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FORMULATION AND *IN-VITRO* EVALUATION OF ANTI-AGING FACE CREAM CONTAINING LACTIC ACID AND COCOA BUTTER

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ABSTRACT: The main aim of the current research is to formulate and evaluate the anti-aging cream containing lactic acid and cocoa butter. Skin health and beauty is regarded as one of the most important variables influencing general "well-being" and "health" perception in humans because of that, various anti-aging techniques have been created in recent years. Uneven pigmentation, increased wrinkles, loss of elasticity, dryness, and roughness are all signs of skin ageing. Lactic acid dissolves the connections that hold old, dull cells together on the skin's surface, allowing them to be removed. Exfoliation is the term for this procedure. Lactic acid helps your skin shed old cells and replace them with new ones by speeding up cell turnover and stimulating cell renewal. As a result of exfoliation, lactic acid prevents and delay new fine lines and wrinkles from developing. Cocoa butter used in the formulation, gives the skin moisture and helps to remove the dark spots because of its anti-oxidant properties.

INTRODUCTION: Cosmetics are used to improve a person's appearance. For hundreds of years, makeup has been utilised. Egyptians were the first people known to employ cosmetics to improve their appearance. Makeup now plays an essential role in the lives of both men and women. Cosmetics have become more important as more individuals aspire to seem young and appealing. Both women and men use cosmetics such as creams, lotions, gels, and colognes every day. Creams can be used as a face cleanser in a variety of situations. Anti-ageing creams have recently been developed that help keep your skin appearing younger for many years ¹. AHAs are organic acids that have one hydroxyl group in the alpha position.

Glycolic acid, lactic acid, malic acid, tartaric acid, and citric acid are all AHAs that are commonly utilised in cosmetic formulations. In dermatology, AHAs have been utilised as superficial peeling agents as well as to reduce the appearance of keratoses and acne ². Although the specific mechanism of topical AHA activity is uncertain, the most commonly accepted notion is that AHAs chelate calcium ions from epidermal cell adhesions. This causes the intercellular adhesions to weaken, resulting in the shedding and flaking of dead and dry cells, which produces an exfoliating effect.

Reduced calcium levels also encourage cell proliferation while decreasing cell differentiation, reducing wrinkles and making skin seem younger. Lactic acid is known for its anti-aging properties which is generally found in the milk. It is said that Cleopatra, Queen of the Ptolemaic Kingdom of Egypt from 51 to 30 BC, used to bathe in sour milk, which contains lactic acid, to keep her skin looking young in ancient times ³. Lactic acid bacteria are good for human health.

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Lactic acid bacteria have a wide range of applications in the food, cosmetics, and pharmaceutical industries due to their GRAS (Generally Recognize as Safe) status and a variety of medicinal and functional qualities. Previous research has shown that lactic acid bacteria, their extracts, and ferments have significant impacts on skin health, including improved skin conditions and the avoidance of skin disorders⁴.

The skin becomes firmer and thicker when lactic acid is used at a concentration of 12 %. As a result, there are less fine lines and deep wrinkles and an overall smoother appearance. These findings reveal that lactic acid can penetrate the skin's deeper layers at such high quantities. Lower doses of 5% have no effect on the skin's middle layer. You'll notice a brighter, smoother complexion after utilising lactic acid. This AHA exfoliant aids in the treatment of moderate to severe hyperpigmentation, which occurs when particular areas of your skin have an excess of melanin. It can remove age spots and wrinkles, as well as diminish the appearance of large pores. Because lactic acid is one of the mildest AHAs, it's safe to use on sensitive skin in the right proportions. But at times, lactic acid may have some side effects like, Redness, Swelling, Burning sensation, Severe itching, Noticeable peeling⁵.

Using cocoa butter to overcome the above mentioned effects will help to reduce the side effects of lactic acid. Cocoa butter preserve the skin from ageing prematurely by preventing damage and prevents it from the early skin aging. Cocoa butter absorbs quickly and penetrates deeply into the skin's layers. It moisturises, calms, hydrates, nourishes, and balances the skin because it contains the bromine, phenyl ethylamine, essential fatty acids, natural antioxidants, vitamins and minerals, and a considerable amount of collagen and elastin⁶.

Cocoa butter effectively reduces itching because of its high moisture content and protective oil basis.

Antioxidants are present in large amount in cocoa butter. Cocoa butter can scavenge free radicals and neutralise them due to the presence of oleic acid, palmitic acid, and stearic acid. Cocoa butter, when used in skincare, can assist to reduce fine lines and wrinkles⁷.

MATERIALS AND METHODS:

Collection of Chemical: Lactic acid used in the formulation was supplied by New Neeta Chemicals, Pune. Cocoa butter used in the formulation was supplied by Kelkar Foods and Fragrances.

Formulation Method: Semisolid emulsion-based creams based on oil in water (O/W) emulsions were developed. The ingredients for the oil phase (Part A) and the aqueous phase (Part B) of the formulations as mentioned below are weighed into two separate beakers. The mixture was homogenized in a mortar and pestle after being heated in a water bath (75°C).

Oil phase ingredients (Part A):

- ❖ Emulsifying wax
- ❖ Coconut oil
- ❖ Cocoa butter

Aqueous phase ingredients (Part B):

- ❖ Distilled water
- ❖ Lactic acid
- ❖ PEG
- ❖ Methyl paraben
- ❖ Propyl paraben
- ❖ Perfume

Various formulations of the compositions reported in **Table 1** were generated using Design of Expert software (DOE).

TABLE 1: BATCHES OBTAINED FROM DESIGN OF EXPERT SOFTWARE (DOE)

Sr. no.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	Emulsifying wax(gm)	1.6	0.6	0.185	1.6	1.6	2.6	2.0	0.6	1.6	3.0
2	Coconut oil(ml)	3	2	3	3	1.5	2	4	4	4.4	3
3	Cocoa butter(gm)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
4	Lactic acid(ml)	2	2	2	2	2	2	2	2	2	2

5	PEG 600(ml)	2	2	2	2	2	2	2	2	2	2
6	Distilled water(ml)	10	10	10	10	10	10	10	10	10	10
7	Methyl paraben(gm)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
8	Propyl paraben(gm)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
9	Perfume	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.



FIG. 1: FORMULATION BATCHES OF CREAM

Evaluation of Cream:

Organoleptic Characteristics: Physical appearance, colour, texture, phase separation and homogeneity were examined for all formulations. Visual observation was used to assess these features. By pressing a little amount of the prepared cream and gels between the thumb and index finger, homogeneity and texture were determined. The texture and homogeneity of the formulations were assessed using the consistency of the formulations and the presence of coarse particles. The immediate skin feel was also assessed (including stiffness, grittiness and greasiness) ⁸.

Spreadability: Two horizontal glass plates were used to measure the spreadability of cream formulations. 1gm of sample was applied to the glass plate and standard weight of 25gm was placed to the upper plate. After one minute, the spreading diameter of 1 gm of sample between two horizontal glass plates was measured to determine the spreadability of the formulations ^{8,9}.

$$\text{Spreadability} = m \times l / t$$

Where, m= Standard weight which is tied to or placed over the upper slide (25g), l= length of a glass slide (5 cm), t= time taken in seconds.

Wash Ability: A small amount of cream was applied on the hand and it is then washed with tap water ⁹.

pH Values: With the use of standard buffer solutions, the pH meter was calibrated. About 5gm of the cream was weighed and dissolved in 50ml of

distilled water in a beaker, and its pH was measured ¹⁰.

Viscosity: Viscosity of cream was done by using Brooke field viscometer at a temperature of 25°C using spindle No. 04 at 60 RPM ¹¹.

Centrifugation Test: 5gm of cream was filled in a test tube and test tube was placed in centrifugation machine for 10 min ¹².

Freeze Thaw Test: Cream was kept in freeze at a low temperature and then brought to room temperature in freeze thaw testing. This cycle was repeated five times, with eye monitoring of the changes ¹³.

Sun Exposure Test: In the sun exposure evaluation, the cream was exposed to the sun for 24 hours and the changes were noted visually ¹³.

Irritancy: On the left hand dorsal surface, make a 1 cm² mark. The cream was then administered to the affected area, and the time was recorded. Then, for up to 24 hours, it is evaluated for irritancy, erythema, and edoema ¹³.

Drug Diffusion Test: Franz diffusion cell with cellophane membrane is used to carry out drug diffusion for all 6 batches. The Franz diffusion cell has two compartments, one for the donor and the other for the receiver. A cellophane membrane is inserted between the two compartments ¹².

Compatibility Study of Lactic Acid with Other Excipients: For the compatibility Study, the FTIR of lactic acid with cocoa butter and coconut oil was performed ¹³.

Accelerated Stability Study of Face Cream: The stability test was carried out in accordance with ICH guidelines. The formulated face cream was placed into tightly sealed container and stored for three months at various temperatures. After 3 months, samples were obtained and examined for appearance, pH, homogeneity, spreadability, after feel, removal of cream, type of smear and irritation ^{13,14}.

RESULT AND DISCUSSIONS:

Organoleptic Characteristics: Organoleptic properties for all 10 formulation batches was observed and f4 batch was found to have good homogeneity and immediate skin feel among all the 10 formulation batches. The observed results for f4 batch is mentioned in the **Table 2**.

TABLE 2: ORGANOLEPTIC CHARACTERISTICS OF CREAM

Sr. no.	Organoleptic property	Result
1	Physical appearance	Opaque
2	Colour	White
3	Texture	Smooth
4	Homogeneity	Homogeneous
5	Immediate skin feel	Moisturizing, no grittiness, light, not greasy

Spreadability: The spreadability of all the formulations were measured and then it is observed that the spreadability of formulation f4 was greater than as compared to others. As mentioned in the table 3 spreadability of formulation f4 was found to be 6.93.

pH values: The pH of all the formulations were measured. As mentioned in the table no. 3 pH of formulation f4 was found to be 5.8.

TABLE 3: EVALUATION RESULTS FOR ALL BATCH

Formulation batches	Spread ability	pH value	Wash-ability	Viscosity (mPas)	Centrifugation test	Freeze and thaw test	Sun exposure test	Irritancy	Drug diffusion test
F1	22.8	5.8	Easily washable	6550.2	Passes	Passes	Passes	Passes	91.18
F2	21.04	5.4	Easily washable	1132	Passes	Passes	Passes	Passes	80.07
F3	20.29	5.7	Not easily washable	3084.21	Failed	Passes	Passes	Passes	81.79
F4	22.8	5.8	Easily washable	6550.2	Passes	Passes	Passes	Passes	91.18
F5	15.79	5.5	Easily washable	2712.32	Passes	Passes	Passes	Passes	87.41
F6	12.95	6	Easily washable	4045	Passes	Passes	Passes	Passes	85.46
F7	39.21	5.3	Easily washable	2700.26	Passes	Passes	Passes	Passes	83.09
F8	19.92	5	Not easily washable	7560.02	Failed	Passes	Passes	Passes	77.69
F9	32.65	5.5	Not easily washable	8200.25	Failed	Passes	Passes	Passes	86.22
F10	33.02	5.2	Not easily washable	3207.03	Failed	Passes	Passes	Passes	84.28

Compatibility Study of Lactic Acid with Other Excipients: From fig. 2, 3, 4 we can say that lactic

Wash-ability: Batch f1, f4 and f7 showed better wash ability as compared with others.

Viscosity: **Table 3** shows the viscosities of all of the formulations, which ranged from 1029 to 5234.5 mPas at 60rpm.

Centrifugation Test: Centrifugation test was carried out to observe the phase separation after 10 min.

Freeze and Thaw Test: No physical changes were observed after removing from the freeze. As mentioned in **Table 3** the test passes.

Sun Exposure Test: After exposing to the sun for 24 hours the cream was observed visually and there were no changes observed in the formulation.

Irritancy: After application of cream for 24 hours, no signs irritancy, erythema, and edoema were observed.

Drug Diffusion Test: Drug diffusion test was carried out for all the 10 batches of cream formulation. Batch f4 showed the best results with 91% of drug diffusion.

acid is compatible with cocoa butter, coconut oil and PEG. The peaks of Lactic Acid are shown in

Table 4, peaks of Lactic Acid + cocoa butter + PEG mixture are shown in **Table 5** and peaks of the Lactic Acid + coconut oil + PEG mixture were shown in **Table 6**.

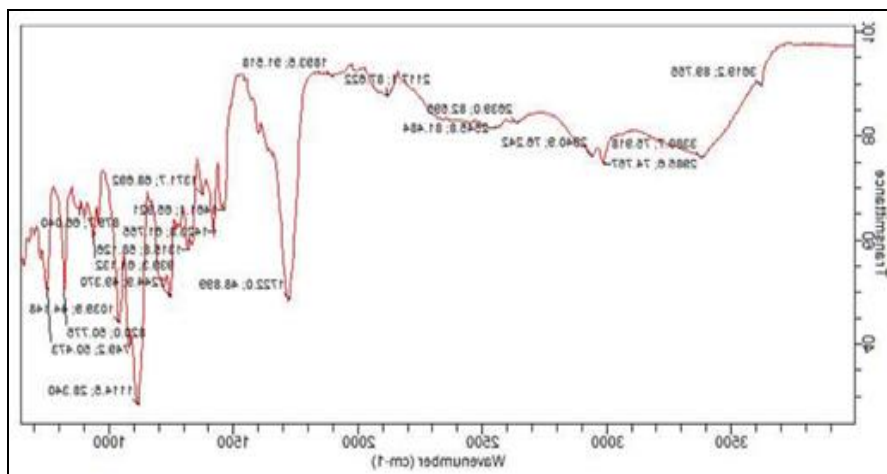


FIG. 2: FTIR OF LACTIC ACID

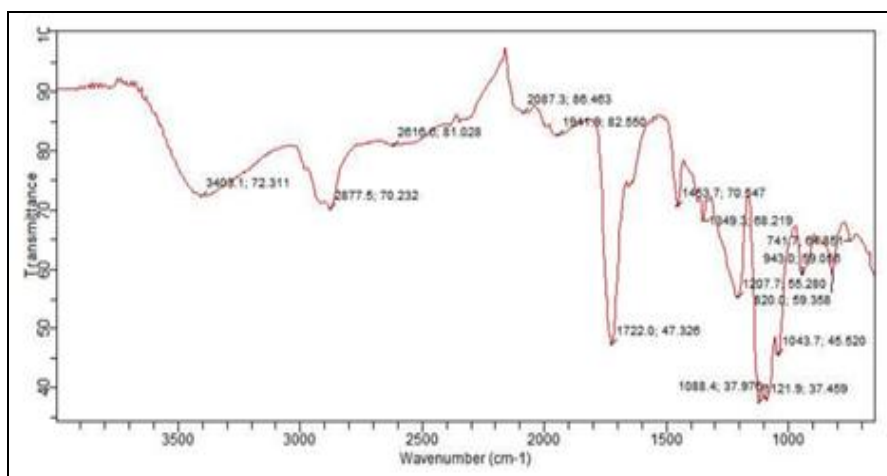


FIG. 3: LACTIC ACID+COCOA BUTTER+PEG

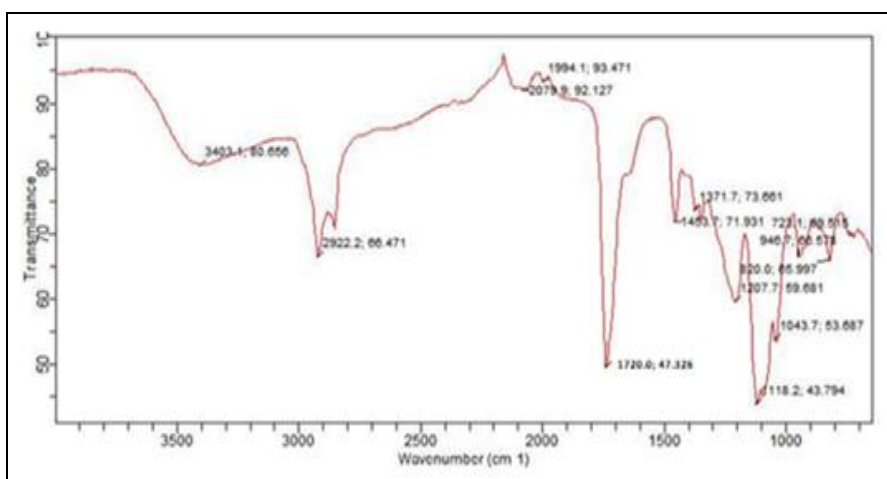


FIG. 4: FOR LACTIC ACID+COCONUT OIL+PEG

TABLE 4: FT-IR SPECTRAL ANALYSIS FOR PURE LACTIC ACID

Sr. no.	Functional group	Standard frequency(cm ⁻¹)	Lactic acid (cm ⁻¹)
1	C=O	1725-1700	1722.0
2	C-H	2962-2853	2840.9
3	C-O	1300-1000	1244.9
4	O-H	3650-3590	3619.2

TABLE 5: FT-IR SPECTRAL ANALYSIS FOR LACTIC ACID+COCOA BUTTER+PEG

Sr. no.	Functional group	Standard frequency(cm ⁻¹)	Lactic acid (cm ⁻¹)	LACTIC ACID+COCOA BUTTER+PEG
1	C=O	1725-1700	1722.0	1722.0
2	C-H	2962-2853	2840.9	2877.5
3	C-O	1300-1000	1244.9	1207.5
4	O-H	3650-3590	3619.2	3403.1

TABLE 6: FT-IR SPECTRAL ANALYSIS FOR LACTIC ACID+COCONUT OIL+PEG

Sr. no.	Functional group	Standard frequency (cm ⁻¹)	Lactic acid (cm ⁻¹)	Lactic Acid+Coconut OIL+PEG
1	C=O	1725-1700	1722.0	1720.0
2	C-H	2962-2853	2840.9	2877.5
3	C-O	1300-1000	1244.9	1207.5
4	O-H	3650-3590	3619.2	3403.1

Accelerated Stability Study of Face Cream: The accelerated stability study was conducted for 3 months. After each month the samples were obtained and examined for appearance, pH,

homogeneity, spreadability, after feel, removal of cream, type of smear and irritation. The results obtained from the accelerated stability study are mentioned in the **Table 7**.

TABLE 7: ACCELERATED STABILITY STUDY OF FACE CREAM

Time	Temp	Appearance	pH	Homogeneity	spreadability	After feel	Type of smear	Removal	Irritation
Initial Month	4-8	No change in colour	5.2±0.25	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
	25	No change in colour	5.4±0.40	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
	40	No change in colour	5.1±0.35	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
After 1 month	4-8	No change in colour	5.7±0.25	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
	25	No change in colour	5.3±0.40	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
	40	No change in colour	5.6±0.30	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
After 2 month	4-8	No change in colour	5.2±0.25	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
	25	No change in colour	5.5±0.55	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
	40	No change in colour	5.3±0.25	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
After 3 month	4-8	No change in colour	5.0±0.40	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
	25	No change in colour	5.9±0.35	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil
	40	No change in colour	5.8±0.35	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil

CONCLUSION: Anti-aging cream containing Lactic Acid and Cocoa Butter was formulated. According to FTIR study performed it is shown that all the ingredients were compatible with each other. The findings showed that the prepared antiaging cream and their contents were of uniform quality and easy to use. Based on the aforesaid findings, it can be concluded that Lactic Acid and

Cocoa Butter containing formulations are safe and suitable for use on the skin.

CONFLICTS OF INTEREST: Nil

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