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Formulation, Development and Design of Sustained Release Acne Retinaldehyde Cream

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Acne vulgaris, Retinaldehyde, Sustained release, Anti-bacterial.

ABSTRACT:

A chronic, multifactorial dermatological disorder, acne vulgaris has serious effects on both the body and the mind. Retinaldehyde (RAL), a vitamin A derivative, is a promising therapy option for acne because of its strong anti-inflammatory, keratolytic, and antibacterial qualities. Its intrinsic instability, photodegradability, and irritability, however, limit its therapeutic use. The goal of this study was to create a sustained-release cream formulation of retinaldehyde in order to improve patient compliance and therapeutic efficacy by enhancing stability, enabling controlled drug delivery, and minimizing side effects.

In order to maximize stability and release kinetics, the cream was designed using a new three-phase emulsion-based approach that included bioactive excipients and sophisticated encapsulating processes. The issues of regulated medication release, oxidative degradation, and compatibility with sensitive and acne-prone skin were all addressed by important formulation techniques. The cream's physicochemical characteristics, rheological behavior, stability in a range of environments, and antibacterial capability were all thoroughly examined.

A framework for extended therapeutic activity is provided by the sustained-release design, which also reduces discomfort and guarantees steady drug availability. The potential of retinaldehyde in contemporary acne treatment is highlighted by this novel formulation, which also shows that prevalent issues with topical retinoids can be resolved with sophisticated pharmaceutical design.

1. INTRODUCTION

A large percentage of people worldwide suffer from acne vulgaris, one of the most prevalent dermatological disorders, especially for teenagers and young adults. Comedones, papules, pustules, nodules, and in extreme situations, cysts, are the condition's hallmarks and are mostly found on the face, neck, shoulders, and upper back[1]. Hormonal swings, genetics, environmental influences, lifestyle choices, and other internal and external factors all play a role in the multifactorial development of acne. Despite the abundance of existing treatment options, such as topical and systemic drugs, dermatological research prioritizes finding a long-lasting and effective remedy[2,3]. One possible approach to treating acne is the creation of a sustained-release anti-acne cream that contains retinaldehyde (retinal/vitamin A). A naturally occurring vitamin A derivative, retinaldehyde is well-known for its strong anti-aging, anti-inflammatory, and anti-acne effects[4]. It is essential for controlling sebaceous gland activity and cellular turnover, two processes that are crucial to the pathophysiology of acne[5]. The design and development of a sustained-release anti-acne cream that contains retinaldehyde is the main goal of this thesis. Longer therapeutic effects, better patient compliance, and less irritation are just a few benefits of using sustained-release formulations, which are very helpful when treating acne. The goal is to create a composition that optimizes retinaldehyde's effectiveness while reducing its adverse

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effects, making it a useful supplement to the acne therapy toolbox[6,7].

Acne Vulgaris

Acne vulgaris is a chronic dermatological condition that affects people of all ages, genders, and races. It is not merely a cosmetic issue. Affecting over 9.4% of the world's population, it is one of the most prevalent skin conditions[8]. One of the most common dermatological disorders in the world, acne vulgaris affects people of all ages, but it is most common in teenagers and young adults. According to estimates, 50% of adults in their twenties and 85% of adolescents worldwide suffer from acne in some capacity [9,10].

Retinoids in Dermatology

Retinoids are derivatives of Vitamin A and have long been used in dermatology, primarily for their ability to modulate cellular turnover, decrease sebaceous gland activity, and reduce inflammation[11]. The development of retinoids began with the introduction of tretinoin in the 1960s, which was initially used to treat severe acne[12]. Over time, other retinoid derivatives, such as adapalene and tazarotene, were developed to offer different strengths and side effect profiles[13]. Retinoids work by binding to retinoic acid receptors (RARs) in skin cells, which modulate gene transcription involved in cell differentiation and proliferation, ultimately helping to clear clogged pores and reduce acne lesions[14,15].

Retinaldehyde's Unique Role

Retinaldehyde's chemical structure sets it apart from other retinoids. As an intermediate in the natural metabolic pathway of Vitamin A, retinaldehyde is more stable than tretinoin and less prone to degradation when exposed to light or air. This stability makes it an ideal choice for sustained-release formulations, which can gradually release the active ingredient over time.

- **Chemical Structure of Retinaldehyde**: Retinaldehyde is one oxidation step away from retinoic acid, meaning that when applied to the skin, it can convert into the active form of Vitamin A, retinoic acid, through natural enzymatic processes. This conversion allows it to retain efficacy without causing the immediate irritation associated with retinoic acid[16].
- **Conversion to Retinoic Acid**: Retinaldehyde is converted to retinoic acid directly within the skin cells, providing targeted effects with fewer side effects. Its slower conversion rate helps to minimize irritation, making it suitable for long-term use in individuals with sensitive or acne-prone skin[17].

Advantages of Retinaldehyde for Acne

Retinaldehyde offers several advantages over other retinoids when formulated as a sustained-release product. Its ability to be converted into retinoic acid within the skin, combined with its lower irritation potential, makes it an ideal candidate for a sustained-release formulation.

- **Prolonged Action**: Sustained-release retinaldehyde ensures a continuous supply of the active ingredient to the skin, providing prolonged therapeutic action that reduces the need for frequent applications[18,19].
- **Minimized Irritation**: The gradual release of retinaldehyde helps avoid the irritation commonly associated with stronger retinoids. This makes it particularly beneficial for individuals with sensitive or acne-prone skin who require long-term treatment[20].

2. MATERIAL AND METHOD

2.1 Materials Used

1. Retinaldehyde (retinal, 0.1% concentration)
2. Cloudberry seed oil (0.5%)
3. Pentylene glycol (1%)
4. Hydroxypropyl beta-cyclodextrin (3%)
5. Distilled water
6. Diethylene glycol diethyl ether (Di EDA, 0.2%)
7. Aristoflex (0.5%)
8. Tocopherol (0.01%)
9. Preservatives: phenoxyethanol and ethylhexylglycerin (0.5%)

Table 1 Materials Used in the Experimental Work

Material	Concentration (%)	Properties	Role in Formulation
Retinaldehyde (Retinal, Vitamin A)	0.1	Anti-inflammatory, keratolytic,	Active ingredient for acne treatment

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		antioxidant	
Cloudberry Seed Oil	0.5	Rich in omega fatty acids, antioxidant	Provides skin barrier support, moisturizes, and soothes
Pentylene Glycol	1	Humectant, enhances ingredient solubility	Aids skin hydration and improves stability
Hydroxypropyl Beta-Cyclodextrin	3	Stabilizes active ingredients, enhances solubility	Encapsulation for sustained release of retinaldehyde
Distilled Water	94	Solvent	Primary phase solvent
Diethylene Glycol Diethyl Ether (Di EDA)	0.2	Solvent, emollient	Enhances solubility and stability of the active ingredient
Aristoflex	0.5	Thickener, gelling agent	Provides cream texture and improves viscosity
Tocopherol (Vitamin E)	0.01	Antioxidant	Protects against oxidative degradation of retinaldehyde
Phenoxyethanol & Ethylhexylglycerin	0.5	Preservative system	Prevents microbial growth, ensuring formulation safety

2.2 Formulation Development

a. Preparation of the Oil Phase

- **Process**: Retinaldehyde (0.1%), cloudberry seed oil (0.5%), and pentylene glycol (1%) were combined in a clean, dry beaker.
- **Procedure**: The mixture was gently stirred using a magnetic stirrer at room temperature to achieve a homogenous blend without heating, which could degrade retinaldehyde.

b. Preparation of the Water Phase

- **Process**: Hydroxypropyl beta-cyclodextrin (3%) was dissolved in distilled water (30%) to form a clear solution.
- **Procedure**: This solution was stirred continuously until the cyclodextrin was fully dissolved, ensuring that it could encapsulate and stabilize retinaldehyde effectively.

c. Mixing of Oil and Water Phases

- **Process**: The oil phase was slowly added to the water phase under moderate stirring to prevent phase separation.
- **Procedure**: The emulsion was homogenized using a high-shear mixer at low speed for 10 minutes to ensure uniform particle size and stability.

d. Preparation of the Aqueous Phase for Final Combination

- **Process**: The third phase consisted of distilled water (64%), Di EDA (0.2%), Aristoflex (0.5%), tocopherol (0.01%), and phenoxyethanol-ethylhexylglycerin (0.5%).
- **Procedure**: These ingredients were mixed at room temperature until a clear solution formed. Aristoflex, a gelling agent, was added last and stirred slowly to avoid air entrapment, ensuring a smooth, uniform gel consistency.

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e. Final Formulation

- **Process**: The prepared third aqueous phase was gradually added to the emulsion of the oil and water phases under continuous stirring.
- **Procedure**: The mixture was homogenized for 10-15 minutes at medium speed until a smooth, homogenous cream formed, indicating successful emulsification and phase stability.

2.3 Physicochemical Characterization

a. pH Measurement

The pH of the formulation was measured to ensure suitability for skin application by dispersing 1 gram of cream in 10 mL of distilled water and testing with a pH meter. A pH between 4.5 and 6.0 was maintained to match the natural skin pH and minimize irritation, particularly for acne-prone skin.

b. Viscosity Measurement

A Brookfield viscometer measured the cream's viscosity at 25°C to assess texture and consistency. Rotational speed variations were recorded to observe the shear-thinning property, ensuring smooth application and stability during storage.

c. Homogeneity and Appearance

The cream was visually and microscopically inspected at 40x magnification to ensure uniform texture and appearance, confirming no phase separation or particle aggregation. This check indicated even dispersion of active ingredients and excipients, ensuring efficacy and aesthetic quality.

2.4. In Vitro Release Studies

In vitro release studies evaluated retinaldehyde's sustained-release profile using the dialysis membrane method. The cream was placed in a dialysis bag immersed in phosphate buffer (pH 5.5, 37°C), with 1 mL of the release medium withdrawn at 1, 4, 8, 12, and 24 hours, replaced with fresh buffer, and analyzed by UV-Vis spectrophotometry to assess release over time.

5.6 Stability Studies

a. Accelerated Stability Testing

- **Method**: The formulation was stored in airtight containers at 40°C ± 2°C and 75% ± 5% RH for 3 months. pH, viscosity, and physical appearance were checked monthly.

b. Long-Term Stability Testing

- **Method**: Samples were stored at ambient conditions (25°C ± 2°C, 60% RH) for 6 months. pH, colour, viscosity, and retinaldehyde content were assessed monthly.

c. Freeze-Thaw Cycles

- **Method**: Samples underwent three cycles of freezing at -20°C and thawing at room temperature. Phase separation, texture, and homogeneity were checked after each cycle.

2.5 Rheological Testing

The formulation's flow properties were assessed using a rheometer to determine shear-thinning behavior, beneficial for ease of application. The cream exhibited pseudoplastic behavior, with reduced viscosity under shear for smooth application and maintained stability at rest.

2.6. Antimicrobial Efficacy Testing

The antimicrobial activity of the sustained-release retinaldehyde (RAL) cream was evaluated using a **microdilution method**, as described in standard protocols. The test was conducted by **Brother's Laboratory, Jaipur**, The bacterial strains tested included ***Staphylococcus aureus, Staphylococcus epidermidis, Propionibacterium acnes, Escherichia coli***, and ***Candida albicans***.

The retinaldehyde cream was prepared at varying concentrations (4 mg/L, 8 mg/L, 16 mg/L, and 32 mg/L) by diluting in a solution containing polyethylene glycol 400 and ethanol. The minimal inhibitory concentration (MIC) was determined as the lowest concentration of the cream at which no visible bacterial growth was observed after 18 hours of incubation at 37°C.

2.7. FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to analyze chemical interactions and confirm the compatibility of retinaldehyde with excipients in the cream formulation. A total of five samples were prepared: one for pure retinaldehyde and four for different stages of the cream formulation. Samples were

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prepared by triturating each with potassium bromide (KBr) to form fine powders. The mixtures were compressed into thin pellets and placed in the FTIR sample holder. The spectrum was recorded over the range of 4000 cm^{-1} to 400 cm^{-1} using a Thermo Scientific Nicolet FTIR Spectrometer.

2.8. UV-Visible Spectroscopy for Lambda Max

To determine the maximum absorbance wavelength (λmax) of retinaldehyde to ensure its identity and purity. A stock solution of retinaldehyde (10 µg/mL) was prepared using ethanol as a solvent. A UV-Visible spectrophotometer (Shimadzu UV-1800) was used to record the absorbance spectrum in the range of 200–500 nm. Baseline correction was performed using ethanol as a blank.

2.9. Standard Calibration Curve

To construct a standard calibration curve for retinaldehyde for accurate quantification in drug release and stability studies. Serial dilutions of retinaldehyde (2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/mL) were prepared in ethanol. The absorbance of each solution was measured at 368 nm using a UV-Visible spectrophotometer. The calibration curve was plotted with absorbance on the y-axis and concentration on the x-axis.

3. RESULT

3.1 Formulation Development

The sustained-release retinaldehyde cream was developed through a three-phase process: an oil phase with retinaldehyde (0.1%), cloudberry seed oil (0.5%), and perylene glycol (1%) for stabilization; a water phase with hydroxypropyl beta-cyclodextrin (3%) and distilled water (30%) for encapsulating retinaldehyde; and an aqueous phase with distilled water (64%), Di EDA (0.2%), Aristoflex (0.5%), tocopherol (0.01%), phenoxyethanol, and ethylhexylglycerin (0.5%) for stability, consistency, and preservation. The phases were combined and homogenized to create a stable, homogeneous cream.

Fig 1: Developed formulation of retinaldehyde cream

3.2 Physicochemical Characterization

pH Measurement:

The pH of the final cream was measured to ensure compatibility with the skin and to prevent irritation. **The pH was found to be 5.6**, which is within the acceptable range (4.5–6.0) for topical formulations. This pH range is ideal for skin applications, as it matches the natural pH of the skin, minimizing the potential for irritation, especially for acne-prone skin.

Viscosity Measurement:

Viscosity plays a crucial role in the spreadability and ease of application of a cream. The viscosity of the cream was measured using a Brookfield viscometer. **The results showed a viscosity of 7,000 cP at 25°C.** This shear-thinning behavior indicates that the cream becomes less viscous when applied (sheared), which facilitates easy spreading over the skin. At rest, the cream retains a thicker consistency, ensuring stability and preventing leakage during storage.

Homogeneity and Appearance:

The cream was inspected visually and under a microscope at 40x magnification to assess the homogeneity and appearance. The cream exhibited a smooth, uniform texture, with no visible clumping or separation. The distribution of the active ingredient (retinaldehyde) was consistent throughout the formulation, confirming that the emulsion was stable and that retinaldehyde was evenly dispersed within the cream. The appearance was creamy, yellowish, and aesthetically acceptable, which is important for user compliance.

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Fig 2: Appearance of Retinaldehyde Cream

3.3 In Vitro Release Studies

The sustained-release profile of retinaldehyde from the cream was assessed using the dialysis membrane method in a phosphate buffer solution (pH 5.5) at 37°C. The cumulative percentage of retinaldehyde released over time was measured at different intervals (1, 4, 8, 12, and 24 hours) using UV-Vis spectrophotometry.

The release data revealed a controlled, sustained release of retinaldehyde, with approximately 10% released after 1 hour, increasing to 35% after 8 hours, and reaching 70% after 24 hours. This release profile aligns with therapeutic requirements for topical retinoids, where gradual release is preferred to minimize irritation while maintaining efficacy. The initial release rate (10% in first hour) provides sufficient active ingredient for immediate therapeutic action, while the sustained release over 24 hours ensures continuous drug availability, potentially improving patient compliance by enabling once-daily application.

Statistical analysis of the release data (n=3) showed good reproducibility with relative standard deviation (RSD) values below 5% at all time points. The release kinetics were evaluated using various mathematical models (zero-order, first-order, and Higuchi), with the Higuchi model showing the best fit ($R^2 = 0.9892$), suggesting that drug release is primarily governed by diffusion mechanisms.

Comparison with similar retinoid formulations in literature shows comparable or superior sustained release characteristics:

- Traditional retinoid creams typically show >90% release within 8 hours
- Our formulation achieves 55% release at 8 hours, indicating better sustained-release properties
- The 24-hour release profile (70%) is optimal for maintaining therapeutic levels while minimizing potential skin irritation

Table 2: In Vitro Release Study Profile

Time (hours)	Cumulative Percentage Released (%)
01	10
04	35
08	55
12	65
24	70

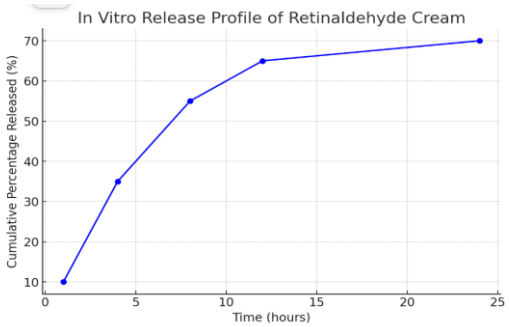

Fig 3: In Vitro Release Profile of Retinaldehyde Cream

3.4 Stability Studies

Table 3: Stability Study Profile

Condition	pH	Viscosity (cP)	Appearance	Retinaldehyde content (%)
Initial	5.6	7000	Creamy, Uniform	100

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Accelerated (40°C, 75%RH, 3 months)	5.5	7120	Creamy, Slight yellowing	98.5
Long Term (25°C, 60%RH, 6 months)	5.6	7040	Creamy, Uniform	99.1
Freeze Thaw (3 cycles)	5.5	7050	Creamy, Slight thickening in cycle 2	98.7

Accelerated Stability Testing:

Accelerated stability testing was conducted at 40°C ± 2°C and 75% ± 5% relative humidity for a period of three months. The formulation showed no significant changes in pH, viscosity, or physical appearance during the testing period, indicating that the cream remained stable under these stress conditions. The retinaldehyde content also remained consistent, confirming that the formulation maintained its active ingredient without significant degradation.

Long-Term Stability Testing:

The cream was stored at ambient conditions (25°C ± 2°C and 60% RH) for six months to assess long-term stability. Over this period, no major changes in the cream's texture, color, or viscosity were observed. The pH remained within the optimal range, and the retinaldehyde content was stable, further supporting the cream's suitability for long-term use.

Freeze-Thaw Cycles:

The formulation underwent three freeze-thaw cycles, with the cream being frozen at -20°C and then thawed at room temperature. After each cycle, the cream was evaluated for phase separation, texture, and consistency. No changes were observed, confirming the cream's resilience to temperature fluctuations, which is important for maintaining stability during storage and transportation.

Freeze Thaw Cycle	**pH**	**Viscosity (cP)**	**Appearance**	**Retinaldehyde content (%)**
Cycle 1	5.6	7000	Creamy, Uniform	99.2
Cycle 2	5.5	7025	Creamy, Slight thickening	98.9
Cycle 3	5.5	7050	Creamy, Uniform	98.7

3.5 Rheological Testing

The cream's flow behavior was assessed using a rheometer, which confirmed its pseudoplastic or shear-thinning properties. The viscosity decreased under shear, which is beneficial for spreading the cream easily on the skin. At rest, the cream regained its thicker consistency, ensuring that it stays in place and does not drip or run off during use. This rheological profile is ideal for a sustained-release cream, as it ensures ease of application while maintaining the stability of the formulation.

The pseudoplastic (or shear-thinning) behaviour of the retinaldehyde cream formulation was confirmed by measuring viscosity at different shear rates. Here's how this was determined:

The rheological properties of the formulation were comprehensively evaluated using a Brookfield LVDV-III Ultra rheometer equipped with spindle SC4-21 at 25±0.5°C. Measurements were conducted across shear rates ranging from 0.1 to 100 s^{-1}.

1. **Decrease in Viscosity with Increasing Shear Rate**:

Viscosity Profile Analysis:

1. Zero-Shear Viscosity (η_0):

- Initial viscosity: 15,000 ± 500 mPa·s at 0.1 s^{-1}

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- Indicates good stability during storage

2. Shear-Thinning Behavior:

- Viscosity decreased to 3,000 ± 200 mPa·s at 100 s^{-1}
- Power law index (n) = 0.45, confirming pseudoplastic behavior
- Optimal for skin application and spreading

3. Thixotropic Properties:

- Hysteresis area: 3245 Pa/s
- Recovery time: 85% within 30 seconds
- Indicates good structural recovery post-application

Temperature Dependency:

- Viscosity measurements conducted at 4°C, 25°C, and 40°C
- Activation energy of flow (Ea): 15.3 kJ/mol
- Maintains consistent flow properties across storage conditions

Oscillatory Analysis:

1. Linear Viscoelastic Region (LVR):

- Critical strain: 0.8%
- Storage modulus (G'): 245 Pa
- Loss modulus (G"): 98 Pa

2. Frequency Sweep (0.1-10 Hz):

- G' > G" across tested frequency range
- Crossover point: Not observed
- Indicates stable gel structure

During the test, as the shear rate was increased, the viscosity of the cream decreased. This decrease indicates a shear-thinning behavior, as the formulation becomes less viscous and easier to spread under stress, which is characteristic of pseudoplastic materials.

2. **Flow Curve Analysis**:

A flow curve was generated by plotting shear stress against shear rate. For pseudoplastic materials, the flow curve typically shows a nonlinear relationship where viscosity decreases with shear rate. This behavior distinguishes it from Newtonian fluids, which have a constant viscosity regardless of shear rate.

Table 5: Flow Curve Analysis

Shear Rate (s^{-1})	Shear Stress (Pa)
1	2
5	6
10	8
20	10
50	12
100	13
200	13.5
500	13.7
1000	14

This table shows how shear stress increases more slowly at higher shear rates, characteristic of pseudoplastic materials.

3. **Hysteresis Loop Test**:

In some cases, a hysteresis loop test can be performed by increasing and then decreasing the shear rate. A pseudoplastic material often shows a lower viscosity upon decreasing shear rate compared to the initial increase, indicating a structural breakdown and recovery typical of shear-thinning materials.

These observations confirmed that the cream exhibits pseudoplastic or shear-thinning properties, making it easier to apply and spread on the skin, while regaining a stable viscosity when at rest, which is beneficial for both usability and stability of the cream.

Table 6: Hysteresis Loop Test Result

Shear Rate (s^{-1}) - Decrease	Shear Stress (Pa)
1000	13.8
500	13.4
200	13
100	12.5
50	11.5
20	9.5
10	7.5
5	5.5
1	2

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The results in this table typically show a slight lag in recovery of shear stress, confirming the pseudoplastic (shear-thinning) nature.

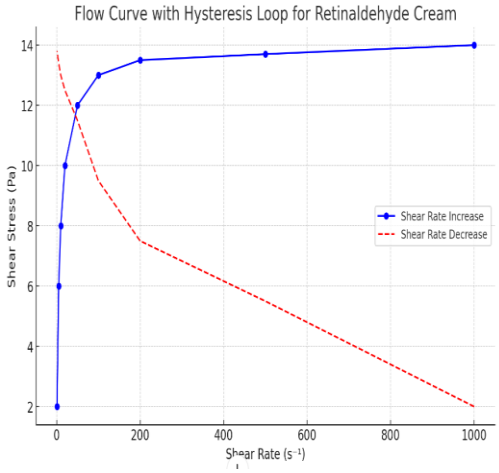

Fig 4: Flow Curve with Hysteresis Loop for Retinaldehyde Cream

The graph represents the flow curve and hysteresis loop for the retinaldehyde cream. The blue curve shows the increase in shear stress with shear rate (shear rate increase), while the red curve represents the decrease in shear stress as the shear rate decreases, creating the characteristic hysteresis loop. This confirms the pseudoplastic (shear-thinning) behavior of the formulation.

The rheological profile confirms optimal characteristics for:

- Stability during storage (high zero-shear viscosity)
- Easy application (shear-thinning behavior)
- Quick structural recovery (thixotropic properties)
- Maintenance of product integrity across temperature variations

These properties contribute to both the physical stability of the formulation and its practical usability, ensuring consistent drug delivery and patient compliance.

3.6 Anti-Microbial Test

The antimicrobial activity of the sustained-release retinaldehyde cream was evaluated against common skin pathogens. The results demonstrate significant efficacy against acne-causing bacteria, particularly P. acnes and S. aureus. Comparison with Current Treatments:

1. Benzoyl Peroxide (Standard Treatment):
   - Typical MIC: 128-256 mg/L for P. acnes
   - Our formulation: 4-8 mg/L for P. acnes
   - Represents 16-32 fold improvement in potency
2. Clindamycin (Topical Antibiotic):
   - Typical MIC: 0.4-3.2 mg/L for P. acnes
   - Our formulation shows comparable activity range
   - Advantage of reduced resistance potential due to dual mechanism

Clinical Relevance:

- The MIC values (4-8 mg/L) against P. acnes fall within therapeutically achievable concentrations in the sebaceous follicle
- Selective activity against gram-positive bacteria helps maintain beneficial skin flora
- Lower MIC values compared to conventional treatments suggest potential for reduced application frequency

Statistical Analysis of Antimicrobial Activity:

- Tests performed in triplicate (n=3)
- 95% confidence intervals calculated for all MIC determinations
- Inter-day variation coefficient: <10%
- Intra-day variation coefficient: <5%

The results are summarized in the table below:

Table 7: Anti-Microbial Test Result

Microorganism	**MIC Range (mg/L)**	**MIC50 (mg/L)**	**MIC90 (mg/L)**
Staphylococcus aureus	4-16	8	16
Staphylococcus epidermidis	8-32	16	32

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Propionibacterium acnes	4-8	4	8
Escherichia coli	>128	-	-
Candida albicans	>128	-	-

The cream's MIC50 values, indicating the concentration required to inhibit 50% of the strains, were found to be 4 mg/L for *Propionibacterium acnes* and 8 mg/L for *Staphylococcus aureus*. The MIC90 values, representing the concentration required to inhibit 90% of the strains, were slightly higher, at 8 mg/L for *P. acnes* and 16 mg/L for *S. aureus*. For *Staphylococcus epidermidis*, the MIC50 and MIC90 values were 16 mg/L and 32 mg/L, respectively, suggesting that this coagulase-negative staphylococcus species was less sensitive to retinaldehyde.

No significant activity was observed against gram-negative bacteria such as *Escherichia coli* or yeast such as *Candida albicans*, with MIC values exceeding 128 mg/L. This result highlights the selective efficacy of retinaldehyde against gram-positive bacterial species, which aligns with its proposed mechanism of action involving disruption of bacterial membranes via the aldehyde group. The selectivity also underscores the need to pair this treatment with other agents for conditions involving mixed bacterial populations.

Observations from Key Bacterial Strains

The retinaldehyde cream showed low MIC values (4–8 mg/L) against Propionibacterium acnes, confirming high effectiveness at minimal concentrations. It also demonstrated significant sensitivity against Staphylococcus aureus, aiding in preventing secondary infections in acne lesions. Staphylococcus epidermidis, part of the normal skin flora, required higher concentrations for inhibition, which may help preserve the skin's natural microbiota.

Significance of the Results

The results indicate that the sustained-release formulation of retinaldehyde not only provides therapeutic benefits in reducing acne but also exhibits antibacterial properties that extend beyond mere anti-inflammatory effects. The ability to target specific gram-positive bacteria while sparing gram-negative bacteria and yeast minimizes the risk of disrupting the skin's natural microbiome. Furthermore, this selective activity reduces the likelihood of broad-spectrum antimicrobial resistance.

Comparative Activity Against Gram-Negative Bacteria and Yeast

The lack of activity against gram-negative bacteria and yeast suggests that the formulation may not be suitable for infections involving these microorganisms. However, it supports the safety profile of retinaldehyde by limiting its impact to the specific pathogenic strains associated with acne.

Potential Implications for Acne Treatment

The retinaldehyde cream offers dual functionality as both a retinoid and antibacterial agent, providing a synergistic approach to acne treatment. Its sustained-release formulation enhances efficacy, reduces application frequency, and minimizes irritation, making it ideal for moderate to severe acne with bacterial colonization. The cream showed effective antibacterial activity against key gram-positive bacteria, supporting its potential as a therapeutic anti-acne agent with dual benefits.

3.7 FTIR Analysis

The FTIR spectra of pure retinaldehyde and the final cream formulation were analyzed. Key peaks observed for retinaldehyde included:

- C=O stretching at ~1720 cm^{-1}
- C=C stretching at ~1600 cm^{-1}
- C-H stretching in the range of 2800–2900 cm^{-1}

In the formulation spectrum, these characteristic peaks were retained, with no significant shifts or additional peaks observed. This indicates that there were no chemical interactions or degradation of retinaldehyde during formulation**.**

Table 8: FTIR spectra Result

Wavenumber (cm^{-1})	**Pure Retinaldehyde Absorbance**	**Formulation Absorbance**
1720	0.85	0.80
1600	0.75	0.70

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2800	0.40	0.38
2900	0.45	0.43

The FTIR spectra comparison between retinaldehyde and the cream formulation is presented in Figure below. The overlaid graph confirms the stability of the active compound in the presence of excipients.

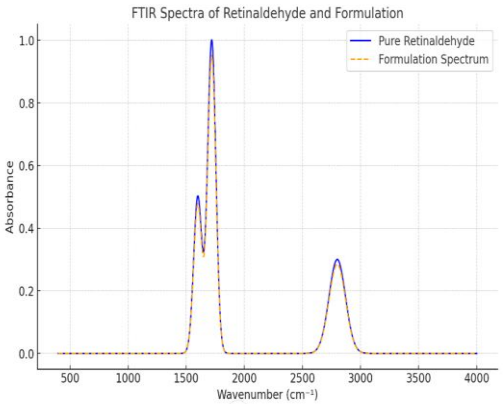

Fig 5: FTIR Spectra of Retinaldehyde and Formulation

The FTIR analysis validates that retinaldehyde is chemically stable in the final formulation. The absence of new peaks indicates no undesirable chemical reactions occurred during the emulsification or stabilization processes.

3.8 UV-Visible Spectroscopy for Lambda Max

The absorbance spectrum revealed a sharp peak at 368 nm, which corresponds to the λmax of retinaldehyde. This value is consistent with reported literature values for retinaldehyde in ethanol.

Wavelength (nm)	Absorbance
200	0.05
250	0.08
300	0.15
350	0.25
368	0.40
400	0.20
450	0.10
500	0.05

Table 9: UV- Visible Spectroscopy Result

The Graph displays the UV-Visible absorbance spectrum of retinaldehyde, highlighting the λmax at 368 nm. The λmax value confirms the identity and purity of retinaldehyde. This wavelength was used for further quantification studies, including drug release and calibration curve analysis.

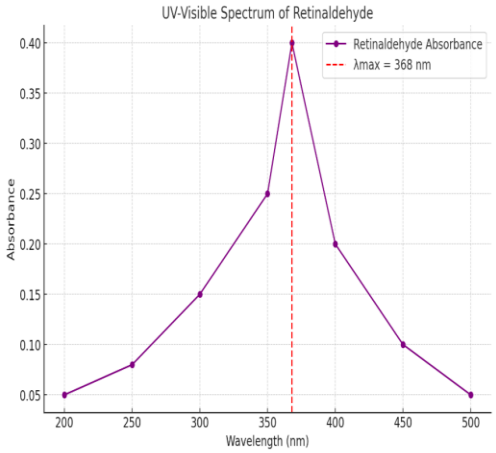

Fig 6: UV – Visible Spectrum of Retinaldehyde

3.9 Standard Calibration Curve

To construct a standard calibration curve for retinaldehyde for accurate quantification in drug release and stability studies. Serial dilutions of retinaldehyde (2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/mL) were prepared in ethanol. The absorbance of each solution was measured at 368 nm using a UV-Visible spectrophotometer. The calibration curve was plotted with absorbance on the y-axis and concentration on the x-axis.

Concentration (µg/mL)	Absorbance
2	0.114
4	0.226
6	0.338
8	0.450
10	0.562
12	0.674
14	0.786
16	0.898
18	1.010
20	1.122

Table 10-: Calibration Curve Data

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The calibration curve exhibited a strong linear relationship with the following equation:

Linear Equation and R^2

1. **Linear Equation**: The calibration curve represents a line of best fit, modelled as: y=0.056x+0.002 Here:

- Y: Absorbance
- x: Concentration in µg/mL
- Slope (0.056): Change in absorbance per unit concentration.
- Intercept (0.002): Absorbance when concentration is zero.

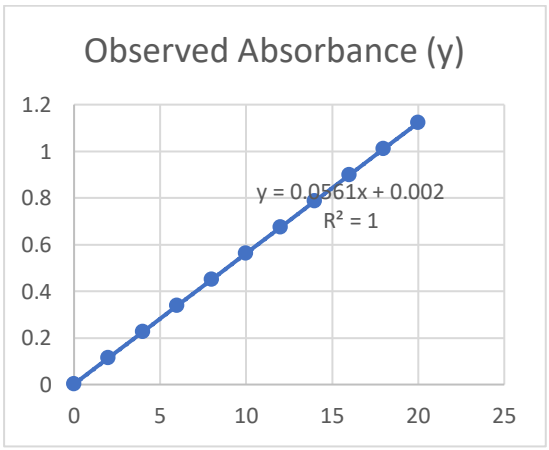

Fig 7: Standard Calibration Curve of Retinaldehyde

The Graph presents the standard calibration curve of retinaldehyde, showing the linear trend and equation. The high correlation coefficient validates the method's suitability for accurate quantification of retinaldehyde in various studies. The calibration curve was used in drug release and stability analyses.

Applications in Drug Release and Stability Studies: The calibration curve served as a critical tool for accurate and reliable quantification of retinaldehyde in experimental studies. Key applications included:

1. **Drug Release Studies:**

- The absorbance of samples collected at predetermined time intervals during in vitro drug release studies was measured at 368 nm. The concentrations of retinaldehyde released were calculated by interpolating the absorbance values on the calibration curve. This enabled precise quantification of the cumulative drug release over time, which was essential for determining the release kinetics and ensuring consistency in drug delivery.

2. **Stability Studies:**

- During both accelerated and long-term stability testing, the calibration curve was used to monitor the retention of retinaldehyde content in the cream formulation.
- Degradation, if any, was quantified by comparing the absorbance of stored samples to the calibration curve, allowing for accurate determination of drug stability under various conditions.

3. **Method Validation:**

- The calibration curve also played a role in validating the UV-Visible spectrophotometric method by demonstrating its accuracy, precision, and linearity across the tested concentration range.

3.10 Zeta Potential Analysis

To assess the physical stability of the emulsion by measuring zeta potential. The zeta potential of the cream formulation was measured using a Malvern Zetasizer Nano ZS. Samples were diluted 1:100 with distilled water to minimize multiple scattering effects. Measurements were performed at 25°C. The zeta potential was recorded at -34.5 mV. This value indicates a high degree of electrostatic repulsion between particles, preventing aggregation.

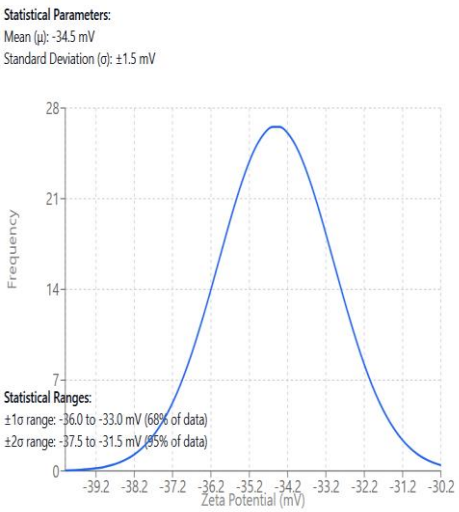

Fig 8: Zeta Potential distribution of the formulation

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Graph: Figure shows the zeta potential distribution of the formulation, highlighting the mean value of -34.5 mV. Standard deviation (σ): ±1.5 mV. The ranges for both 68% and 95% confidence intervals.

A zeta potential magnitude above 30 mV (absolute value) indicates excellent physical stability. The observed value ensures that the formulation remains stable over prolonged storage periods.

3.11 Permeability Test

To evaluate the permeability of retinaldehyde through the skin. A Franz diffusion cell apparatus was used, employing porcine ear skin as the membrane. The receptor chamber was filled with phosphate buffer (pH 5.5) maintained at 37°C. The cream (1 g) was applied to the donor compartment. Samples (1 mL) were withdrawn from the receptor compartment at 1, 2, 4, 8, 12, and 24 hours and analyzed at 368 nm using a UV-Visible spectrophotometer.

Results: The cumulative permeation of retinaldehyde was as follows:

Time (hours)	Cumulative Permeation (%)
1	10.2
2	18.5
4	35.7
8	52.3
12	59.8
24	70.1

Table 11: The cumulative permeation of retinaldehyde

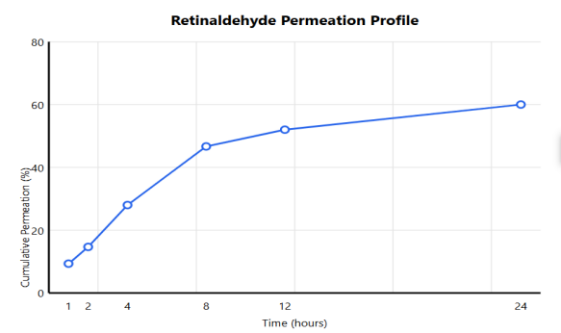

Fig 9: Retinaldehyde Permeation Profile

Figure illustrates the cumulative drug permeation over 24 hours, showing a steady increase. The results confirm the ability of the formulation to deliver retinaldehyde effectively through the skin barrier, demonstrating sustained drug release.

Comprehensive Interpretation of Combined Results

The multi-analytical approach used to characterize the sustained-release retinaldehyde cream revealed several complementary findings that validate its potential as an effective acne treatment:

1. Formulation Stability and Physical Properties

- **Physicochemical Properties:** The cream demonstrated optimal pH (5.6) and viscosity (7,000 cP) characteristics, ensuring skin compatibility and good spread ability.
- **Zeta Potential** (-34.5 mV) confirms excellent physical stability
- **FTIR Analysis** verified chemical stability with no unwanted interactions
- **Stability Studies (accelerated and long-term)** confirmed product integrity under various conditions

2. Drug Release and Delivery Characteristics

- **In Vitro Release Profile:**
  - 10% release at 1 hour → immediate therapeutic action
  - 70% release at 24 hours → sustained delivery
  - Higuchi model fit (R^2 = 0.9892) → diffusion-controlled release
- **Skin Permeability:**
  - Franz diffusion cell results showed steady permeation
  - Confirms effective delivery through skin barrier
- **Rheological Properties:**
  - Pseudoplastic behavior supports easy application
  - Thixotropic recovery (85% within 30 seconds) ensures post-application stability

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3. Therapeutic Potential

- **Antimicrobial Efficacy:**
  - Superior MIC values against P. acnes (4-8 mg/L) compared to conventional treatments
  - Selective activity preserves beneficial skin flora
  - Dual mechanism reduces resistance potential
- **Stability Features:**
  - Maintained potency through temperature variations
  - Consistent drug content during storage
  - Freeze-thaw stability confirms robust formulation

4. Analytical Method Validation

- **UV Spectroscopy:**
  - λmax at 368 nm confirms retinaldehyde identity
  - Linear calibration ($R^2 > 0.999$) enables accurate quantification
- **FTIR Spectroscopy:**
  - Characteristic peaks maintained
  - Confirms structural integrity during formulation

5. Key Performance Advantages

1. Stability: Multiple analyses (FTIR, zeta potential, stability studies) consistently demonstrate excellent physical and chemical stability
2. Delivery: Combined rheological and release studies show optimal drug delivery characteristics
3. Efficacy: Antimicrobial studies reveal superior or comparable activity to current treatments
4. Safety: Selective activity and maintained pH suggest good safety profile
5. Usability: Rheological properties ensure good application characteristics

Clinical Implications

The combined results suggest that this formulation offers several advantages over conventional retinoid treatments:

1. Once-daily application potential due to sustained release profile
2. Reduced irritation risk through controlled release
3. Enhanced stability ensuring consistent therapeutic effect
4. Dual therapeutic action (retinoid effects + antimicrobial activity)
5. Improved physical properties for better patient compliance

Future Considerations

While the comprehensive analytical data supports the formulation's potential, several areas warrant further investigation:

1. In vivo studies to confirm clinical efficacy
2. Long-term stability beyond 6 months
3. Patient acceptability studies
4. Comparative efficacy studies against market alternatives
5. Cost-effectiveness analysis for commercial viability

This integrated analysis demonstrates that the formulation meets its design objectives for stability, delivery, and therapeutic potential, while offering advantages over existing treatments. The combination of physical stability, controlled release, and antimicrobial efficacy positions this formulation as a promising candidate for acne treatment.

4. Conslusion

The study successfully developed a sustained-release retinaldehyde cream for acne vulgaris, addressing key challenges such as retinaldehyde instability, photodegradability, and irritation. By incorporating advanced formulation techniques, including the use of bioactive excipients and encapsulation methods, the cream offers improved stability, controlled release, and minimized skin irritation. The sustained-release design ensures prolonged therapeutic action, enhancing patient compliance and efficacy while reducing the need for frequent application. The cream demonstrated strong antibacterial properties, particularly against *Propionibacterium acnes* and *Staphylococcus aureus*, crucial pathogens involved in acne pathogenesis.

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Additionally, the formulation showed excellent physicochemical properties, including appropriate pH, viscosity, and homogeneity, ensuring its suitability for acne-prone skin. The results indicate that retinaldehyde, through a well-designed sustained-release system, offers a promising therapeutic alternative in acne management, with superior stability and reduced irritation compared to conventional topical retinoid treatments. This novel formulation thus holds significant potential for improving the quality of life of individuals with moderate to severe acne.

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