliglobules in gel after one month was found to be 0.152 gm. It was concluded that the milliglobules are stable and compatible at the gel base. No amount of active was released by the milliglobules in the gel in the time span of one month. From the obtained results of the corneometer it might be concluded that the gel containing 10% Vitamin E milliglobules provides sufficient amount of the moisturization to the skin of subjects i.e, greater than 45 upto 1.5 hours. After 1.5 hours there might be decrease in the moisturization level. From the microbial analysis of the gel it was concluded that the obtained total microbial count values are within the limits and the product is stable and safe to use.

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