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Formulation and characterization of anti-acne cream of clascoterone and tazarotene (combination therapy)

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ABSTRACT:

The study successfully developed and characterized a stable O/W cream combining clascoterone and tazarotene for acne treatment. Preformulation studies confirmed the drugs' organoleptic properties, solubility, and compatibility with selected excipients. Zeta potential measurements indicated colloidal stability, and in vitro release studies demonstrated a controlled drug release profile, with clascoterone releasing faster and tazarotene exhibiting prolonged retention, optimizing therapeutic efficacy. Accelerated stability testing confirmed the formulation's robustness under stress conditions, with no significant changes in pH, appearance, or drug content over four weeks. These findings suggest that the developed clascoterone-tazarotene O/W cream is a promising, stable, and effective topical treatment for acne, targeting multiple pathogenic pathways while minimizing irritation. Future studies should focus on ex vivo permeation assays and clinical trials to further validate its therapeutic potential. This formulation represents a significant advancement in acne therapy, combining novel mechanisms of action with enhanced patient compliance through optimized delivery.

Acne:

Acne vulgaris is a long-term inflammatory condition that affects the pilosebaceous units of the skin, often involving excess oil production and bacterial growth. It's one of the most common skin conditions among teenagers and young adults. In the United States alone, more than 50 million new cases are reported each year. Globally, acne affects an estimated 9.4% of the population. Between 1990 and 2019, worldwide cases rose significantly—from about 79.7 million to 117.4 million. Beyond the physical symptoms, acne can leave behind scars and dark spots, which often contribute to emotional and psychological challenges. Individuals with acne tend to report higher levels of low self-esteem, anxiety, and depression compared to those without the condition. (Basendwh, M.A. et al. 2024)

Acne vulgaris is a chronic inflammatory skin disease with a multifactorial pathogenesis involving disordered keratinization, androgens resulting in sebum overproduction, and microbial colonization with Cutibacterium acnes. Although a number of acne treatments areavailable, efforts to reduce side effects such as skin irritation, dryness, and photosensitivity and to improve efficacy via improved formulations and drugs with novel mechanisms of action are underway. Emerging treatments with novel mechanisms of action and improved formulations target various points along acne's multifactorial pathogenesis (John,J.J.H.A. et al. 2021)

Pathophysiology of acne:

Acne is a long-lasting inflammatory condition affecting the pilosebaceous unit.Its underlying causes include excessive oil production, irregular shedding of skin cells within hair follicles, and the growth of *Propionibacterium acnes* (now known as *Cutibacterium acnes*). Recent studies have provided new insights into the role of the sebaceous glands and the inflammatory impact of the skin's microbiome. During puberty, changes in the composition of skin oils—a condition known as dysseborrhoea—along with stress, cosmetic use, skin irritation, and possibly diet, can trigger inflammation and lead to the formation of various acne lesions (Dreno, B. et al.2017)

At the start of puberty, rising levels of circulating androgens stimulate the sebaceous glands to produce more sebum within the pilosebaceous unit. This increase in oil, along with other changes, creates a favorable environment for the growth of *Propionibacterium acnes* (now known as *Cutibacterium acnes*), a normally present skin bacterium. As it multiplies, *P. acnes* releases inflammatory substances and signaling molecules that trigger and sustain inflammation in the area. These factors may also contribute to an increase in keratinocyte production, further contributing to acne formation (Webster,

G.F. et al.2005)

Several biological processes are involved in the onset and progression of acne. It typically starts with a rise in androgen levels before puberty, which sets off a sequence of changes. These include disrupted shedding of skin cells and abnormal keratin buildup in the hair follicles, overgrowth of sebaceous gland cells (sebocytes), enlargement of the sebaceous glands, and an overall increase in sebum production (Bergfeld, W.f. et al.2004)

Acne is a multifactorial condition marked by different types of lesions, and its development involves a combination of genetic, hormonal, microbial, and inflammatory influences. This summary explores both current and emerging approaches to acne treatment, covering topical and systemic therapies, in-office procedures, and the role of diet. Common topical treatments include retinoids, benzoyl peroxide, antibiotics, and other targeted agents. For more severe cases, systemic therapies such as oral antibiotics, hormonal treatments, and isotretinoin are often effective. In addition, procedures like laser therapy, photodynamic therapy, chemical peels, and intralesional injections can help manage symptoms and reduce scarring. New research is also pointing toward innovative solutions such as biologics, bacteriophage therapy, probiotics, and peptides.

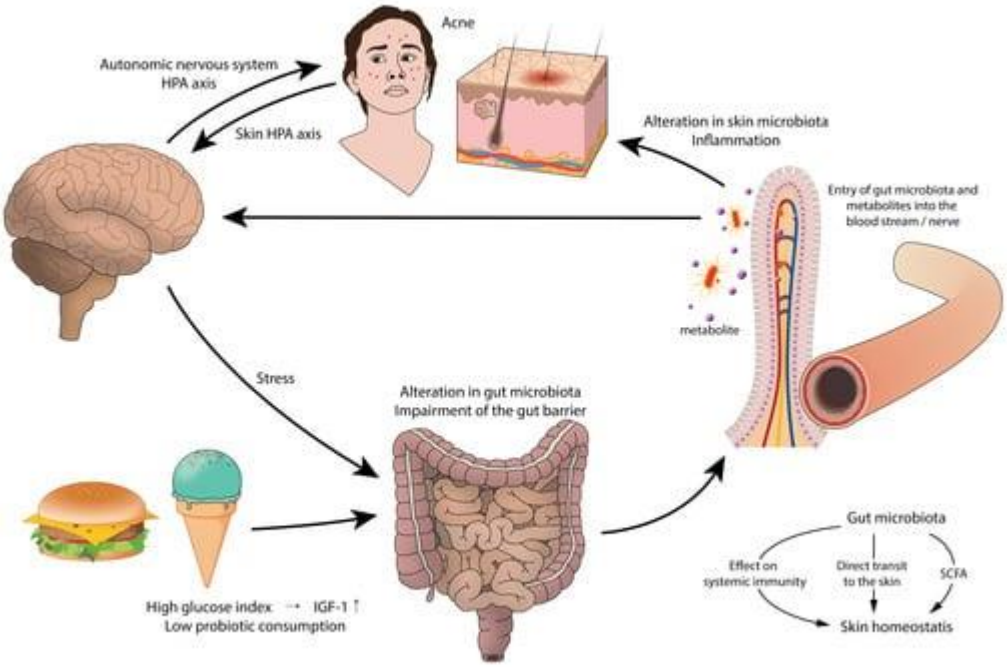


Figure 1: Pathophysiology of Acne (Lee, Y. B., Byun, E. J., & Kim, H. S. (2019)

Material and Method:

b) **Solubility studies in solvents and excipients**: In 1 millilitre of solvent (methanol, ethanol, Dimethyl sulfoxide (DMSO)) and excipients (oleic acid, corn oil, tween 80, propylene glycol, and polyethylene glycol 400), 10 milligrams of excess clascoterone and tazarotene were added separately. At room temperature, all of the samples were shaken for 24 hours. Subsequently, the samples underwent a 10-minute centrifugation at 10,000 rpm, and the supernatant was taken out and filtered using a 0.45-micron filter. At the lambda max, samples were examined using UV spectroscopy after appropriate dilutions were made using appropriate solvents. Solubility was computed using the noted absorbance.

c) **Identification of drug and analytical methodology:** UV spectrophotometry was used as an analytical method of clascoterone and tazarotene for identification and to quantify the drugs for different studies including solubility studies, partition coefficient, in vitro drug release studies. Methanol and PBS (pH 5.5) were used.

1) Determination of Absorption maxima (λmax) and construction of Calibration curve of Clascoterone and tazarotene

Preparation of stock solution and dilutions: 10 mg of tazarotene was dissolved in 10 ml methanol to create a stock solution containing one milligram of tazarotene per millilitre that is 1000 ppm. The stock solution was then diluted ten times to create a working standard of 100 ppm. It was then diluted once more to create a solution with a concentration of 10 ppm. Using this method, further dilutions were made by taking suitable quantities from 10

ppm solution and adding methanol respectively, to achieve the resultant dilutions of 2, 4, 6, 8, 10 ppm, respectively.

Determination of absorption maxima (λmax) and Calibration curve construction:

• Using a UV spectrophotometer, the produced solution was scanned between 200 and 400 nm to determine the absorption maxima (λmax) of tazarotene, which was then compared with published data. The produced dilutions were then scanned using a UV spectrophotometer at the determined absorption maxima (λmax), which is 310 nm to determine the absorbance. The absorbance and concentration of the drug was correlated to create a calibration curve, and linear regression analysis was used to determine the best-fit line.

d) Drug – excipient interaction by visual observation:

Several important factors that should be considered while doing visual observations to identify drug-excipient and excipient-excipient interactions include whether there are any physical changes, such as colour changes, phase separation, precipitation, or crystallisation.

e) Melting point: The melting point of clascoterone and tazarotene were determined using the capillary method. It was filled in a small quantity in a capillary which was inserted in melting point apparatus (MR-VIS, Labindia, Mumbai, India) and as the temperature raised the melting point of the drug was noted.

f) Partition coefficient: To evaluate the drug's hydrophilicity and lipophilicity, the partition coefficient must be determined. For this, the Shake Flask method was applied.. The samples were scanned at their lambda max. With the obtained absorbance, the concentration was calculated. Using the following formula, the partition coefficient (K) was determined: (Cumming H and Rucker C, 2017)

$K_{o/w} = C_o / C_w$ (Eq 4.1)

Where, K- partition coefficient

Co - concentration of clascoteronein organic solvent

Cw - concentration of clascoteronein distilled water.

1.4 Formulation of Clascoterone and tazarotene loaded emulsion:

The emulsion was prepared using coconut oil as oil phase, Tween 20 as surfactant, Propylene Glycol as co-surfactant and water as continuous phase. The emulsion prepared was an O/W emulsion. Tween 20 and Propylene Glycol were mixed in equal ratio that is 1:1 to get a mixture of surfactant: co-surfactant which is called as Smix. The coconut oil was taken first and Smix was added in it in a certain quantity and mixed properly. 2 to 3 drops of rose water (fragrance) were added during this stirring time to give good fragrance to the emulsion. Then the water was added slowly in the required quantity with continuous magnetic stirring at 500 rpm to make an emulsion.

Table 3: Different batches of the formulation made

Formulation	Oil (coconut oil) (%)	Smix (1:1) (%)	Water (%)
F1	5	25	60
F2	7	30	67
F3	7.5	28	64.5
F4	10	30	60
F5	15	25	60

1.5 Characterization of the prepared cream (w/o emulsion) (F1 to F5)

1.5.1 **pH determination**:

The pH of the prepared emulsion was determined with a digital pH meter. The electrode was fully immersed in the emulsion to ensure complete coverage. Measurements were conducted in triplicate, and the average value of these readings was recorded.

Dilution test. It was performed by diluting the formulated emulsion sample (Emulsions A and B) with oil or water. This test depends on the fact that no phase separation is possible when a dispersion medium is added to an Emulsion.

Dye solubility test. Two dyes were used: amaranth (water-soluble dye) and scarlet red (oil-soluble dye). This test is based on the principle that the dye can disperse uniformly throughout the phase in which it is more soluble, i.e., amaranth in the aqueous phase, whereas scarlet red in the oil phase.

Cobalt (II) chloride (CoC12)/filter paper test. This test involved the use of filter paper. The filter paper was first impregnated with CoC12 and dried (it appeared to be blue). This dried filter paper was then dipped in the formulated emulsion sample (A and B) and observed for any color change, if any. The color change from blue to pink indicates an o/w type of Emulsion. This test may fail if the Emulsion is unstable or break in the presence of an electrolyte.

1.5.2 **Thermodynamic Stability and Precipitation assessment**

1.5.2.1 Centrifugation test:

Accelerated ageing was done using centrifugation and freeze-thaw cycles to evaluate thermodynamic stability. The formulations that had "good"

dispersion properties were centrifuged for 30 minutes at 5,000 rpm, and any indications of phase separation, including creaming or cracking, were noted. For the ensuing freeze-thaw testing, only the formulations that passed the centrifugation test were chosen.

1.5.2.2 Freeze – Thaw test:

The chosen formulations were made to go through four freeze-thaw cycles, each lasting 24 hours, with the freezing phase lasting at -4°C and the thawing phase lasting at 40°C and the changes were noted

1.5.2.3 Precipitation assessment:

After 24 hours, precipitation was detected by visually examining the resultant emulsion. The formulations were categorised as non-clear (turbid), clear (transparent or transparent with a bluish tinge), stable (no precipitation after 24 hours) and unstable (precipitation detected within 24 hours) (Afzal O et al., 2023)

4.2.6.4 Stability studies:

In order to make sure that the formulation created is stable and safe, three samples of the prepared emulsion were prepared and all the samples were subjected to a high temperature of 40°C for a period of 1 month. The samples were withdrawn from each of the three developed emulsion at regular intervals of time that is at 0,2 and 4 weeks, to check for appearance, pH and concentration of drug to ensure that there are no major changes in these characteristics. UV Spectrophotometry was used to perform the drug assay of formulations using methanol as solvent. Appearance was checked visually and 81 the pH was checked using a digital pH meter (IG-10PH, PH Meter, IGene Labserve Pvt. Ltd.) (Afzal O et al., 2023, Harshal M et al., 2011).

RESULTS AND DISCUSSION

5.1 Preformulation studies

5.1.1 Organoleptic properties The drug powder was physically examined and the following observations were recorded. The recorded observations of physical state, colour and powder odour of the drug were found to be similar to the reference reported in official literature.

Table: Observed organoleptic properties of Clascoterone

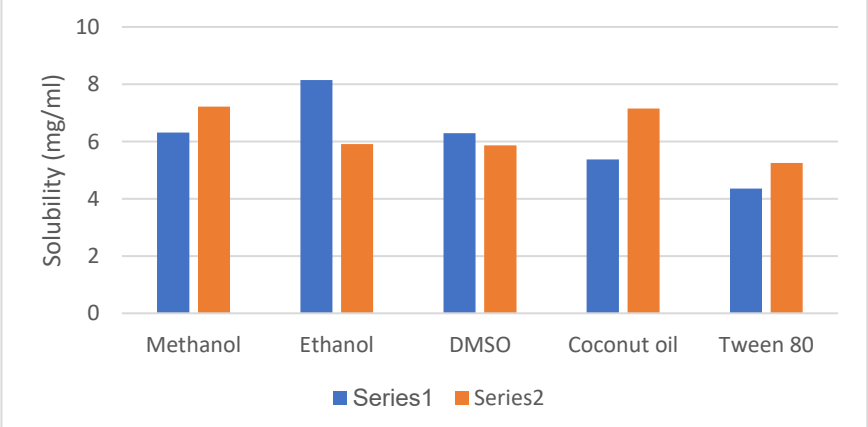
Properties	**Clascoterone**	**Tazarotene**
Physical form	Amorphous powder	Amorphous powder
Colour	White to off white	Yellow to orange
Odor	Odourless	Odouless

5.1.2 Solubility studies: In solvents: It was experimentally found that clascoterone had highly soluble in methanol and the resultant order of solubility is reported in the table. In excipients: Using UV/VIS spectroscopy, the solubility of clascoterone and tazarotene in a variety of excipients. Drug was found to be soluble in corn oil, and tween 80, hence these excipients are selected to be used in formulation development.

Table: Experimentally obtained solubility values of clascoterone and tazarotene in different excipients

Excipients	**Clascoterone**	**Tazarotene**
Methanol	6.316 ± 0.41 mg/ml	7.215 ± 0.29 mg/ml
Ethanol	8.147 ± 0.35 mg/ml	5.914 ± 0.26 mg/ml
DMSO	6.289 ± 0.29 mg/ml	5.863 ± 0.18 mg/ml
Coconut oil	5.374 ± 0.61 mg/ml	7.153 ± 0.57 mg/ml
Tween 80	4.418 ± 0.23 mg/ml	4.278 ± 0.31 mg/ml

Figure: Solubility of clascoterone and tazarotene



Determination of Absorption maxima (λmax) and construction of Calibration curve of Clascoterone:

5.1.3.1.A Determination of Absorption maxima (λmax) of Clascoterone:

Lambda max of clascoterone was identified using UV-Vis spectroscopy and was found to be 304 nm as depicted by the figure. The wavelength of 220 nm was chosen for the λ max because it is the point on a bell-shaped peak where the maximum absorption occurs. Selecting a peak with a bell shape is beneficial since the absorbance of a solution changes quickly with small wavelength differences on its steep sides. If there is even a slight variation in the wavelength setting of the instrument, this quick change can result in significant measurement inaccuracies. As a result, a bell-shaped peak reduces the possibility of appreciable errors in absorbance readings, guaranteeing more accurate and consistent observations.

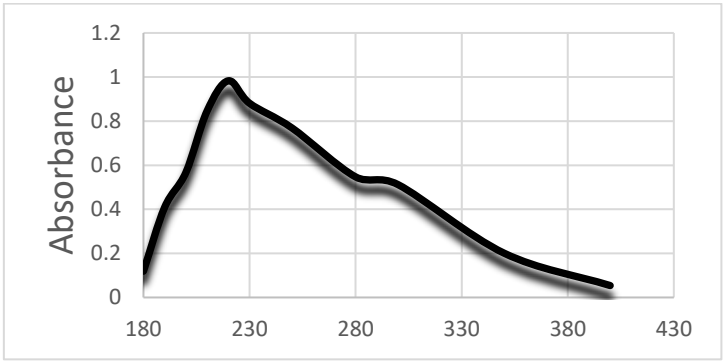


Figure: Lambda max of clascoterone

5.1.3.1.B Construction of calibration curve of Clascoterone:

Using the different dilutions that were made, absorbance values of Clascoterone at different concentrations were determined and these values along with concentration values were plotted on a graph to get the calibration curve. The regression value was calculated and was found to be 0.9828.

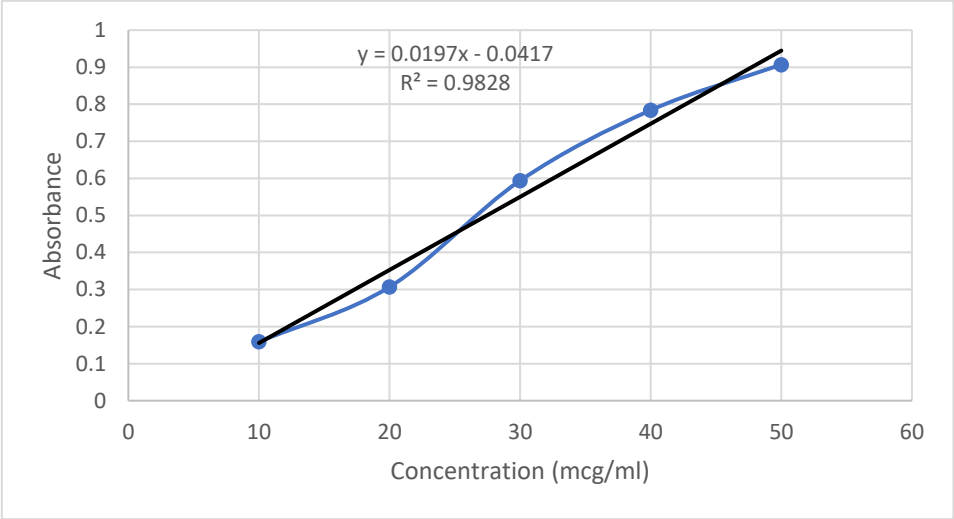


Figure: Calibration curve of Clascoterone in methanol.

2. Determination of Absorption maxima (λmax) and construction of Calibration curve of Tazarotene :

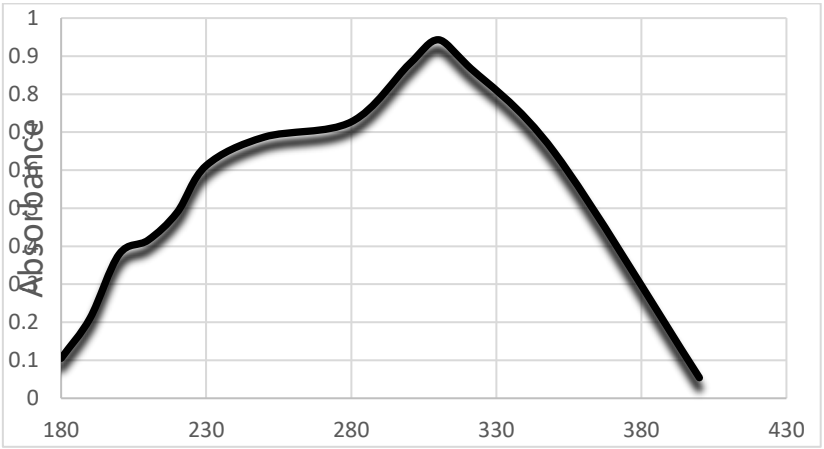


Figure : Absorption maxima of tazarotene in Methanol Solvent

Construction of calibration curve of tazarotene:

Using the different dilutions that were made, absorbance values of tazarotene at different concentrations were determined and these values along with concentration values were plotted on a graph to get the calibration curve. The regression value was calculated and was found to be 0.985.

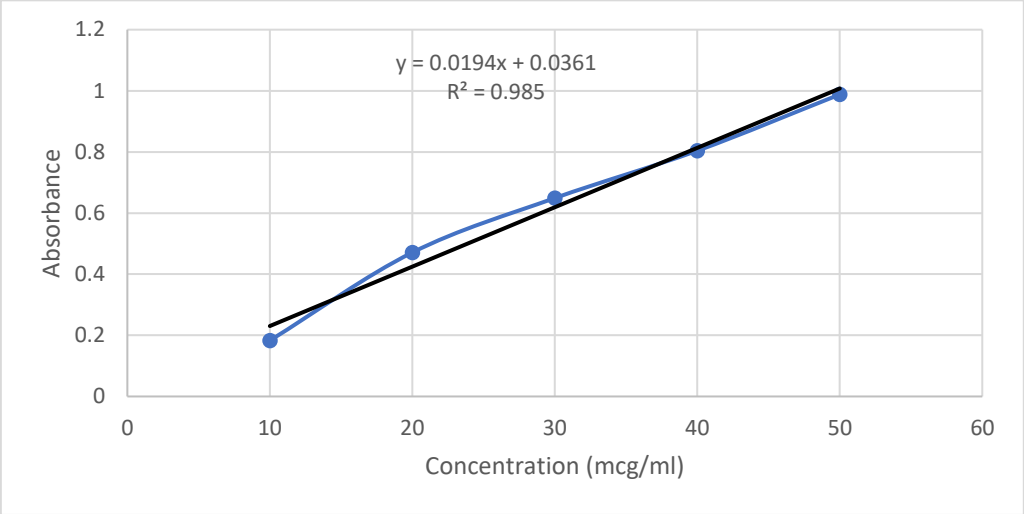


Figure: depicts the generated calibration curve of tazarotene in methanol.

5.1.4 Drug – excipient interaction by visual observation:

There were no physical changes, such as colour changes, phase separation, precipitation, or crystallisation. Texture and consistency were also same. No aggregates formation was there. Any new odour was not developed and there was no change in odour with absence of sedimentation or phase separation. These results suggested that the developed formulation is free of any kind of potential interactions and is stable and safe.

5.1.5 Melting point:

The melting point of pure Clascoterone and tazarotene were found to be 180 ± 2°C and 98 ± 1°C, respectively. Since the experimentally obtained melting point is found to be near the actual melting point of both the drugs, it can be suggested that the compound is pure and this is also likely to be confirming the identity of the compound.

5.1.6 Partition coefficient:

To evaluate the drug's hydrophilicity and lipophilicity, the partition coefficient was determined because it is a fundamental parameter which can influence various parameters of formulation development like, solubility, permeability, stability, distribution and more. Absorbance values were determined using UV spectrophotometer of Clascoterone in octanol and Clascoterone in water, which was used to calculate concentration of Clascoterone in these solvents and the formula was used to determine partition coefficient, which was found to be 3.17 ± 0.08 and 4.46 ± 0.09.

Table : Mean absorbance with obtained concentration and calculated partition coefficient

Solvents	**Concentration ± SD(N=3) of clascoterone**	**Concentration ± SD(N=3) of tazarotene**
Octanol	18.94 ± 0.002	16.79 ± 0.13
Water	5.97 ± 0.004	3.76 ± 0.07
Calculated partition coefficient (Log P)	3.17 ± 0.08	4.46 ± 0.09

pH determination:

The pH of the prepared emulsion and its different batches were recorded in triplicate and the results are presented in the following table:

Table: Observed pH of the given prepared formulation

Formulation	**pH**
F1	5.0
F2	6.0
F3	6.5
F4	7.5
F5	5.5

Dilution test. It was performed by diluting the formulated emulsion sample (Emulsions A and B) with oil or water. This test depends on the fact that no phase separation is possible when a dispersion medium is added to an Emulsion. For example, when water is added to o/w (oil in water) Emulsion, it is freely miscible with the Emulsion, and no phase separation occurs. Similarly, the addition of oil to water in oil (w/o) Emulsion shows miscibility in the case of the o/w type of Emulsion; if it is diluted with water, it will remain stable as water is the dispersion medium. Still, if it was diluted with oil, the Emulsion will break as oil and water will not be miscible with each other.

Table: Results of the dilution test

Formulation	**Result of dilution test**
F1	No phase separation
F2	No phase separation
F3	No phase separation
F4	**Phase separation**
F5	No phase separation

Dye solubility test. Two dyes were used: amaranth (water-soluble dye) and scarlet red (oil-soluble dye). This test is based on the principle that the dye can disperse uniformly throughout the phase in which it is more soluble, i.e., amaranth in the aqueous phase, whereas scarlet red in the oil phase.

Table: Results of dye test on various formulation

Formulation	Dye used	Observation	Inference
F1	Amaranth/Scarlet red	External phase turns red/Internal globules turn red	o/w emulsion
F2	Amaranth/Scarlet red	External phase turns red/Internal globules turn red	o/w emulsion
F3	Amaranth/Scarlet red	External phase turns red/Internal globules turn red	o/w emulsion
F4	**Amaranth/Scarlet red**	**No distinct color**	**Broken emulsion**
F5	Amaranth/Scarlet red	External phase turns red/Internal globules turn red	o/w emulsion

Cobalt (II) chloride (CoC12)/filter paper test. This test involved the use of filter paper. The filter paper was first impregnated with CoC12 and dried (it appeared to be blue). This dried filter paper was then dipped in the formulated emulsion sample (A and B) and observed for any color change, if any. The color change from blue to pink indicates an o/w type of Emulsion. This test may fail if the Emulsion is unstable or break in the presence of an electrolyte.

Table: Results of filter paper on the prepared formulations

Formulation	**Dye used**	**Observation**	**Inference**
F1	Blue cobalt chloride paper	Pink Color	o/w emulsion
F2	Blue cobalt chloride paper	Pink Color	o/w emulsion
F3	Blue cobalt chloride paper	Pink Color	o/w emulsion
F4	Blue cobalt chloride paper	**No color change**	**Broken emulsion**
F5	Blue cobalt chloride paper	Pink Color	o/w emulsion

1.5.2 **Thermodynamic Stability and Precipitation assessment**

1.5.2.1 Centrifugation test:

Accelerated ageing was done using centrifugation and freeze-thaw cycles to evaluate thermodynamic stability. The formulations that had "good" dispersion properties were centrifuged for 30 minutes at 5,000 rpm, and any indications of phase separation, including creaming or cracking, were noted. For the ensuing freeze-thaw testing, only the formulations that passed the centrifugation test were chosen (Afzal O et al., 2023, Harshal M et al., 2011).

1.5.2.2 Freeze – Thaw test:

The chosen formulations were made to go through four freeze-thaw cycles, each lasting 24 hours, with the freezing phase lasting at -4°C and the thawing phase lasting at 40°C and the changes were noted (Afzal O et al., 2023, Harshal M et al., 2011).

1.5.2.3 Precipitation assessment:

After 24 hours, precipitation was detected by visually examining the resultant emulsion. The formulations were categorised as non-clear (turbid), clear (transparent or transparent with a bluish tinge), stable (no precipitation after 24 hours) and unstable (precipitation detected within 24 hours) (Afzal O et al., 2023, Kumar Gupta S,)

Table: Results of different characterization tests done on the formulations

Formulation	**Centrifugation test**	**Freeze-thaw test**	**Precipitation test**
F1	Passed	**Did not pass**	Passed
F2	Passed	Passed	Passed
F3	Passed	Passed	Passed
F4	**Did not pass**	**Did not pass**	**Did not pass**
F5	Passed	Passed	**Did not pass**

Zeta potential

The surface charge and colloidal stability of the prepared emulsioln was assessed by measuring their zeta potential. The results revealed a negative zeta potential of **−10.59 mV**, likely due to the anionic nature of phospholipids and other components in the lipid bilayer. This negative charge plays a crucial role in stabilizing the formulation by promoting electrostatic repulsion between particles, thereby minimizing aggregation and enhancing dispersion stability.

While a zeta potential magnitude exceeding **±30 mV** is typically required for optimal colloidal stability, the observed value suggests that the prepared emulsion still maintain sufficient stability to resist coalescence and sedimentation. Furthermore, the surface charge may influence interactions with biological membranes, potentially affecting cellular uptake and the overall bioavailability of clascoterone.

Formulation	**Zeta Potential**
F2	-11.35
F3	-12.5

Table: Zeta potential of the final formulations

In-vitro drug release study:

- Clascoterone exhibited a sustained release profile, with ~50% released within 4hours. This suggests that the O/W emulsion effectively modulates its release, which is desirable for prolonged topical action in acne treatment.
- Tazarotene, being highly lipophilic (log P ~5.7), showed a slower initial release (~10% in 2 hours) but reached ~90% release by 24 hours. This aligns with its tendency to partition into the oil phase, requiring gradual diffusion into the aqueous receptor medium.
- The difference in release rates between the two drugs can be attributed to their distinct physicochemical properties:
    - Clascoterone (log P ~4.5) has relatively better solubility in the aqueous phase, facilitating faster diffusion.
    - Tazarotene's higher lipophilicity delays its release, which may enhance skin retention and reduce systemic absorption.

2. Influence of Emulsion Composition

- The oil phase (e.g., coconut oil) played a critical role in solubilizing tazarotene, while the aqueous phase (with surfactants like Tween 20) facilitated clascoterone dispersion.
- Optimized surfactant concentration ensured emulsion stability without hindering drug release, as evidenced by minimal droplet coalescence during the study.

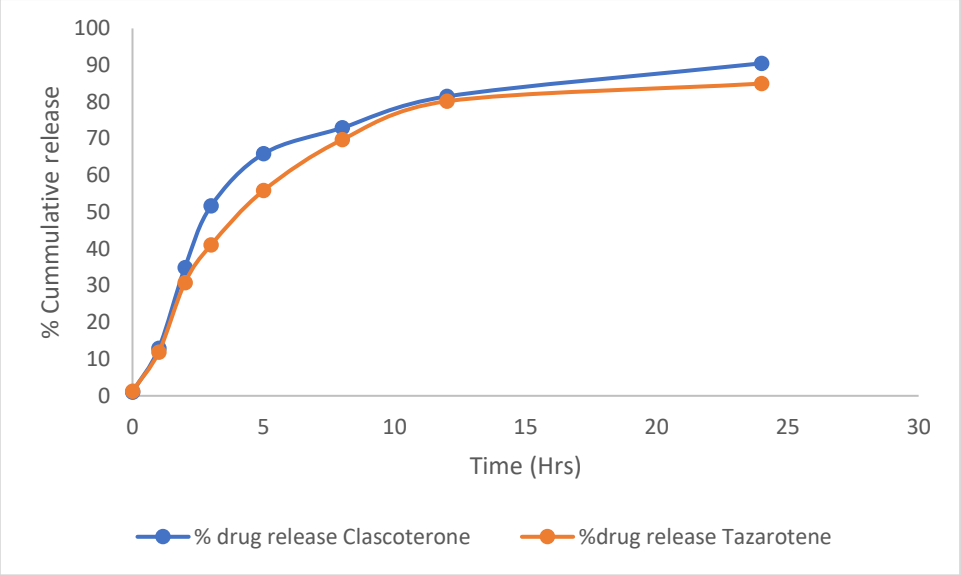


Figure: percentage cumulative release of the cream

Stability study

Stability study of three samples of developed formulation was done at a high temperature of 40°C and it was found that the pH of the formulation was consistent with negligible change, appearance does not changed in any manner to suggest any instability in the developed formulation as it was consistent throughout the test period and no change in colour, odour and consistency. Assay of drug was done and it was found that a very minor variation in the concentration of drug was there which did not suggest any degradation of the drug. The results suggests that all the samples of developed formulation did not show any major variations in pH, appearance and drug concentration which provides us evidence to report that the developed formulation is stable and it can be concluded that it is safe to use and its efficacy is also ascertained.

Table: Results of stability studies

Parameter	Time point	Zeta potential	Phase separation
Set 1 (4°C)	**Week 0**	-10.36	No
	Week 2	-11.87	No
	Week 4	-16.71	No
Set 2 (25° C ± 2° C 60% RH ± 5% RH)	**Week 0**	-11.76	No
	Week 2	-19.42	No
	Week 4	-18.13	No
Set 3 (40° C ± 2° C 75% RH ± 5% RH)	**Week 0**	-15.91	No
	Week 2	-16.94	No
	Week 4	-17.12	No

Summary and conclusion:

The preformulation studies for the development of an oil-in-water (O/W) cream containing clascoterone and tazarotene were conducted to assess organoleptic properties, solubility, absorption maxima (λmax), calibration curves, drug-excipient compatibility, melting point, partition coefficient, and emulsion stability. Clascoterone appeared as an amorphous white to off-white powder, while tazarotene was an amorphous yellow to orange powder, both odorless. Solubility studies revealed that clascoterone was highly soluble in methanol (6.316 ± 0.41 mg/mL) and ethanol (8.147 ± 0.35 mg/mL), while tazarotene showed good solubility in methanol (7.215 ± 0.29 mg/mL) and coconut oil (7.153 ± 0.57 mg/mL). Based on these findings, coconut oil and Tween 80 were selected for formulation development due to their favorable solubility profiles. The partition coefficient (log P) was determined to assess hydrophilicity and lipophilicity, with clascoterone at 3.17 ± 0.08 and tazarotene at 4.46 ± 0.09, indicating moderate lipophilicity for skin retention. pH determination of the formulations (F1-F5) showed values ranging from 5.0 to 7.5, with F3 (pH 6.5) being the most skin-compatible. Dilution and dye tests confirmed the O/W nature of the emulsions, with F1, F2, F3, and F5 showing stability, while F4 exhibited phase separation. The cobalt chloride test further validated the O/W structure, with formulations turning pink upon testing.

Thermodynamic stability studies, including centrifugation and freeze-thaw cycles, indicated that F2 and F3 were the most stable, with no phase separation or precipitation. Zeta potential measurements revealed a negative surface charge (-10.59 mV to -12.5 mV), contributing to colloidal stability by preventing particle aggregation. In vitro drug release studies demonstrated sustained release profiles, with clascoterone showing faster initial release due to its moderate lipophilicity, while tazarotene exhibited prolonged release, enhancing skin retention. Stability studies under accelerated conditions (40°C, 75% RH) confirmed that the formulation maintained consistent pH, appearance, and drug content over four weeks, with no significant degradation. The zeta potential remained stable, further supporting long-term emulsion stability. These results indicate that the developed O/W cream is physically and chemically stable, ensuring efficacy and safety for topical acne treatment.

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