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Development and evaluation of medicated cosmetic cream to produce triple effect on skin for the treatment of uneven skin tone

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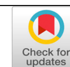
ABSTRACT



Combinations of drugs are available in the market only to produce a single effect. To overcome this, a combination of three drugs namely α-arbutin, glutathione, and grape seed extract, are used to benefit the triple effect on the skin. The set forth study to formulate and evaluate medicated cosmetic cream in an effective curative strategy for derma to produce a triple effect. Medicated cosmetic cream containing triple bioactive agents formulated by the O/W dispersion method. Fourier transform infrared (FT-IR) and differential scanning calorimetry (DSC) was carried out for characterization. The formulated cream assessed for physical appearance, homogeneity, pH, viscosity, particle size analysis, spreadability, percentage yield, drug content, diffusion studies, release kinetics, acute dermal toxicity studies, and short-term stability studies. FT-IR and DSC studies revealed that no chemical interaction existed between the drug and excipients used. The optimized formula A5 revealed to be off white, semisolid, pleasant odor, smooth & slippery texture, non-greasy smear, easy to remove, and homogenous. The A5 cream exhibited a pH of 5.89, Viscosity of 1597.73 CPS, Spreadability of 20.86 g.cm/sec., the Particle size of 0.237%, Percentage yield of 98.8%, Drug content of 99.22 at 289 nm, 99.75 at 200 nm and 100.1 at 286 nm. The optimized cream demonstrated prolonged drug release 94.41% at 289 nm, 92.68 at 200 nm, and 92.78 at 286 nm up to 12 hrs. The optimized cream remained stable after the heating-cooling cycle at 4° C, 45° C and at room temperature. Furthermore, acute dermal toxicity studies showed the optimized formulation to be non-irritant, with no erythema, and no edema. The present study signified that the multiple combinations showed a better synergistic effect, superior efficacy, and multiple activities that resulted in the best, smooth, and stable medicated cosmetic cream.

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INTRODUCTION

Humans are always fascinated by the color and appearance of the skin. To look stunning and beautiful is a natural aspiration. Men and women always harbor a hidden desire to change their skin appearance. This increased awareness of looking good has also brought in more sensitivity to problems of the skin, especially the face. The ability to alter the statement of pigment and wrinkle content in the skin, to advance an even-looking skin tone and a progressively young appearance, profoundly wanted in the present Society. Indians are seeing a change

in outlook from conventional techniques for utilizing home items to present-day strategies by utilizing medicated cosmetic cream to become fair (Berry, 2016).

Medicated cosmetics are cosmetic products with bioactive ingredients purported to have medical benefits. They are used to ensure skin against the harmful exogenous and endogenous substances and improve the excellence and allure of skin (Mohanty, 2012). Utilization of beautifiers building up an alluring outer appearance, however, towards accomplishing life span of good wellbeing by lessening skin issue.

The ingredients, namely synthetic or natural present, supports the texture, the health of the skin, skin integrity, moisturizing, and the elasticity is also maintained by reducing the type I collagen and photo-protection, etc. This behavior of cosmetic is due to the constituents in skincare formulation it reduces the free radicals produced by the skin and deal with the properties of skin for a long time. The cosmetic products are superlative to diminish skin issues such as hyper-pigmentation, skin aging, skin wrinkling and rough skin texture (Whittaker, 2008).

Skin care cosmetics are being used for several functions like anti-oxidants, anti-inflammatory, anti-aging, anti-microbial, antiseptic, skin whitening agents, etc. Vitamins are also added in skincare cosmetic formulation (Saraf and Kaur, 2010). Triple combination activity includes one skin whitening agent that can be synergistically boosted by the inclusion of UV-inhibitor or UV-absorber, one antioxidant activity and a bioactive agent having anti-aging benefits. (Datta and Paramesh, 2010; Smit *et al.*, 2009).

The α-Arbutin is a glycosylated form of hydroquinones; they are used as a skin whitening agent. It has been accounted for the inhibition effect of tyrosinase and melanin synthesis hence, it plays a significant role in the whitening of the skin. It is quicker and successful than kojic acid (skin lightener, which is commonly used), and the hydroquinone is left in the shade. Additionally, it has sun protection properties. The melanogenesis is hindered by reversibly or competitively with the help of binding to tyrosinase that causes tyrosinase for the mRNA transcription (Ros *et al.*, 1993).

Glutathione is small molecules with molecular weight is low, water-soluble thiol-tripeptide formed by these amino acids (glutamate, cysteine, and glycine) compound found in our bodies. It is used to reduce stress-induced wrinkles. Besides, it has likewise been embroiled in skin lightening. It additionally counters oxidative stress.

The tyrosinase glycosylation inhibition, the maturation is blocked, and the GERL (Golgi endoplasmic reticulum lysosome) is transferred to the melanosome. It also quenches peroxides and the present free radicals the tyrosinase activation and the formation of melanin (Sonthalia and Sarkar, 2017; Singhal *et al.*, 2013; Baskar *et al.*, 2018).

Grape (Vitis vinifera) seed extract is an excellent anti-oxidants that enhances the skin texture and acts as anti-aging (Shi *et al.*, 2003). They contain important constituents, namely the phenols, oligomeric proanthocyanidin complexes, vitamin C, and E are sourced from grape seeds belongs to the family of flavonoids. Proanthrocynidin shows greater oxygen quenching ability when compared to vitamin E and vitamin C. Besides; they increase the sun protection factor on application (Lin *et al.*, 2008).

Therefore, the purpose of this study was to formulate medicated cosmetic cream by mixing the triple bioactive agents to produce multipurpose effects on the skin, such as whitening with additive sunscreen, anti-aging, and anti-wrinkle properties and as an anti-oxidant.

MATERIALS AND METHODS

α-Arbutin was procured from Indchem International, Maharashtra; oxidized glutathione was procured from Kyowa Hakko Bio India Pvt. Ltd., Mumbai, Maharashtra and grape seed extract was extracted in Department of Pharmacognosy, JSS College of Pharmacy, JSS AHER, Mysuru; Ceto-stearyl alcohol from Godrej Industries Ltd., Maharashtra; Cetomacrogol-1000 B. P from Croda, Maharashtra; Light Liquid Paraffin, Propylene Glycol I. P, Disodium EDTA, Benzyl Alcohol I.P. purchased from Merck; White Soft Paraffin from Unicorn Petroleum Industries Pvt Ltd; Sepineo P600 from SEPPIC, Paris, France; The other excipients and chemicals used in the study area of laboratory and analytical grade.

Preformulation studies

The following preformulation studies were performed for α-arbutin, oxidized glutathione, and Grape seed extract the pure drug.

Characterization of Pure drug

UV visible (Ultraviolet-visible) spectrophotometer

λ_{max} of α-arbutin, oxidized glutathione, and grape seed extract was done in Phosphate buffer of pH 5.5 by simultaneous estimation method.

Fourier transform infrared (FT-IR) studies

FT-IR spectroscopic analysis was carried out for pure drugs and polymers for their compatibility

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evaluation. The three API was mixed with KBr separately by using mortar and pestle and compressed to form a KBr pellet by using a KBr press (Technosearch Instruments, Mumbai, India) at 5 tons of pressure for about 5 min. The results were recorded using the FT-IR spectrophotometer (8400S, Shimadzu, Kyoto, Japan).

Differential Scanning Calorimeter (DSC)

Differential Scanning Calorimeter (DSC-60, Shimadzu, Kyoto, Japan) used to carry out the thermal analysis for confirming the compatibility between pure drugs and polymers. Taken 5 mg of samples sealed hermetically in aluminum pans and heated from 20 to 300°C and the heating rate of 20°C/min under nitrogen atmosphere.

Preparation of cream base

The required quantity of ceto-stearyl alcohol, cetomacrogol-1000 B.P., Light Liquid Paraffin, soft paraffin (white), propylene glycol I.P., sepineo P600, and disodium EDTA was accurately weighed in a beaker and heated gently to a temperature of 70-75^{0}C using the hot plate in varying concentrations as shown in Table 1.

Simultaneously, the required quantity of aqueous phase (water) was placed on a heating mantle at a temperature 70-75^{0}C using a hot plate and added to the above step (Villarama and Maibach, 2005).

Formulation of medicated cosmetic cream

Further α-arbutin, oxidized glutathione, and grape seed extract were weighed and dissolved in little quantity of propylene glycol and purified water and added to the cream base (Chakraborty *et al.*, 1998).

When the cream temperature drops to 40 ^{0}C, preservative (Benzyl Alcohol) was added and stirred for a few more minutes to ensure uniform dispersion, and the pH of the cream was adjusted by triethanolamine as shown in Table 1.

Evaluation of Prepared Cream

Physical Appearance and Homogeneity

The formulations were prepared and evaluated for color, homogeneity, grittiness, and smoothness by visual inspection (Rai *et al.*, 2019).

Measurement of pH

1gm of cream was weighed in a beaker; to it, 100ml of distilled water was added and stirred using a magnetic stirrer until it forms a uniform solution. Then the pH was recorded using probe pH meter (Burger *et al.*, 2016).

Viscosity measurement

It was carried out for all the formulations was measured using Brookfield Viscometer (DV II+ Pro model) using spindle no. T-F with ± 50 torque at 30-50 rpm (Rai *et al.*, 2019).

Particle size analysis

Measurement of globule size was performed by zeta sizer 3000 (Malvern Instruments, Malvern, UK). The prepared cream was diluted in (1:1000) with deionized water and measured the particle diameter range. The number of photon counts per second was evaluated at room temperature.

Spreadability

1gm of the cream was sandwiched between the two glass plates (20cm x 20cm). 1kg of the weight was kept on the upper glass plate for 5mins to expel the air. The hook and spring help to pull the 100gms from the top plate. The time taken was noted, which covers the distance. The spreadability was calculated using the following equation and was expressed as gms.cm.sec^{-}1 (Allemann and Baumann, 2008; Rai *et al.*, 2019).

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

Where,

S = Spreadability (cm.g.s-1)

M=Weight (g) in the pan (tied to the upper slide)

L = Length (cm) moved by the glass slide.

T = Time (in a sec) taken to separate the slide completely.

Percentage yield

The percentage yield was calculated to determine the percent yield or efficiency of any method. The production yield of cream was determined by weighing accurately the initial weight of raw materials and the final weight of cream recovered by using below formula:

% Product Yield =

$$\frac{Practical\ yield\ of\ cream}{Theoretical\ yield\ of\ cream} \times 100$$

Drug content

For determining the drug content, 0.1gm of the formulation was dissolved in PBS pH 5.5, and volume was made up to 100 ml with the same solvent. After appropriate dilutions, absorbance was measured using a UV-Visible spectrophotometer at 289, 200, and 286 nm, respectively.

The percentage of drug content was determined following the below-given equation. The results were done triplicate and represented as mean±SD (Taha *et al.*, 2016).

Table 1: Formulation chart of medicated cosmetic cream

Sl. No	Ingredients (%W/W)	A1	A2	A3	A4	A5	A6	A7
1.	α-Arbutin	2.000	2.000	2.000	2.000	2.000	2.000	2.000
2.	Oxidized Glutathione	2.000	2.000	2.000	2.000	2.000	2.000	2.000
3.	Grape seed extract	0.200	0.200	0.200	0.200	0.200	0.200	0.200
4.	Ceto-stearyl Alcohol	9.000	9.250	9.500	9.750	10.000	10.250	10.500
5.	Cetomacrogol-1000 B.P.	3.500	3.800	4.000	3.500	4.000	3.800	4.000
6.	Light Liquid Paraffin	5.000	5.000	4.500	4.000	5.000	4.000	4.500
7.	White Soft Paraffin	7.000	7.000	7.000	7.000	7.000	7.000	7.000
8.	Propylene Glycol I.P.	16.800	16.000	15.500	15.500	15.000	14.500	13.000
9.	Disodium EDTA	0.050	0.050	0.050	0.050	0.050	0.050	0.050
10.	Sepineo P600	1.300	1.350	1.350	1.400	1.500	1.500	1.550
11.	Benzyl Alcohol I.P.	1.000	1.000	1.000	1.000	1.000	1.000	1.000
12.	Triethanolamine BP	QS	QS	QS	QS	QS	QS	QS
13.	Deionized Water Upto	100	100	100	100	100	100	100

QS = QuantitySufficient

The drug content was determined using the below formula:

% Drug content =

$$\frac{Actual\ Content}{Theoretical\ Content} \times 100$$

In-vitro drug diffusion studies and release kinetics

Franz diffusion cell (Perme Gear Inc., Bethlehem, PA, USA) was used to perform in-vitro diffusion study. 12 ml of phosphate buffer solution of pH 5.5 was filled in the receptor compartment and a magnetic stirrer rotating at 75 RPM. Dialysis membrane with pore size 0.65 μm was clamped between the receptor & donor compartment and was in contact with the receptor compartment media. In the donor compartment, 100mg of the sample was directly weighed into the membrane.

The entire system was kept at 37 $\pm$ 1°C by temperature regulating the water jacket. The test was carried out for 12 hrs. 1 ml of sample was withdrawn at a time intervals of 1, 2, 4, 6, 8, 10, and 12 hrs from the receiver compartment and was replenished with fresh PBS to conserve sink condition. Then the samples were analyzed using a UV-visible spectrophotometer at 289, 200, and 286 nm, respectively (Solano *et al.*, 2006).

Release Kinetics

BCP Software is used to obtain mathematical models such as Zero-order release, First order release, Higuchi, and Korsmeyer-Peppas model by using cumulative drug release data.

Acute Dermal Toxicity Studies using experimental animals

Nine Wistar rats with intact skin were used to evaluate acute dermal toxicity studies. All experimental animals were acclimatized for the laboratory condition for a period of one week prior to the initiation of the experiment. 24hr prior to the test, hair present on the dorsal side of the trunk was removed and applied with 100mg of 1% cream. It was covered with a cotton bandage for 24 hrs and washed using distilled water and made it dried. After the exposure period, the animals were observed for scoring the signs of the toxicity at 30min, 4 hr and 24hr as below (Mahtab *et al.*, 2016).

0 —No reaction

1 —Redness

2 — Erythema

3 — Ulceration (OCED, 2005)

Accelerated physical st ability studies

The medicated cosmetic cream was subjected to heating-cooling cycle to determine the stability of pH and viscosity (Pa. s) at room temperature, by keeping them at 4° C and 45° C for 6 cycles for 24 h (Imokawa, 1989).

RESULTS AND DISCUSSION

Characterization of pure drug

UV visible (Ultraviolet-visible) spectrophotometer studies

Absorption maxima of α-arbutin, oxidized glutathione, and grape seed extract in Phosphate buffer pH 5.5 is shown in Table 2 and Figure 1, Figure 2 and Figure 3.

Calibration curve

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Table 2: Maximum absorbance wavelength (λmax)of α-arbutin, oxidized glutathione, and grape seed extract

Solvent	Active Pharmaceutical Ingred	Wavelengths of maximum absorbance, λmax (nm)	
		Observed	Reported
Phosphate buffer pH of 5.5	α-arbutin	288.5	289
	oxidized glutathione	199.5	200
	Grape seed extract	285.3	286

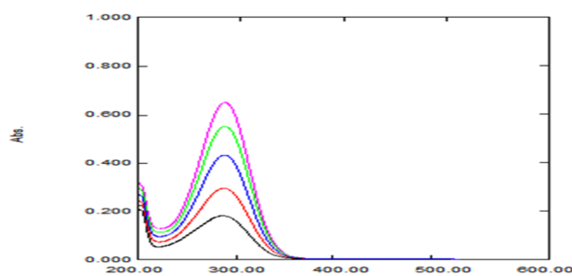

Figure 1: UV spectrum of α-arbutin in Phosphate buffer pH 5.5

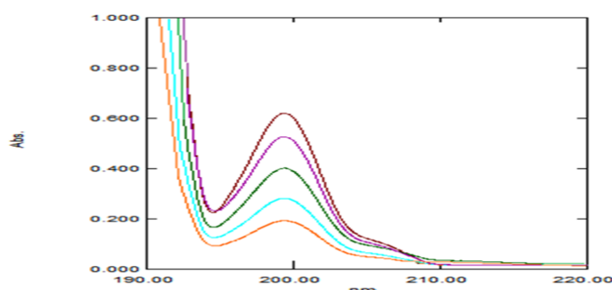

Figure 2: UV spectrum of oxidized glutathione in Phosphate buffer pH 5.5

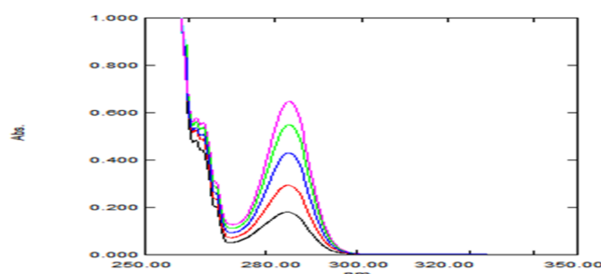

Figure 3: UV spectrum of grape seed extract in Phosphate buffer pH 5

The calibration curve of α-arbutin, oxidized glutathione, and grape seed extract was plotted in Phosphate buffer (pH 5.5), values, and graphs obtained for it are depicted in Tables 3, 4 and 5 and Figures 4, 5 and 6 respectively.

α -arbutin

Regression coefficient of 0.9971 with slope value 0.0114 and 0.0088 as Y-intercept value.

Oxidized glutathione

The regression coefficient was 0.999, with a slope value 0.0607 and 0.001 as the Y-intercept value.

Grape seed extract

The regression coefficient of grape seed extract was 0.9988, with a slope value 0.0022 and 0.0131 as the Y-intercept value.

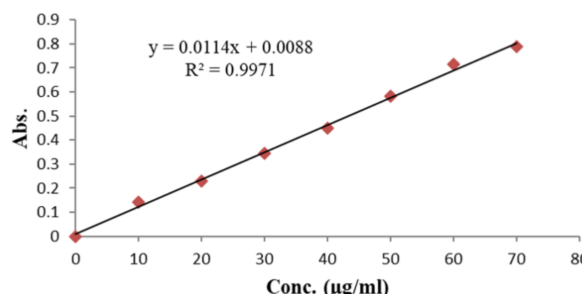

Figure 4: Calibration curve of α-arbutin in Phosphate buffer pH 5.5

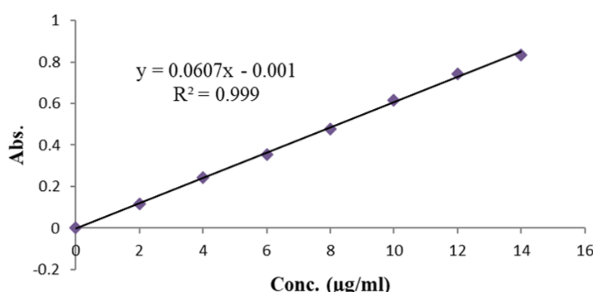

Figure 5: Calibration curve of oxidized glutathione in Phosphate buffer pH 5.5

Fourier transform infrared (FT-IR) studies

The FT-IR spectrum of the pure drug was compared with those of the FT-IR spectra of drugs with excipients, as shown in Figure 7. The Functional groups obtained for drugs and physical mixture of drugs and excipient was found to be in correlation. FT-IR spectroscopic interpretation results showed no interactions between drug mixture and polymer occurred because no change in the peaks was seen. Hence, drug mixture and selected polymer were

Table 3: Absorbancevalues for different concentrations of α-arbutinin Phosphate buffer pH 5.5

Sl. No.	The concentration of FE-B (μg/ml)	Absorbance
1.	0	0.000
2.	10	0.143±0.04
3.	20	0.228±0.01
4.	30	0.344±0.02
5.	40	0.451±0.02
6.	50	0.582±0.05
7.	60	0.714±0.04
8.	70	0.788±0.007

Standard Deviation, Mean N= 3

Table 4: Absorbance values for different concentrations of oxidized glutathione in Phosphate buffer pH 5.5

Sl. No.	The concentration of FE-G (μg/ml)	Absorbance
1.	0	0.000
2.	2	0.118±0.05
3.	4	0.245±0.05
4.	6	0.356±0.04
5.	8	0.479±0.02
6.	10	0.615±0.02
7.	12	0.742±0.03
8.	14	0.834±0.04

Standard Deviation, Mean N= 3

Table 5: Absorbance values for different concentrations of grape seed extract in Phosphate buffer pH 5.5

Sl. No.	The concentration of FE-A (μg/ml)	Absorbance
1.	0	0.00
2.	50	0.132±0.09
3.	100	0.239±0.08
4.	150	0.337±0.01
5.	200	0.463±0.09
6.	250	0.561±0.07
7.	300	0.659±0.03
8.	350	0.783±0.05

Standard Deviation, Mean N= 3

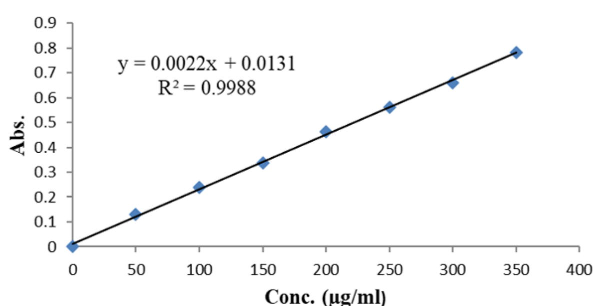

Figure 6: Calibration curve of grape seed extract in Phosphate buffer pH 5.5

compatible with each other.

Differential Scanning Calorimeter (DSC)

The DSC thermograms of Arbutin, Oxidized glutathione, grape seed extract, and their physical mixture are depicted in Figure 8. Endotherm corresponding to melting of Arbutin, Oxidized glutathione, Grape seed extract was observed in respective thermograms at 208.3°C, 191.91°C, and 120.09°C, respectively. Compatibility analysis via DSC revealed an endothermic peak at 108.12°C, represents the peak of Grape seed extract, and 123.59°C

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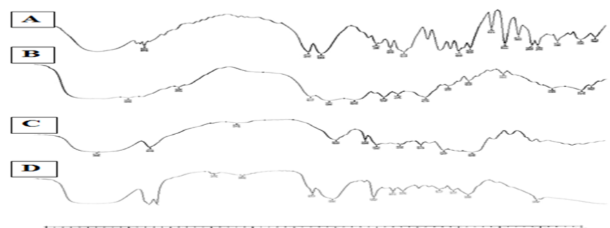

Figure 7: Overlain FT-IR spectra of (A) α-Arbutin pure drug (B) Oxidized glutathione pure drug(C) Grape seedextract pure drug (D)Physical Mixture of α-Arbutin + Oxidized glutathione+ grape seed extract+ Ceto-stearyl Alcohol+Cetomacrogol-1000 B.P.+Sepineo P600

represents the peak of α-Arbutin and Oxidized glutathione in DSC thermogram of the physical mixture. As the corresponding endothermic peaks of the drug as well as excipients were adequately retained in their physical mixture, it was inferred that the drug did not interact while in contact with the excipients, suggesting good compatibility among them.

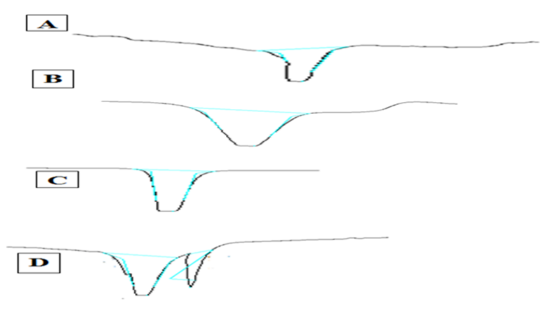

Figure 8: Overlain DSC thermograms of (A) α-Arbutin (B) Oxidized glutathione (C) Grape seedextract(D) Physical Mixture of α-Arbutin+ Oxidized glutathione+ grape seed extract +Ceto-stearyl Alcohol+Cetomacrogol-1000 B.P+Sepineo P600

Evaluation of Prepared Cream

Physical Appearance & Homogeneity

The topical cream prepared was off-white in color and opaque without any lumps, aggregates, particles, etc. indicating excellent homogeneity of the formulation, as shown in Figure 9. Even the same was observed in the stability batch of the formulation.

Measurement of pH

The pH of formulated medicated cosmetic cream was noted between 5.67 to 6.9. The pH of optimized formulation (A 5) was 5.89 that lay in the normal skin pH range, which was acceptable. The results are shown in Table 6.

Measurement of viscosity

Figure 9: Physical appearance of the cream

The viscosity of formulated cream was noted between 738.75 to 5634.45 CPS. It was found to be dependent on polymeric and emulsifier concentration in cream formulations. The viscosity value of the optimized formulation was measured to be 1597.73 CPS are shown in Table 6.

Particle size analysis

The PDI value of all samples was found to be within 0 to 1, and Z-avg values (Harmonic intensity averaged particle diameter) were found between 293.9 to 753.7 nm, indicating homogeneous dispersion of globules. The results obtained for particle size analysis are shown in Table 6.

Spreadability

The spreadability results were in the range of 20.24-25.85. The formulations A 1 showed the highest spreadability, whereas A 1 showed the least spreadability as it was more viscous. Though there are no acceptance limits for spreadability, it is evident from the literature that the lesser the time taken for the top plate to cover the distance better is the spreadability. Hence, spreadability of all the formulations was acceptable are shown in Table 6.

Percentage yield

The optimized cream A 5 showed the highest percentage yield, which was calculated based on the theoretical weight and practical weight of the product that had been obtained are shown in Table 6.

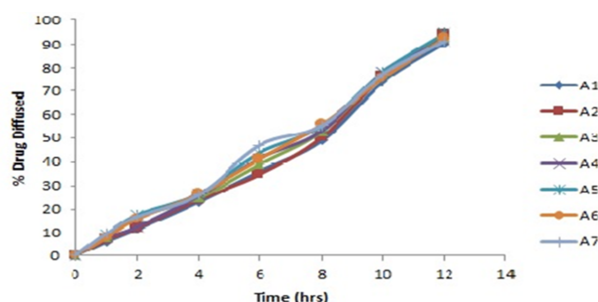

Figure 10: Graph showing % drug diffused of formulation at 289 nm

Drug content

Table 6: Evaluation of medicated cosmetic cream

Formulation Code	pH	Viscosity (CPS)	Particle size PDI %	Spreadability (g.cm/sec)	Percentage Yield
A1	5.67±0.005	738.75±0.725	0.532	25.85±0.36	93.6±0.06
A2	5.68±0.009	907.24±0.132	0.833	25.42±0.48	96.9±0.05
A3	5.69±0.009	1004.57±0.307	0.545	23.73±0.23	95.3±0.01
A4	5.68±0.005	1203.24±0.502	0.582	22.97±0.25	96.2±0.02
A5	5.89±0.000	1597.73±0.701	0.237	20.86±0.25	98.8±0.02
A6	6.2±0.020	2708.94±0.39	0.461	20.29±0.19	97.1±0.09
A7	6.9±0.005	5634.45±0.19	0.407	20.24±0.00	97.4±0.10

* Standard Deviation, Mean N= 3

Table 7: Data for drug content of formulations

Formulation Code	Assay % ± SD Mean ± SD*		
	289 nm	200nm	286nm
A 1	101.72 ± 0.11	100.9 ± 0.36	99.8 ± 0.48
A 2	98.92 ± 0.52	100.1 ± 0.28	99.8 ± 0.3
A 3	98.85 ± 0.20	98.7 ± 0.12	99.1 ± 0.65
A 4	98.12 ± 0.4	97.92 ± 0.6	98.5 ± 0.78
A 5	99.22 ± 0.34	99.75 ± 0.15	100.1 ± 0.46
A 6	98.9 ± 0.6	98.32 ± 0.65	98.8 ± 0.9
A 7	97.80 ± 0.43	97.13 ± 0.33	97.54 ± 0.6

*Standard Deviation, Mean N=3

Table 8: Data for in vitro drug diffusion studies at 289 nm

Time (hrs)	% Drug diffused (w/w) Mean ±SD*						
	A1	A2	A3	A4	A5	A6	A7
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	6.18 ± 0.02	6.88 ±0.01	8.01 ± 0.01	7.88 ±0.01	9.35 ±0.02	8.02 ±0.01	9.86 ±0.02
2	11.57 ± 0.01	12.02 ±0.02	16.64 ±0.02	12.62 ±0.01	17.63 ±0.01	15.92 ±0.02	16.87 ±0.03
4	23.11 ±0.03	24.04 ±0.01	24.82 ±0.01	25.62 ±0.02	26.53 ±0.026	26.43 ±0.01	26.02 ±0.03
6	35.94 ±0.045	34.65 ±0.02	38.83 ±0.01	41.33 ±0.01	43.64 ±0.02	41.02 ±0.02	46.88 ±0.08
8	48.83 ±0.02	50.22 ±0.02	52.72 ±0.02	52.96 ±0.01	55.02 ±0.02	55.72 ±0.02	55.23 ±0.04
10	73.86 ±0.02	76.46 ±0.02	77.23 ±0.03	78.23 ±0.02	78.10 ±0.03	75.42 ±0.02	77.22 ±0.02
12	90.42 ±0.02	93.54 ±0.05	92.18 ±0.01	91.41 ±0.03	94.41 ±0.02	92.13 ±0.01	91.20 ±0.03

*Standard Deviation, Mean N=3

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Table 9: Data for in vitro drug diffusion studies at 200nm

Time (hrs)	% Drug diffused (w/w) Mean ±SD*						
	A1	A2	A3	A4	A5	A6	A7
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	6.94 ±0.01	7.60 ±0.03	7.21 ±0.02	7.04 ±0.02	8.98 ±0.01	8.46 ±0.021	8.27 ±0.05
2	14.77 ±0.03	15.65 ±0.01	14.27 ±0.01	15.30 ±0.01	15.70 ±0.02	15.68 ±0.02	14.60 ±0.01
4	23.64 ±0.03	26.42 ±0.01	26.18 ±0.03	23.87 ±0.03	26.56 ±0.02	27.88 ±0.01	27.05 ±0.02
6	44.32 ±0.01	45.16 ±0.03	41.76 ±0.02	45.62 ±0.03	46.23 ±0.01	46.22 ±0.02	46.21 ±0.01
8	61.37 ±0.01	60.12 ±0.03	61.89 ±0.03	62.16 ±0.02	62.63 ±0.01	62.34 ±0.03	60.21 ±0.02
10	76.22 ±0.01	76.16 ±0.03	78.32 ±0.02	78.16 ±0.01	79.39 ±0.01	78.43 ±0.03	78.72 ±0.02
12	88.03 ±0.03	88.28 ±0.03	91.06 ±0.02	91.74 ±0.03	92.68 ±0.03	91.56 ±0.02	90.16 ±0.04

*Standard Deviation, Mean N=3

Table 10: Data for in vitro drug diffusion studies at 286nm

Time (hrs)	% Drug diffused (w/w Mean ±SD*						
	A1	A2	A3	A4	A5	A6	A7
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	8.33 ±0.04	8.98 ±0.02	9.54 ±0.01	10.35 ±0.02	11.96 ±0.01	10.59 ±0.02	10.66 ±0.03
2	13.78 ±0.01	13.85 ±0.01	14.01 ±0.01	14.49 ±0.01	15.55 ±0.02	15.37 ±0.01	15.42 ±0.01
4	24.65 ±0.02	24.67 ±0.01	24.26 ±0.02	23.43 ±0.03	25.49 ±0.01	24.08 ±0.02	25.09 ±0.03
6	37.58 ±0.03	37.92 ±0.01	38.86 ±0.02	38.16 ±0.03	39.39 ±0.01	39.12 ±0.02	39.89 ±0.02
8	54.45 ±0.01	54.66 ±0.02	54.67 ±0.01	54.74 ±0.02	55.27 ±0.01	55.65 ±0.02	55.27 ±0.03
10	77.87 ±0.02	76.26 ±0.01	77.47 ±0.02	77.62 ±0.01	79.56 ±0.02	78.34 ±0.01	78.24 ±0.02
12	90.32 ±0.03	90.56 ±0.02	90.27 ±0.01	91.46 ±0.01	92.78 ±0.02	91.40 ±0.02	91.45 ±0.05

*Standard Deviation, Mean N=3

Table 11: Release Kinetics at 289 nm, 200 nm & 286 nm

Mathematical Model	R2 of A5 Optimized Formulation		
	289 nm	200 nm	286 nm
Zero order release	0.994	0.9984	0.9908
First order release	0.8504	0.9084	0.8698
Higuchi Model	0.8859	0.8997	0.8855
Korsmeyer Peppas Model	0.8759	0.8852	0.8625

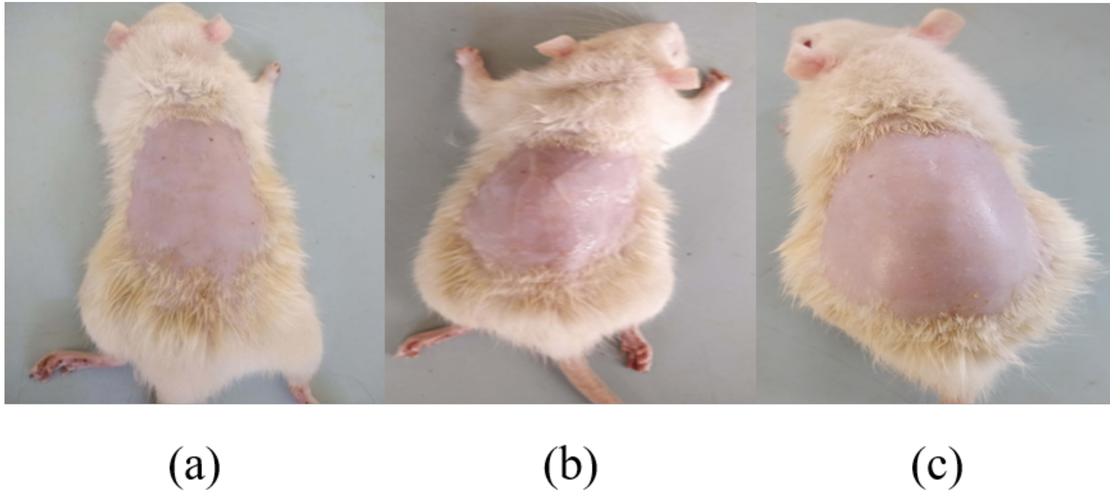

Figure 13: Images of dorsal skin of mice (a) Control (b) Treated with Medicated Cosmetic Cream (c) After 24 hrs of application

Table 12: pH and viscosity accelerated stability studies (Heating and cooling cycle) of the cream

Formulation Code	Condition for 24 hours					
	pH			Viscosity		
	4° C	45° C	Room Temp.	4° C	45° C	Room Temperature
A1	5.66 ±0.005	5.64 ±0.002	5.67 ±0.001	725.42 ±0.002	721.32 ±0.003	729.52 ±0.005
A2	5.67 ±0.004	5.67 ±0.007	5.68 ±0.008	900.42 ±0.003	895.14 ±0.003	902.12 ±0.002
A3	5.66 ±0.004	5.64 ±0.005	5.66 ±0.002	1000.1 ±0.001	996.24 ±0.004	1002.5 ±0.004
A4	5.67 ±0.002	5.67 ±0.002	5.68 ±0.001	1202.51 ±0.05	1997.62 ±0.005	1211.20 ±0.008
A5	5.83 ±0.008	5.80 ±0.003	5.84 ±0.003	1532.02 ±0.008	1523.34 ±0.001	1540.84 ±0.007
A6	6.0 ±0.005	5.9 ±0.001	6.1 ±0.003	2704.31 ±0.007	2696.10 ±0.003	2709.42 ±0.003
A7	6.7 ±0.006	6.4 ±0.002	6.8 ±0.005	5629.11 ±0.004	5617.33 ±0.002	5633.54 ±0.009

Mean±SD, n=3

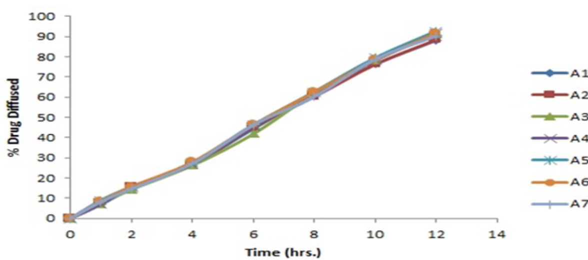

Figure 11: Graph showing % drug diffused of formulation at 200 nm

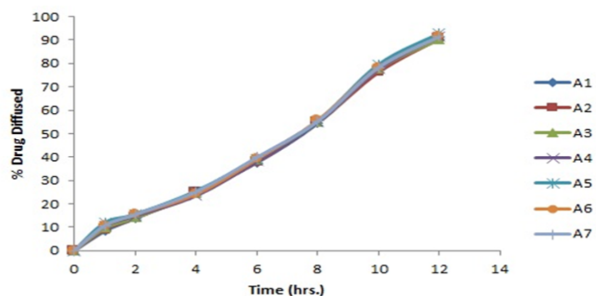

Figure 12: Graph showing % drug diffused of formulation at 286 nm

The percentage of drug content in different formulations was calculated using the formula:

% Drug content =

$$\frac{Practical\ Amount\ of\ drug\ concentration}{Theoretical\ amount\ of\ drug\ concentration} \times 100$$

The assay for the prepared formulation of cream was found between 97.13 to 101.72 % and showed that there were no distinct changes in the assay content of formulations, as shown in Table 7.

In-vitro drug diffusion studies and release kinetics

The results are shown in Tables 8, 9 and 10 and the corresponding graph is shown in Figures 10, 11 and 12.

Release Kinetics

Comparatively, A5 showed the highest percentage of drug release. Therefore, release kinetics is done for A5 formulation. The release kinetic profile of the optimized cream is depicted in Table 11. The highest drug release was noted in optimized formula A5 with having drug content 99-100 % showed drug release of 92-94% following zero-order kinetics. This is attained because of smaller drug particles than the thickness of the system, and the release of the drug occurs in a dimensional way. Thereof the release profile followed the diffusion process as Higuchi model.

Acute Dermal Toxicity Studies using experimental animals

The prepared cream was evaluated for its skin irritancy test as per the procedure, there was no erythema and edema and any kind of reaction starting from 0th-14th days of study, and scores are given respectively. Thus, the cream was found to be safer for topical use. The observations were shown in Figure 13 and scores are shown in Table 12.

Accelerated Stability studies

The formulated cream appeared to be off-white with satisfactory physical characteristics and consistency in texture, viscosity, and pH. The stability test results at the accelerated conditions of 4° C, 45° C and room temperature showed that there were no changes to the physical properties of the cream in terms of pH, the phase of separation and color, although there was slightly changed in viscosity as showed in Table 12. Nevertheless, the overall evaluation, the cream demonstrated the potential for a prototype product leading to the large-scale production of cosmetic cream.

CONCLUSIONS

In the current scenario, triple combinations are used to promote multiple benefits, not for single benefits. Selecting the most effective medications is very much important for its therapy. Therefore, there is a need to develop a formulation with multiple benefits. Multiple combination with different mechanism of action acting at different sites of the skin, giving synergistic action with the benefit of patient compliance and cost-effectiveness, which is not available in the market. Thus, the study demonstrated that the formulated cream has a great demand for the convenient treatment of uneven skin tone.

Ethics approval

Ethics approval from Institutional Animals Ethics Committee, JSS Academy of Higher Education And Research, JSS College of Pharmacy, Mysuru-570015

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Conflicts of Interest

The author confirms that this article content has no conflicts of interest.

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