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FORMULATION AND EVALUATION OF MICROEMULSION BASED HYDROGEL FOR TOPICAL DRUG DELIVERY SYSTEM OF KETOCONAZOLE

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ABSTRACT

The goal of the current study was to create a hydrogel formulation based on microemulsions for the topical application of ketoconazole that has increased microbiological activity while avoiding systemic side effects. A medication with antifungal properties. Oleic acid was chosen after several oils were evaluated for drug solubility. To create microemulsions, various cosurfactants and surfactants were evaluated. Specifically, Propylene Glycol and Twee 80. The hydrogel based on microemulsion was created using Carbopol 934 as a gel matrix to increase the viscosity of the microemulsion for topical application. Zeta Potential, pH, visual inspection, thermodynamic stability (heating and cooling cycle), and infrared spectroscopy were all used to assess the improved microemulsion. Following the test, the microemulsion we had chosen proved to be completely stable.

KEYWORDS: Microemulsion, ketoconazole, Propylene Glycol, Hydrogel, Oleic acid, Carbopol 934.

INTRODUCTION

The synthetic broad-spectrum antifungal drug ketoconazole (KTZ) was introduced to clinical usage in 1977. Ketoconazole, which works well against yeasts, dimorphic fungus, dermatophytes, and Malassezia species. The primary way that ketoconazole works is by preventing the formation of a vital lipid found in fungal membranes. It disrupts the production of ergosterol, which modifies the permeability of the fungal cell membrane in susceptible organisms. This antimicrobial drug exhibits broad range efficacy as a fungistatic. The gastrointestinal side effects of KTZ, such as nausea or anorexia, are by far the most common. Hepatic and cardiovascular side effects have also been documented.[1,2] Patients with pre-existing liver disease cannot get KTZ since it exhibits hepatotoxicity.[3] Because topical therapy is non-invasive and may direct medication to the site of action, it is extremely desirable as it can reduce systemic side effects and increase patient compliance.[4]

In comparison to conventional creams and lotions, topical drug delivery microemulsions (MEs) have advantages. Drugs were solubilized and topical medication availability was increased with their application.[5]

The enhancement of topical KTZ's antifungal efficacy through the use of microemulsion- based hydrogel systems was the study's main objective. After screening a variety of oils, surfactants, and cosurfactants to determine the best component ratio for KTZ microemulsion based hydrogel formulation, MEs containing KTZ were created. Several techniques were used to evaluate the prepared formulae's stability, microbiological activity, and physical characteristics.[6]

DESIRABLE CHARACTERISTICS OF TOPICAL DRUG DELIVERY SYSTEMS

Topical formulations have three main functions:

- Because of their emollient properties topical formulations helped to hydrate skin.
- Protection from external environment or heal an intact or injured area of the skin.
- Delivering medication to the skin.[7]

Advantages of Topical Drug Administration

1. Gastro intestinal pH, enzymatic activity and drug interactions with food, drink, and other orally administered drugs are major cause of GI infection which causes difficulties in drug absorption can be avoided by topical drug administration.
2. As an alternative to other administration routes (such as oral administration or intravenous injection) in cases when such routes are inappropriate, such as in cases of diarrhea, vomiting, swallowing issues, or resistant youngsters.
3. A wide range of medications can be dissolved with different chemical properties, which leads to making different combination therapy with one transdermal cream.
4. Provides extended therapy with a single application, improving compliance.
5. Less greasy and can be easily removed from the skin.[8,9]

MATERIAL AND METHOD

Ketoconazole API were purchased From Yarrow chem Products Private limited, Excipients Oleic acid (Oa), Propylene Glycol (Pg), Tween 80, Isopropyl Myristate (IPM), Glycerine, Tween 20, Butanol, Ethanol Was Obtained From LBYP college of pharmacy, Pathri.

Preformulation studies

A] Solubility study

The maximum solubility of Ketoconazole (KTZ) was determined in different oils by adding an excess amount of the drug in 2 mL of each solvent (oleic acid, Propylene Glycol, Tween 80, Isopropyl Myristate, Glycerine, Twee 20, Butanol, Ethanol, Distilled Water. Solubility test shown in (Fig.no-5)

Characterization of ketoconazole

Physical examination like colour, Odour and melting point was determined (Fig no- 1). Fourier transform infrared spectrophotometer measured ketoconazole infrared spectrum. IR platform received a small sample. Spectra were scanned at 4 cm-1 resolution from 4000 to 400 cm-1.(Fig no-2)

B] Uv spectroscopy

a. Determination of λmax of ketoconazole

STOCK 1-10 mg ketoconazole was dissolved in 2 ml of methanol and make up the volume upto 10 ml with phosphate buffer [6.8]

STOCK 2- use STOCK 1 solution and add in 100 ml of volumetric flask then adjust the volume upto 100 ml with phosphate buffer [6.8] Finally, 9 ml Phosphate buffer solution [6.8] and 1 ml Stock II solution were added to a 10 ml volumetric flask UV spectrophotometric study (200-800 nm) determined λmax for the solution.(Fig no. 3)shows the λmax of ketoconazole in methanol Phosphate buffer [6.8].[10]

b. Calibration curve of ketoconazole in Phosphate buffer solution (pH 6.8)

STOCK 1-10 mg ketoconazole was dissolved in 2 ml of methanol and make up the volume upto 10 ml with phosphate buffer [6.8]

STOCK 2 – STOCK 1 solution and add in 100 ml of volumetric flask then adjust the volume upto 100 ml with phosphate buffer [6.8] Calibration curve of ketoconazole in Phosphate buffer solution (pH 6.8) (shown in Fig no.4)

Table 1: Dilutions of phosphate buffer solution.

1.	Take out 1 ml from stock 2 +	9 ml of phosphate buffer 6.8 which gives	10 µg/mL.
2.	Take out 2 ml from stock 2 +	8 ml of phosphate buffer 6.8 which gives	20 µg/mL.
3.	Take out 3 ml from stock 2 +	7 ml of phosphate buffer 6.8 which gives	30 µg/mL.
4.	Take out 4 ml from stock 2 +	6 ml of phosphate buffer 6.8 which gives	40 µg/mL.
5.	Take out 5 ml from stock 2 +	5 ml of phosphate buffer 6.8 which gives	50 µg/mL.

All solutions were scanned with a Shimadzu UV1800 spectrophotometer as blank of isotonic PBS [pH 6.8]

C]. Selection of oils, surfactants and co-surfactant for formulation study[11,12,13,14]

In order to prepare an optimized Microemulsion, it was great importance to select an appropriate oil, surfactant and co-surfactant combination that had a good solubilizing capacity for ketoconazole drug. Oleic acid dissolved ketoconazole well. Solubilizing microemulsion improved dermal flow. Oleic acid increased stratum corneum lipid fluidity and permeability in ketoconazole microemulsion.

Surfactant selection

Hydrophillic emulsifier {Tween} –use in case of O/W Emulsion

Lipophillic emulsifier {Span}—use in case of W/O Emulsion

Co-surfactant selection

The selected co-surfactant was combined with four different solubilizers as co- surfactants, namely isopropanol, ethanol, PEG 400, Propylene Glycol.

From the above data we use

Oleic acid as a oil, Tween 80 as a Surfactant and Propylene Glycol as a Co- surfactant

Preparation of Ketoconazole Microemulsion systems

KTZ ME formulae were selected at different component ratios according to the microemulsion areas in phase diagrams. The drug (appropriate amount to prepare MEs containing 2 % KTZ) was dissolved in oil by stirring for 5 min, then

S/Cos mixture was added and stirring was continued for a further 30 min. Finally, water was added and the system was stirred to attain equilibrium for 10 min. The prepared KTZ MEs were stored in tightly closed glass containers for three days to attain equilibrium before subjection to further evaluation tests.[15]

This below Table helps in formulation of optimum microemulsion.

Table 2: Preparation of Ketoconazole Microemulsion.

Batches		Oil (5ml)	Surfactant	Co- Surfactant	Aqueous phase (40ml)
	Drug	Oleic acid	Tween 80	Propylene glycol	Water
A	2%	5	27.5	27.5	40
B	2%	5	18.33	36.66	40
C	2%	5	13.75	41.25	40
D	2%	5	11	44	40
E	2%	5	44	11	40
F	2%	5	41.25	13.75	40
G	2%	5	36.66	18.33	40

Characterization of Ketoconazole Microemulsion[16]

The prepared Ketoconazole micro emulsion was subjected to the following tests.

1] Visual inspection

The formulae were inspected for optical transparency and homogeneity by visual observation against strong light. The systems were also checked for the presence of undissolved drug, fluidity, and phase separation Results shown in (Fig no:6).

2] Fourier Transform Infrared Spectrometer (FTIR)

Fourier Transform Infrared Spectrometer is a versatile analytical technique used to study the chemical composition, molecular structure, and bonding characteristics of substances by measuring their interaction with infrared radiation. Results shown in (Fig no: 7) Identification of function group Quantitative analysis Structural Information.

3] Zeta potential

The zeta potential for microemulsion was determined using zetasizer (Malvarn instrument) sample were place in clear disposable zeta cells and result were recorded. Cuvettes were rinsed with the sample to be measured before each experiment, and they were cleaned with methanol before adding the fresh sample. shown in (Fig no:8).

4] pH measurements

The pH values of the ketoconazole MEs were measured by direct immersion of the electrode of the pH meter in the system. Shown in (Fig No: 9).

5] Assessment of physical stability [Centrifugation and heating and cooling cycle]

In order to eliminate unstable systems, KTZ MEs were centrifuged at 3500 rpm for 30 min. Those formulae that did not show any phase separation were taken for the heating and cooling cycle. Six cycles between refrigerator temperatures of 4 ± 0.5 °C and 45 ± 0.2 °C for 48 h were done. The formulae that were stable at these temperatures were subjected to three freeze-thaw cycles (FTC) between -5 ± 0.5 °C and 25 ± 2 °C. The formulas that passed thermodynamic stability testing were chosen for more research. Results shown in (Fig No:10,11).

After completion of all tests for microemulsion, it was observed that the selected microemulsion batch is totally stable. Then we go for the next process, i.e. Formulation of a Microemulsion-based hydrogel.

Method of preparation of microemulsion-based hydrogel

The Carbopol 934 as a gel matrix was used to construct the microemulsion-based hydrogel for improving the viscosity of microemulsion for topical administration. The hydrogel and microemulsion were stirred slowly together. The viscosity of the system microemulsion-based hydrogel reduced as the microemulsion was added. Hence, to obtain sufficient viscosity of microemulsion-based hydrogel, the hydrogels were prepared at various concentrations (1,1.5, and 2%w/w), and the final concentration was selected on the basis of viscosity and transparency.[17,18]

Evaluation of microemulsion, hydrogel, and microemulsion-based hydrogel

- Physical appearance
- pH Test

RESULT AND DISCUSSION

A] Organoleptic Properties

Table 3: Identification Test.

Identification Test	Observed Result	Reported Result
Appearance	Colourless powder	Colourless powder
Colour	White	White
Odour	Odourless	Odourless

The Ketoconazole was studied for organoleptic character such as Colour, Odour And Apperance Results of organoleptic properties of Ketoconazole were found to be similar as mentioned in literature which is given in (Table No-3).

B] Determination of Melting point of Drug

Melting point the melting point determined by using Digital Melting point Apparatus results are showing in (Table no-4) Compared with standard.

Table 4: Melting of Ketoconazole.

Identification test	Result	Reported Result
Melting point	153°C	148-152°C

Fig 1: Melting Point of Drug.

Results: From the above results, it was concluded that our ketoconazole (API) melting point matches with standard values.

C]. IR Spectra of Ketoconazole

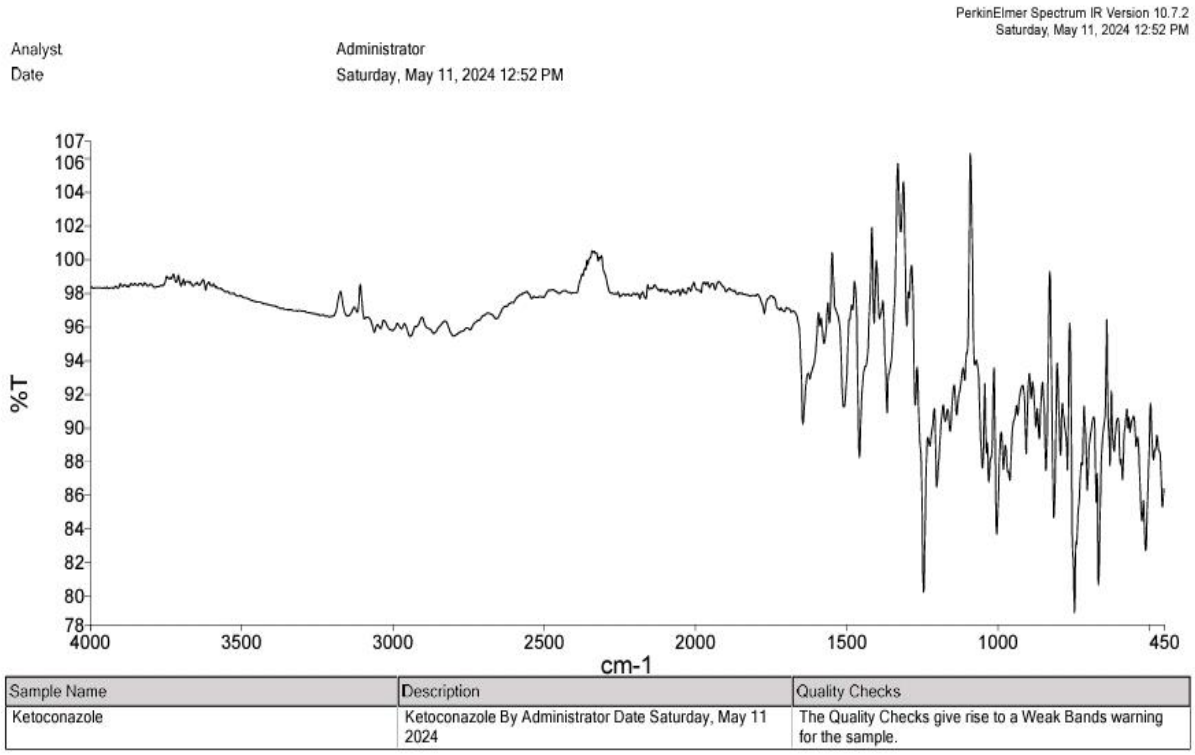

Fig. 2: IR Spectra of ketoconazole.

Table 5: Interpretation of IR (Ketoconazole (API)).

Peak no	Peak	Functional group
1.	1644.31	(C=O) Keto group in ketoconazole.
2.	1509.64	(Aromatic ring stretching vibrations) which indicates Ketoconazole contains an imidazole ring and a phenyl ring.
3	1457.05	(CH3) Ketoconazole has several methyl groups attached to its aromatic rings.
4.	1365.51	(CH3) Presence of methyl groups in ketoconazole.
5.	1244.63	(C-O-C) In ketoconazole, it might relate to the ether linkagem
6.	1200.99	(C-O) Presence of ether or alcohol groups in Ketoconazole.
7.	1050.05	(C-N) Ketoconazole contains an amine group
8.	1028.89	(C-N) Ketoconazole contains an amine group
9.	1002.96	(C-H) Aromatic rings (benzene rings) present in ketoconazole.
10.	905.38	(C-H) Related to the aromatic rings in ketoconazole.

D]. Estimation of ketoconazole by Uv spectroscopy

Determation of absorbance maximum (λ max) of Ketoconazole

Solvent	Observed λ max (nm)	Standard λ max (nm)
Methanol + phosphate buffer solution	**242 nm**	**255.2 nm**

Fig. 3: UV –Visible Spectroscopy.

Result: From the above result, it was concluded that our ketoconazole (API) wavelength matches with the standard value.

Table 6: Calibration curve of ketoconazole in isotonic PBS 6.8.

Sample	Absorbance
1.	0.0425
2.	0.0832
3.	0.1199
4.	0.1602
5.	0.2028

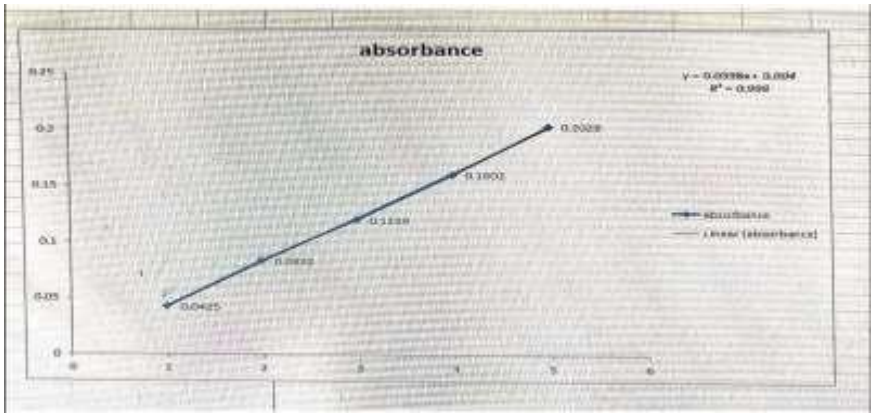

Fig. 4: Calibration curve of ketoconazole in isotonic PBS 6.8.

Intercept	0.0398
R^2	0.998

E] Solubility determination of ketoconazole

Ketoconazole was found to be soluble in Methanol, Chloroform, Oleic acid, Propylene Glycol, Butanol. Sparingly soluble in Distilled water, Insoluble in Glycerine.

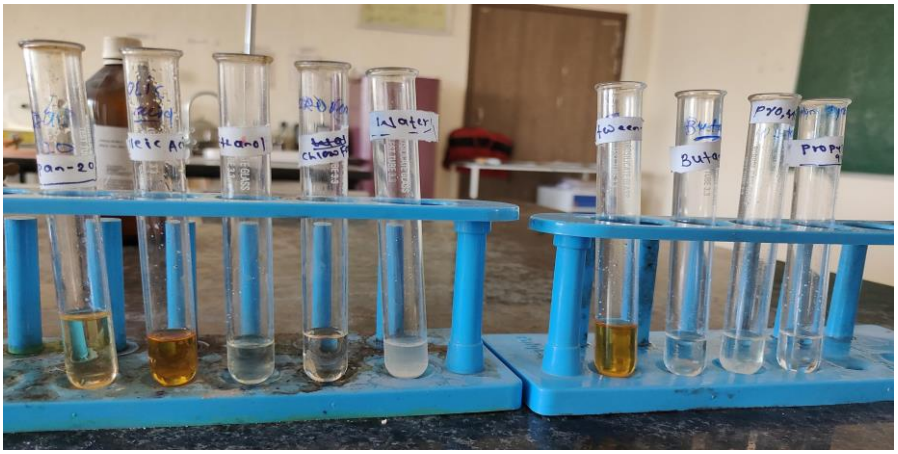

Fig. 5: Solubility profile of Drug.

Results: From the above results, it was concluded that our ketoconazole (API) is completely soluble in methanol, sparingly soluble in distilled water, and insoluble in glycerine.

Physicochemical evaluation of Microemulsion

1. Transparency

The Microemulsion formed were transparent and appeared like a homogenous single- phase liquid, when observed for visual clarity strong light. No traces of undissolved drug or other solid ingredient were found in all sample.

Table 7: Optical transparencies.

Batches	Transparency
A	Transparent
B	Transparent
C	Transparent
D	Transparent
E	Transparent
F	Transparent
G	Transparent

2. Visual Inspection

When the sample appeared as a transparent, readily flowable fluid, it was determined to be a microemulsion.

Fig. 6: Visual Inspection of Microemulsion.

Result: From the above result, it was concluded that the microemulsion is transparent and an easily flowable fluid.

3. IR Spectra of Ketoconazole Microemulsion

This IR spectra shows the Functional group information Present in Microemulsion and Below Spectra match with Standards Results.

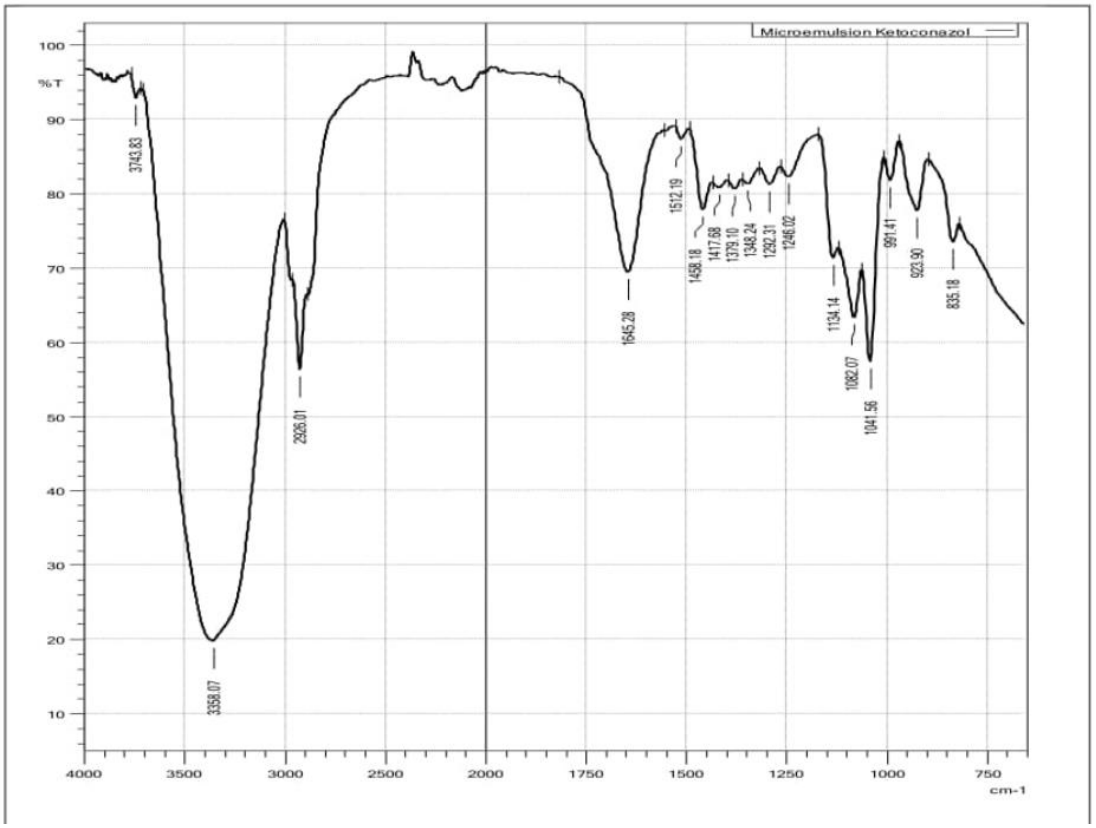

Fig. 7: IR Spectra of Ketoconazole Microemulsion.

Table 8: Interpretation of IR (Ketoconazole Microemulsion).

Peak No	Peak	Functional group
1.	835	C-H stretching in alkanes or aromatic compounds
2.	923	C-H stretching vibrations in benzene rings.
3.	991	C-H stretching vibrations in aromatic compounds
4.	1041	C-N stretching vibrations in amines or amides.
5.	1082	C-O stretching vibrations in alcohols, Phenols, or ethers.
6.	1134	C-N stretching vibrations in amines or Amides.
7.	1246	Stretching vibration of the ether linkage (C-O-C)
8.	1292	C-H stretching vibrations in aromatic compounds.
9.	1348	stretching vibration of C-N bonds in amines Or amides.
10.	1379	C-H stretching vibrations in aromatic compounds.

4. Zeta Potential

Zeta potential was 0.147 mV. This Zeta potential indicate that droplets of the microemulsion having 0.147 mV charge on each globule, and that could responsible for the repulsion of globule from each other and that not allows the globule to settle down for longer period of time, indirectly causing the long stability of the formulations. Zeta potential was determined by using malvernzetasizer.

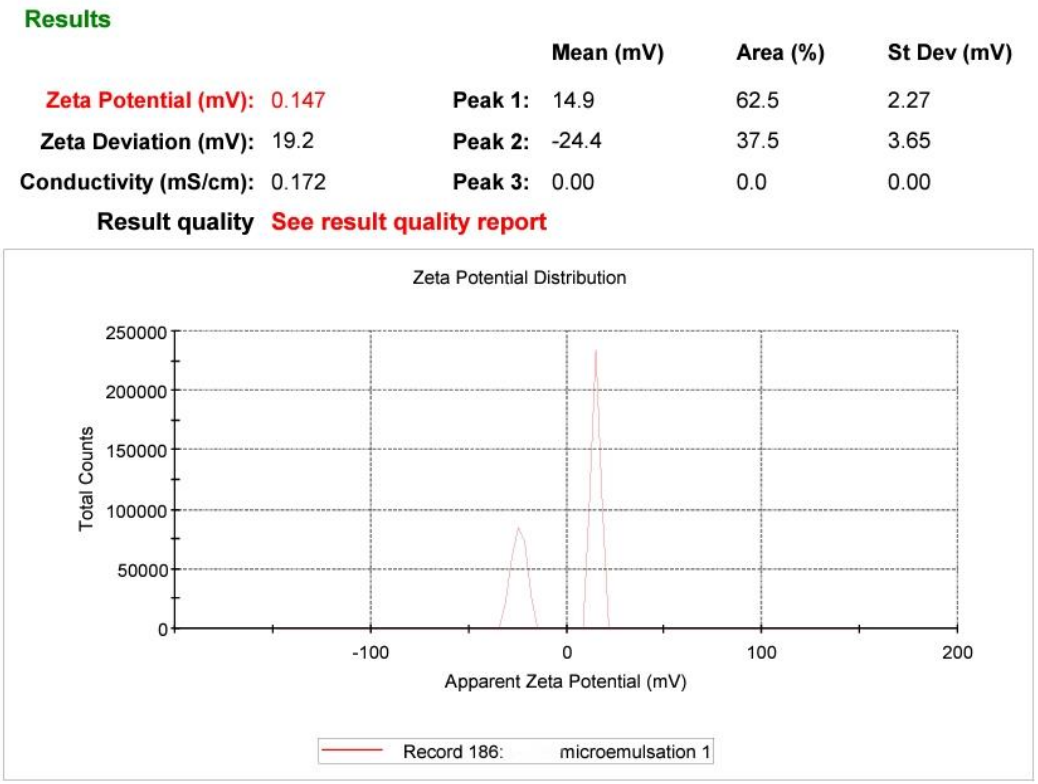

Fig. 8: Zeta Potential of Microemulsion.

5. pH measurement

The values of pH measurements of all formulations were listed in Table No-14 pH of all formulations were found in between 6-6.5 which was acceptable for pH of skin. This is an important parameter as the skin pH ranges between pH 5.5-6.5.

Table 9: pH of microemulsion formulations.

Batches	A	B	C	D	E	F	G
pH	5.84	5.67	5.75	5.59	5.80	5.93	5.70

Fig. 9: pH measurement of Microemulsion.

Result –From the above result it was conclude that the our microemulsion pH get match with skin pH so it is safe to use Topically.

6. Thermodynamic stability

A. Centrifugation Test

All the formulation batches were analyzed for the optical transparency; the results were given in (table No-15.) None of the microemulsion systems showed signs of phase separation on centrifugation at 1000 rpm for 30 minutes. This result provided a rapid and full proof identification of the system as microemulsion, and which was the sign of stability of microemulsion.

Table no-10: Phase separation data

Batches	A	B	C	D	E	F	G
Phase Separation.	No Phase Separation	Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation

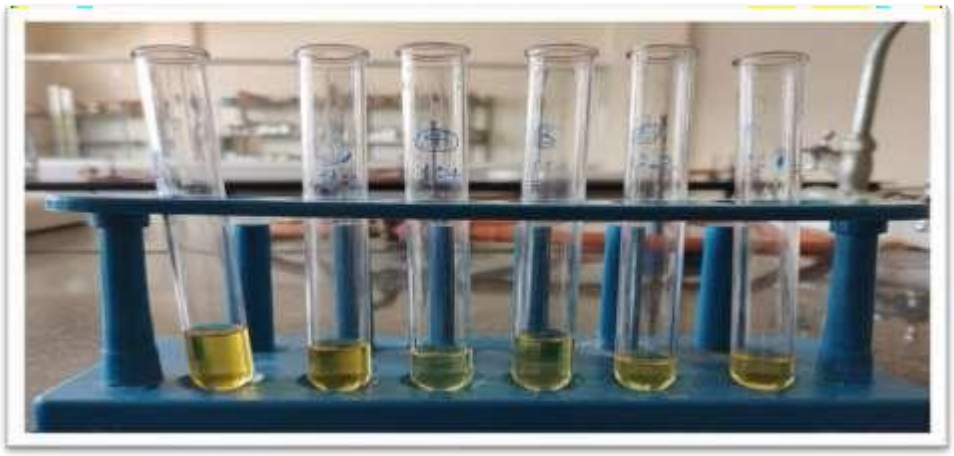

Fig. 10: Centrifugation Test.

From the above result it was observes that all the batches are still stable after centrifugation Test.

B. Stress Test

The transparency and phase separation of the ketoconazole-loaded ME were unaltered during two cycles of heating and cooling. Moreover, there was no evidence of drug precipitation.

There were no indications of phase separation, cracking, or creaming in the drug-loaded ME gel.

Fig. 11: Stress Test.

Physicochemical evaluation of Microemulsion Based Hydrogels

Physical appearance

Conversely, the gel based on ME had a uniform, smooth texture and a shiny look.

Fig. 12: Physical Appearance of Hydrogel.

pH measurement of Hydrogel= pH 6.2

From the above result it was conclude that the ph of Hydrogel is 6.2 which is basically match with our skin pH

CONCLUSION

The developed Microemulsion based Hydrogel formulation showed promising results in terms of enhancing the solubility and stability of Ketoconazole, which is crucial for its effective topical delivery. The Microemulsion based Hydrogel formulation exhibited good physical stability, with no signs of creaming, cracking, or phase separation observed even after stress testing, indicating its suitability for topical application .The study highlighted the importance of preformulation studies in characterizing the physical and chemical properties of the drug and excipients, which are essential for designing an effective drug delivery system .The solubility profile of Ketoconazole in different oils and solvents was determined, providing valuable insights into the drug's solubility behavior, which is crucial for formulating an efficient delivery system. From the above results it is conclude that our Batch B is observed stable during all the tests.

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