DEVELOPMENT AND EVALUATION OF KETOCONAZOLE SERUM

Submitted to

Submitted by

School of Pharmaceutical Sciences

FACULTY OF HEALTH SCIENCES
ATMIYA UNIVERSITY, RAJKOT

By

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8th Semester B.pharm (2023-24)

Under The guidance of

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April-2024

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Certificate

This is to certify that Mr./Ms. ***Detroja Meetkumar Ashokbhai*** *with Enrollment no.* ***200501013*** *of Program* ***B. Pharm****, Semester* ***VIII*** *has satisfactorily completed his/her term work in the Course* ***Project Work*** *, Course Code* ***18BPHCC803*** *for the term ending in the month of* ***April*** *Academic year 20****23*** *-20****24****.*

Remarks:

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Exam No.	*Exam Date*	*Examiner's Name*	*Examiner's Sign*

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Date

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Remarks:

2023/24.
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200501018 | 18/04/2024 | Vijaykumar |
Exam No. | *Exam Date* | *Examiner's Name* | *Examiner's Sign*

DECLARATION

We, all hereby declare the Work is presented in the project report entitled Development and Evaluation of Ketoconazole Skin Serum.

It is an authentic record of work carried out by us during the studying period of semester 8 at and under the guidance of Atmiya University, Rajkot, and is being submitted for partial fulfillment of the requirement for the award of a bachelor's degree in B.pharm. This is not submitted anywhere else for the award of any other degree/diploma.

Detroja Meetkumar Ashokbhai

Dhinoja Meet Mukeshbhai

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Achievement of goal is not a one person's job. It is obtained by guidance and cooperation of others. Gratitude brought to be a way of life, something which we cannot give enough of. It can mean a smile or a thank you gesture or appreciation.

Thank god, heartly, for always standing by us and giving our strength to complete our work to a satisfactory level in a short period of time.

It gives me great pleasure to express our gratitude and obligation to our respected guide **Ms. Hani Jani** Department of health science, Atmiya University, Rajkot. Whose guidance and encouragement helped me to complete our work and also for her immense knowledge and suggestion throughout my review work.

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With whole hearted sincere gratitude, would like to dedicate the entire work of mine to my facility and without whose care, support, guidance and encouragement, it would be impossible.

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Abstract:-

This work is an outlines of development and evaluation of an anti-fungal ketoconazole serum. A skin serum is a concentrated skincare product formulated with high levels of active ingredients designed to address specific skin concerns. Fungal infections pose a significant health threat worldwide, so it requires a good or effective dosage form of antifungal agent. Ketoconazole broad-spectrum anti-fungal agent in this study we develop and evaluate ketoconazole serum for topical use & topical application. Ketoconazole main effect is anti-fungal, dermatitis and anti-dandruff but on skin formulation mainly effect is anti-fungal and concentration of ketoconazole are mainly use is a 1%, 1.5% and 2%. As a formulation, serum contains ketoconazole with Rose oil and we use a minimum excipients an achieve an anti-fungal action for human skin. To formulate a serum we use excipients like a moisturizing agent, humectant, emulsifier, preservatives and stabilizing agents. Then the variety of factors we need to check like stability and compatibility. We use a methanol and rose oil that improve solubility of drug ketoconazole and also they have an individual anti-fungal and anti-microbial activity. Tween 20 used to improve solubility of ketoconazole and also acting as an emulsifier. Glycerine is use as a humectant or moisturizing agent and xanthan gum use as stabilizing agent. To preparation method of serum use as Mechanical starrer 700-800rpm for 15 min. For the stability concern sequence of addition of API & excipient is important application. Evaluation parameter like a pH, general appearance, spread-ability, wash-ability, Absorption time, viscosity, Anti-microbial activity at a laboratory scale and all evaluation parameter was in standard limit.

Keyword: - *Skin Serum, Evaluation, Ketoconazole, Anti-fungal.*

Aim: Development and evaluation of ketoconazole Anti-fungal serum.

Objectives:-

- To formulate a ketoconazole serum using a minimum excipient for topical application.
- To evaluate prepared ketoconazole serum by various evaluation parameters like pH, viscosity, spredebility etc…
- To compare the antimicrobial efficacy of ketoconazole serum with ketoconazole solution.

Introduction:-

Fungal infections continue to present challenges in clinical management due to their prevalence and the emergence of drug resistant strains. Ketoconazole, a synthetic imidazole antifungal agent, has demonstrated broad-spectrum activity against various fungal pathogens. In this project, ketoconazole was formulated into a serum for topical application to enhance its efficacy and facilitate targeted delivery to affected areas.[13]

ANATOMY OF SKIN [11, 12]

Human skin comprises of three but mutually dependent tissues:

The stratified, vascular, cellular called as “epidermis”.

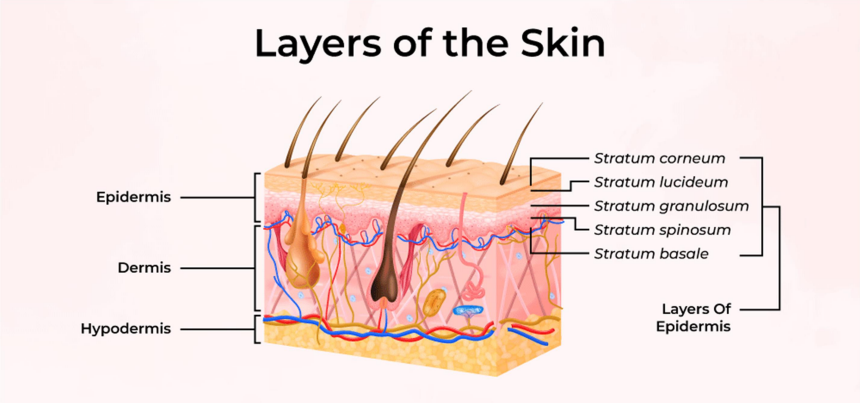


Fig.1 Skin structure

Epidermis

The epidermis of the skin is formed by stratified epithelium, which is made up of5 layers:

1. Stratum corneum.
2. Stratum lucidum
3. Stratum granulosum
4. Stratum spinosum and
5. Stratum germinativum

The absence of blood vessels in the epidermis is its most significant characteristic. The dermis's capillaries supply the nutrients. The top layer of skin, called the epidermis is a

stratified, squamous, keratinizing epithelium. Keratinocytes, which are responsible for the skin's barrier properties, make up more than 90% of the cell population.[11]

Dermis

The next layer of skin, the dermis, is made up primarily of collagen, elastin, and fibril in, which give it its strength and flexibility. The dermis is made up of blood vessels, nerve endings, sweat glands, oil glands, and hair follicles. The dermis is a vascularized, collagen-rich connective tissue that also contains the ground substance, a group of muco poly saccharides. [11]

Hypodermis

The hypodermis is the skin's innermost layer. It is the layer of skin that comes into contact with the body's deeper tissues, like muscles and bone. Sebaceous glands sweat glands, and hair follicles all originate in the dermis but are enclosed in the epidermis. A thin salt solution is injected into the skin's surface by sweat glands. In order to regulate body and skin temperatures, the evaporation of this diluted salt solution cools the skin. The body contains sweat glands all around. The amount of dilutions (sweat) created is influenced by the temperature of the environment, the level of heat-producing skeletal muscle activity, and a variety of emotional aspects. Sebum is an oily substance that enters hair follicles before leaving them and reaching the skin's surface. Sebum defends both hair types.[12]

Function of skin:-[12]

- ✓ Provides a protective barrier against mechanical, thermal and physical injury and hazardous substances.
- ✓ Prevents loss of moisture.
- ✓ Reduces harmful effects of UV radiation.
- ✓ Acts as a sensory organ (touch, detects temperature).
- ✓ Helps regulate temperature.
- ✓ An immune organ to detect infections

Fungus infection:-

Fungus is parasite. The human fungi parasitic relationship results in **mycotic illness**, the majority of which involve superficial invasion of skin or the mucous membranes of body orifices. Fungal diseases are generally called as mycoses. These mycotic infections are caused by ***Tichophyton, Microsporum, Epidermophyton species,*** **Candidiasis** and ***Candida albicans***.

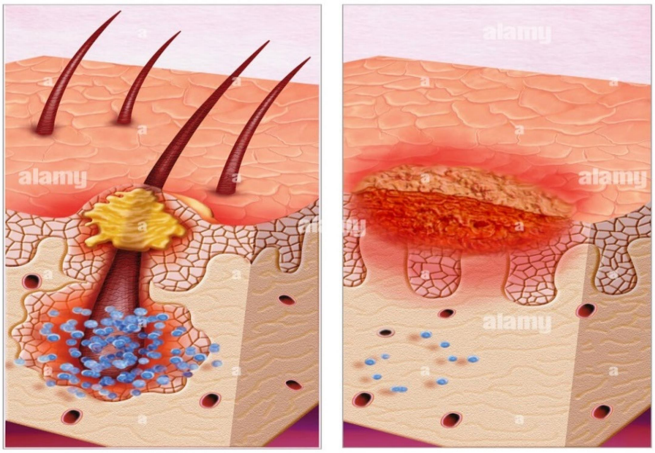


Fig.2 Fungal infection on skin

Here for our project focus on a candidiasis that affects skin and mucous membrane. Our targeted drug is ketoconazole that are a synthetic antifungal agent and include in a azoles class of drug. This class represents **synthetic antifungal agents**. [8]

- **Mechanism:** The cytochrome P-450 class enzyme, **lanosterol α-demethylase**, which is the likely **target for the azoles.** When **DE methylation is inhibited**, the functions of certain membrane-bound enzyme systems such as ATPase and enzymes of the electron transport system, and thus inhibiting the growth of the fungi. [9, 10]

Skin Serum:-[7]

A skin serum is a concentrated skincare product formulated with high levels of active ingredients designed to address specific skin concerns. Unlike traditional moisturizers, serums contain smaller molecules that penetrate deeper into the skin, delivering potent ingredients directly to the targeted areas. Serums are typically lightweight and fast absorbing, making them suitable for use under moisturizers and makeup.

Skin serums are composed of a variety of active ingredients chosen for their specific benefits. Common ingredients including vitamins, hyaluronic acid, peptides, retinoid, botanical extracts anti-oxidant and humectant. These ingredients work synergistically to hydrate, brighten, firm, and protect the skin.

Skin serums serve multiple functions depending on their formulation and ingredients. Some common functions include hydration, anti-aging, brightening, acne treatment, soothing and repairing damaged skin. Each serum is formulated to target specific concerns, allowing for a customized skincare routine tailored to individual needs.

To archive optimal results, proper application of skin serum is crucial. After cleansing and toning the skin, a small amount of serum is dispensed onto the fingertips and gently massaged onto the face and neck using upward, circular motions. Serums are typically applied before moisturizers and sunscreen, allowing for maximum absorption of active ingredients.

Skin serums offer several benefits over traditional skincare products. Their lightweight texture allows for quick absorption, making them suitable for all skin types, including oily and acne-prone skin. Additionally, their high concentration of active ingredients delivers targeted results, effectively addressing specific concerns such as fine lines, wrinkles, hyperpigmentation, and dehydration.

With consistent use, skin serums can deliver noticeable improvements in the overall appearance and health of the skin. However, it is important to remember that results may vary depending on individual skin types and concerns. Patience and adherence to regular skincare routine are keys to achieving desired outcomes.

In conclusion, skin serums are potent skincare products formulated to deliver targeted benefits to the skin. By understanding their composition, functions, application techniques, benefits, and expected results, individuals can incorporate serums into their skincare routines to achieve healthier, more radiant skin.

Types of serums based on their formulation:-[7]

1. **Water – Based Serums:** These serums have a lightweight, watery consistency and are typically formulated with water-soluble for all skin types, especially oily or acne-prone skin, as they provide hydration without adding excess oil to the skin.

Fig. 3 Water based serum

2. **Oil-Based Serums:** These serums contain lipid-soluble active ingredients and have a richer, more nourishing texture. They are ideal for dry or mature skin types, as they help to replenish moisture and improve skin elasticity. Common oils used in oil-based serums include argon oil, jojoba oil, and rose oil.

Fig. 4 Oil-based serum

3. **Emulation serum:** Emulsion serums combine water-based and oil based ingredients to create a hybrid formulation that offers both hydration nourishment. They are suitable for normal to combination skin types and provide a balanced approach to skincare.

Fig. 5 Emulation serum

4. **Gel Serum:** Gel serums have a gel-like texture and are formulated with ingredients like hyaluronic acid or Aloe Vera to provide intense hydration and soothe the skin. They are lightweight and fast-absorbing, making them suitable for all skin types, including sensitive skin.

Fig.6 Gel Serum

5. **Cream Serums:** Cream serums have a creamy texture and are formulated with a blend of water-based and oil-based ingredients. They provide hydration, nourishment, and anti-aging benefits in one product, making them ideal for mature or dehydrated skin types.

Fig.7 Cream serum

6. **Pressed balm serum:** Pressed balm serum is skincare product that combines the properties of balm and a serum. It typically comes in a solid, pressed from like a balm but contains concentrated active ingredients similar to those found in serum. This type of product aims to provide intense hydration, nourishment, and other targeted benefits to the skin. The pressed format makes it convenient to use and travel-friendly.

Fig.8 Pressed balm serum

These different formulations cater to various skin types and concerns, providing options for individuals to find the most suitable serum for their specific needs and preferences.

Ideal Properties of skin serum:-

1. **Hydration:** A good face serum should be hydrating, helping to maintain the skin's moisture barrier. Ingredients like hyaluronic acid are commonly found in serums for their ability to deeply hydrate the skin.
2. **Brightening**: Serums with brightening properties can help even out skin tone and fade dark spots or hyperpigmentation. Ingredients like vitamin C and niacinamide are effective at brightening the skin and promoting a more radiant complexion.
3. **Fast Absorption**: A good serum should have a lightweight texture that absorbs quickly into the skin without leaving a greasy or sticky residue. This allows for easy layering with other skincare products and makeup application.
4. **Suitable for Sensitive Skin**: For individuals with sensitive skin, it's important to choose a serum formulated with gentle ingredients that are less likely to cause irritation or allergic reactions.
5. **Customization**: Ideally, face serums should offer some level of customization to address specific skincare concerns, whether it's targeting wrinkles, dark spots, acne, or dryness. This may involve selecting serums with specific active ingredients tailored to individual needs or using serums in combination with other skincare products for enhanced results.

Advantages of skin serum

1. High concentration of active ingredients.
2. Deep penetration
3. Intense hydration
4. Targeted treatment
5. Lightweight formulation
6. Fast absorbing
7. Visible results

Disadvantages of skin serum

1. Expensive than other skincare product.
2. Allergic reactions in sensitive individuals.

Direction to use of serum:-

Applying an antifungal serum to the skin requires careful consideration and adherence to proper application techniques to ensure effectiveness and minimize any potential side effects. Here's a step-by-step guide on how to apply an antifungal serum to the skin:

1. Cleanse the Skin: Start by washing the affected area with a gentle cleanser and water. This helps to remove any dirt, oils, or impurities from the skin's surface, allowing the antifungal serum to penetrate more effectively.

2. Pat Dry: Gently pat the skin dry with a clean towel. Avoid rubbing, as this can irritate the skin, especially if it's already sensitive due to a fungal infection.

3. Apply a Thin Layer: Using clean hands or a cotton swab, apply a thin layer of the antifungal serum directly to the affected area. Be sure to cover the entire affected area and a small margin of healthy skin surrounding it to prevent the spread of the infection.

4. Massage Gently: Use gentle, circular motions to massage the serum into the skin until it's fully absorbed. Take care not to apply too much pressure, especially if the skin is inflamed or tender.

5. Allow Absorption: Let the serum dry completely before covering the area with clothing or applying any other skincare products. This allows the antifungal medication to penetrate the skin and exert its effects.

6. Repeat as Directed: Follow the instructions provided with the antifungal serum regarding how often to apply it and for how long. It's essential to adhere to the recommended dosage and duration of treatment to achieve the best results and prevent the recurrence of the fungal infection.

7. Maintain Good Hygiene: To prevent the recurrence of fungal infections, practice good hygiene habits such as keeping the skin clean and dry, avoiding sharing personal items like towels and clothing, and wearing breathable fabrics.

By following these steps, you can effectively apply an antifungal serum to the skin to treat fungal infections and promote skin he6yalth.

Table:-1. Common serum ingredients and their use

Uses	Ingredients
Emollient/Humectant	Glycerin,
Preservatives	Sodium benzoate, Methyl Paraben, Ethyl Paraben
Thickener/Stabilizer	Carbopole 940, Acacia Gum, Xanthan gum
pH adjustor	Triethanolamine, lactic acid, citric acid
Fragrance/Perfume Agent	Rose oil, lavender oil , lemon oil, Many flavors
Emulsifier	Span 60, Span 20, Tween 20, Tween 80, PEG 400
Oils	Rose oil, argan oil, tea tree oil, olive oil

Fig.9 Marketed serum images

Material & Methodology

Material & Methodology:-

From the trial and error batches we concluded with following ingredients for final batch respective to its concentration.

Uses	Ingredients	Amount In %
API	Ketoconazole	1%
Emollient/Humectant	Glycerin,	5%
Preservatives	Sodium benzoate	0.2%
Thickener/Stabilizer	Xanthan gum	0.3%
pH adjustor	Triethanolamine	0.01%
Fragrance/Perfume Agent	lavender oil	0.01%
Emulsifier	Tween 20	5%
Oils	Rose oil	1%
Solubilizer	Methanol	5%
Vehicle	Water	Up to 100%

Table: 2. Ingredients and its concentration for final batch

Table: 3. Ketoconazole profile:-

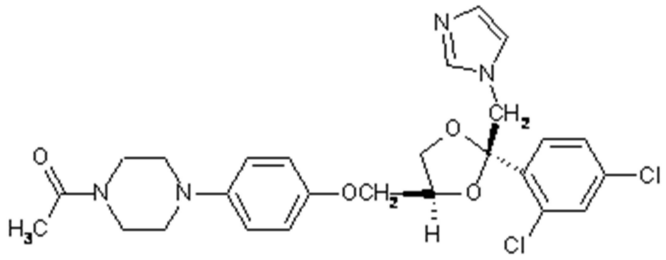
Name of API	Ketoconazole
Physical Description	A white or almost white powder
Mol. Formula	$C_{26}H_{28}C_{l2}N_4O_4$
IUPAC Name	1-[4-[4-[[2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]ethanone
Chemical Structure	
Relative molecular mass	531.4 g·mol−1
Solubility	Practically insoluble in water; freely soluble in dichloromethane R; soluble in methanol R; sparingly soluble in ethanol (~750 g/l) TS
Category	Antifungal drug.
Storage	Ketoconazole should be kept in a well-closed container, protected from light
Mfg.	Dano pharm Gift sample by SG HEALTH CARE PVT LTD

Fig.10 Ketoconazole Gift Sample SG HEALTH CARE PVT LTD

Table: 4. Glycerin profile:-

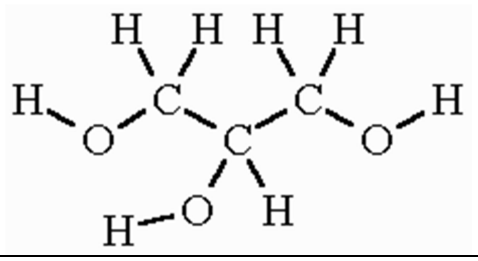
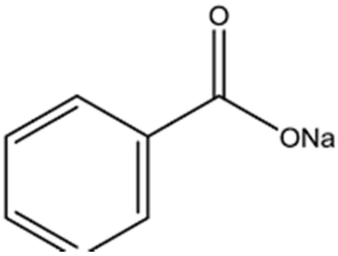
Name of API	**Glycerin**
Physical Description	Colorless hygroscopic liquid
Mol. Formula	$C_3H_8O_3$
IUPAC Name	Glycerol1,2-EthanediolPropane-1,2,3-triol1,2,3-Trihydroxypropane
Chemical Structure	
Relative molecular mass	92.094 g·mol−1
Solubility	Water
Use	Improving smoothness, providing lubrication, and as a humectant
Viscosity	1.412 Pa·s
Mfg.	CDH

Fig.11 Glycerin

Table: 5. Sodium benzoate profile:

Name of API	**Sodium benzoate**
Physical Description	White or colorless crystalline powder
Odor	Odorless
Mol. Formula	$C_7H_5NaO_2$
IUPAC name	Sodium benzoate
Chemical Structure	
Relative molecular mass	144.105 g·mol−1
Solubility	Water, methanol, ethanol, dioxana
Use	Preservative in pharmaceutical and cosmetics
Mfg.	Nulaligens

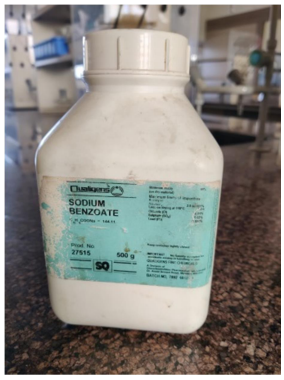
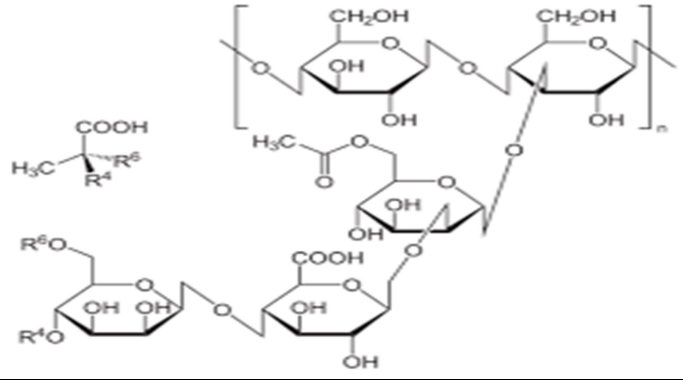


Fig.12 Sodium Benzoate

Table: 6. Xanthan gum profile:

Name of API	Xanthan gum
Physical Description	Light yellow to white flow-able powder
Odor	Slight odor
Mol. Formula	$C_{35}H_{49}O_{29}$ (monomer)
IUPAC name	2-(2,4-diaminophenoxy)ethan-1-ol dihydrochloride
Chemical Structure	
Relative molecular mass	933.748 g·mol−1
Solubility	Cold and hot water
Use	0.1% (by weight). Increasing the concentration of gum gives a thicker, more stable emulsion up to 1%
Mfg.	Qualikems

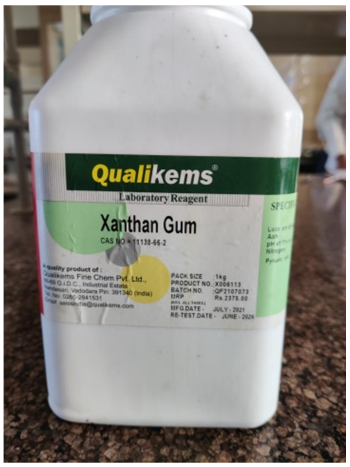
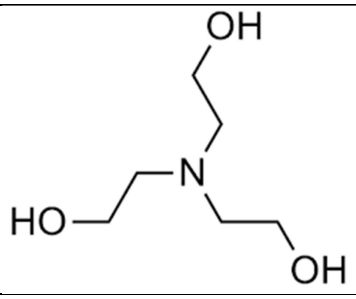


Fig.13 Xanthan gum

Table: 7. Triethanolamine profile:

Name of API	Triethanolamine
Physical Description	Viscous liquid
Color	Colorless
Mol. Formula	$N(CH_2CH_2OH)_3$
IUPAC name	2-[bis(2-hydroxyethyl)amino]ethanol

Chemical Structure	
Relative molecular mass	149.190 g·mol−1
Solubility	Water
Use	Neutralizes, adjusts the pH, emulsifier
Mfg.	Qualikems

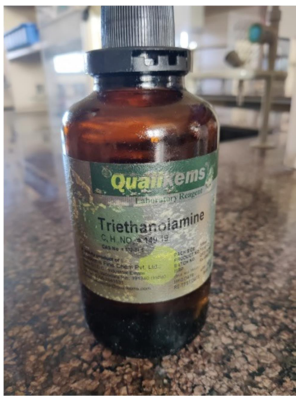
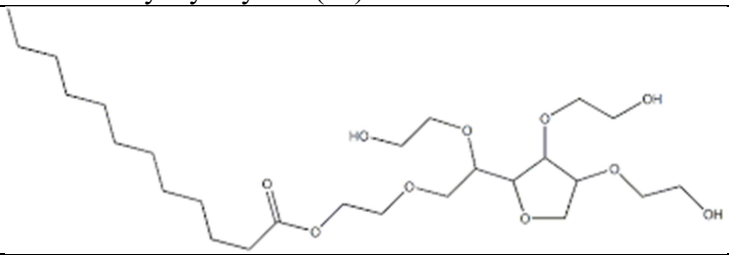


Fig.14 Triethanolamine

Table: 8. Tween 20 profile:

Name of API	**Tween 20**
Physical Description	Clear, viscous liquid
Color	Yellow to yellow-green
Mol. Formula	$N(CH_2CH_2OH)_3$
IUPAC name	Polyoxyethylene (20) sorbitan monolaurate
Chemical Structure	
Relative molecular mass	1226 g·mol−1
Solubility	Miscible with water, alcohol, dioxana, and ethyl acetate
Use	Pharmaceutical applications to stabilize emulsions and suspensions
Mfg.	Qualikems

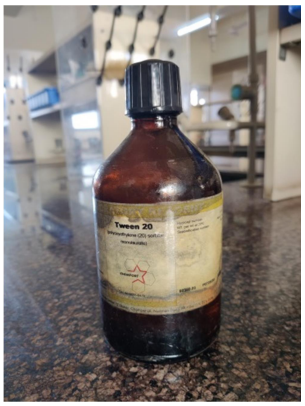


Fig.15 Tween 20

❖ **Rose oil profile:**

- It has a light yellow color.
- It has a strong aroma and sharp balsamic taste.
- Rose oil is known to have a regenerative effect on cell tissue, making it especially beneficial for dry, sensitive or ageing skin. It can keep skin healthy, lubricated.
- Use: For helping to enhance solubility of ketoconazole
- Mfg. Moriax

Fig.16 Rose oil

Table: 9. Methanol profile:

Name of API	Methanol
Odor	Faint and similar to ethanol
Color	Colorless liquid
Mol. Formula	CH_3OH
IUPAC name	Methanol

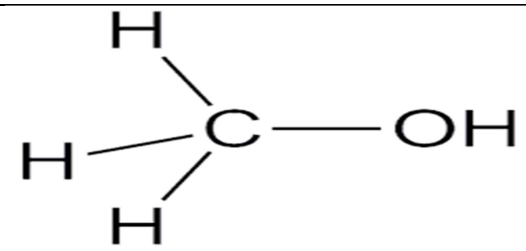
Chemical Structure	
Relative molecular mass	32.04 g·mol−1
Solubility	Water soluble
Use	Making a solution of ketoconazole
Mfg.	Rankem

Fig.17 Methanol

Method of preparation:

Step-1
- 1% ketoconalole +1% rose oil + 5% Methanole mix in beaker .
- Add 5% tween 20 steering proper untile proper mixing.

Step 2
- Add 60% water + 0.3 % xenthan gum
- Add 5% Glycerin + 0.2% sodium benzoate
- Mix in mechanical stirrer 700-800 rpm for 15 min

Step 3
- Up to 100% volume with water and mixing using mechaniccal stirrer 700-800 rpm until uniforme mixtcure get.

Evaluation Parameter of serum

Evaluation Parameter of serum:-

1. **Physical Evaluation:** The formulation's color and appearance were visually assessed. The consistent dispersion of extracts during the formulation process both touch and visual appearance were used to confirm this test.

   The texture, color, and smell of the serum were examined directly to determine its physical properties. All formulas produced products that were milky white in color and were not greasy or oily. The scent of the serum was pleasant compared to the traditional damask rose fragrance. It appeared transparent white in color.

2. **pH Value:** A standard buffer solution was used to calibrate a pH meter. After accurately measuring and dissolving nearly 1 milliliter of the serum in 50 milliliters of pure water, the pH of the mixture was determined. Since the pH of skin serum should range from 4.1 to 6.7, the skin has an acidic range. [2]

3. **Spredebility:-**

   The serum spreads over the filter paper, showing the size of the area it was applied to. The total area of each filter paper (A1) for each of the selected filter paper sizes and its mass are quantified (W1). Select the test formulation, transfer a small amount of milliliters to a BDD 5 mL syringe, and insert 20 droplets into the center of the filter paper. Once the last drop of liquid hits the filter paper, set a stopwatch or timer to run until exactly ten minutes have elapsed. The filter paper will be covered in liquid in a largely constant circular pattern for the duration of the 10-minute test. After ten minutes, carefully cut along the line that separates the wet spread.

   Also, use scissors to dry the filter paper. Weigh the residual dry (unsaturated) filter paper. This weight has to be identified as W2. It is essential to measure the saturated filter paper's diameter. If the spread was not a full circle, measure the diameter multiple times .The average diameter by measuring the spread zone. For this measurement, A2 is the proper input. [1]

   $$\% \text{ Spread by Area} = (A2/A1)100$$

4. **Absorption Time:-**

   After applying the serum to the skin and timing how long it takes it to absorb, note the time. [4]

5. **Wash-ability:-**

   First, place the product on your face. Then, test it on yourself to see how easy and how thoroughly it can be washed off. [5]

6. **Homogeneity:-**

   The homogeneity of the serum was checked both visually (for the absence of any particle Matter) and physically (by contacting the product). The homogeneity of the droplets, size, shape and coalescence could be verified when serum was examined in a microscope. [1]

7. Stability analysis:-

The preparation and development of a medicinal product must not be completed without carefully checking that it is stable in terms of physical and Chemical Stability, so as to verify its safety. For the prediction of stability, a process known as rapid stability analysis is often applied in which materials are heated to high temperatures. In the course of stability investigations, ICH guidelines were followed. A three-month accelerated short-term for the formulation stability studies have been performed. The samples have been stored in a range of temperatures and relative humidity levels, including 25^0C RH=60% and 40^0C ±2%. For three months, RH =75%. Samples were collected and evaluated on a monthly basis. But in laboratory level we check a multiple environment like a directly contact with sunlight and not contact with sunlight we check for 10 day. [3]

8. Viscosity:-

A Brookfield viscometer is used with 100 rpm, 100 ml of serum, to measure the viscosity of the formulation. Putting the spindle dipped in the big mouth container with the serum for about 3 minutes before to the measurement. [3]

9. Microbial study:-

The antibacterial activity of we assessed against Staphylococcus, Proteus vulgaris bacteria species. We use a cup plate method firstly we make agar plate and speeding bacteria on the solid agar plate using a speeder. Then create a pore using a borer and transfer a serum formulation in to pores. Incubate a petri-plate in to incubator for 24 hours at 38ºC ± 2ºC. After 24 h see a petri-plate and find a zone of inhibition. [6]

Result & Discussion

Result & Discussion:-

1. **Physical Evaluation:-**
   To assess its physical characteristics, the texture, color and aroma of the serum were examined in direct. All formulas resulted in products with a white, milky appearance and were smooth viscous. It's a pleasant smell of lavender.

Fig.18 Serum final batch image

2. **pH Value:-**
   The pH value of the formulation was determined to be 5.5, which is close to the predicted value. The product is still unionized, so that it is compatible with the skin's pH, and there are no heavy metals in the formulation. The formulation is not responsible for irritation or allergic reactions.

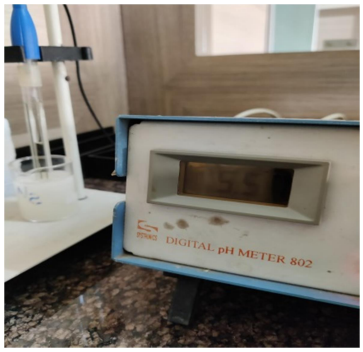


Fig.19 pH meter reading

3. **Spredebility:-**
   It was anticipated that the formulation would have good spredebility. There is a Viscosity and spredebility has a linear relationship in rheological studies; the lower the viscosity, the lower the surface tension, and the higher the spredebility.

$$\% \text{ Spread by Area} = (A2/A1)100$$

Here,
A1= 33.16 cm^2
A2= 4.90 cm^2
These values put in equation than,
% Spread by Area = (A2/A1)100
= (4.90/33.16)100
= (0.1477)100
= 14.77%

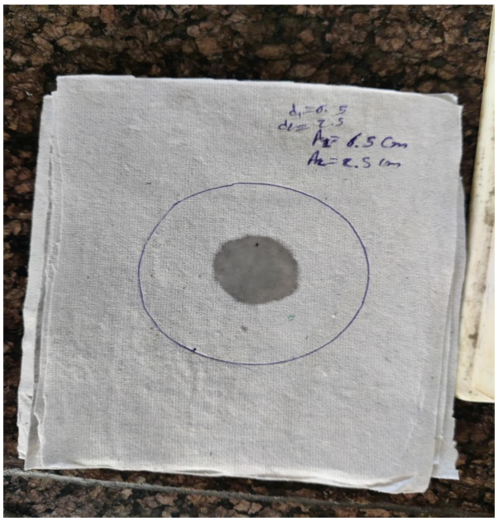


Fig.20 Spredebility testing

4. **Absorption Time:-**
When applied to the skin, serum begins to absorb as soon as it's absorbed and is fully absorbed in 1–2 minutes.

5. **Wash-ability:-**
The face was assessed for the ability of the formula to wash. The skin's been left in perfect condition, bright. After its simple removal, it is moist and fresh.

6. **Homogeneity:-**
You'll apply some serum drops to your hand, and the homogeneity will be monitored as you can see it. The serum is to be used in an even way according to the formulation. Particles are well dispersed with a galling agent.

7. **Stability analysis:-**
Laboratory level we check a multiple environment like a directly contact with sunlight and not contact with sunlight we check for 10 day. Throughout the stability study, the product's quality, safety, and efficacy are maintained.

8. **Viscosity:-**
The viscosity of the formulation was assessed using a Brookfield Viscometer, but our department Brookfield viscometer damage than we can't perform this study.

9. Microbial study:-

The results of antimicrobial studies indicate that agar plate of test inoculums show similar zone of inhibition as compared to standard from 24 h grown culture. The result indicating that the formulation was acting as anti-microbial activity and safe to use.

Fig.21 Microbial Study

Table: 10. Result Summary

SR. No.	Parameters	Result
1	Color	White, milky
2	Appearance	Smooth Viscous
3	Homogeneity	Homogenous mixture
4	pH	5.5
5	Stability	Stable
6	Wash-ability	Washable
7	Absorbance time	1-2 min
8	Spredebility	Very Spreadable
9	Viscosity	---
10	Anti-microbial activity	Satisfactory

Conclusion

Conclusion:-

Developed formulation contain ketoconazole which dissolved in rose oil and methanol due t its good solubility in these solvents. Other ingredients like glycerin act as humectant, xanthan gum is for viscosity enhancer and stabilizer and tween-20 is act as emulsifier in this formulation. After development of serum it was evaluated which parameter like Color, Appearance, Homogeneity, pH, Stability, Wash-ability, Absorbance time, Spredebility, Viscosity, Anti-microbial activity so all tested parameters were satisfactory. The developed formulation was tested for in anti-microbial activity using a *streptococcus* and *proteus vulgaris* by cup plate method and it was shows anti-microbial action. So, it was calculated that prepared serum was well develop & have satisfactory results.

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