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FORMULATION AND EVALUATION OF TRANSFEROSOMES BASED ANTIFUNGAL CREAM CONTAINING KETOCONAZOLE

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ABSTRACT

In 1991, Gregor Cevc popularized the word "transfersome" and its meaning. In its broadest sense, a transfersome is a complex aggregation that is very durable and versatile. Its ideal structure is an aqueous core surrounded by a flexible vesicle with a complicated lipid bilayer. The vesicle may self-regulate and optimize due to the interdependence of the bilayer's composition and structure. Because of this special characteristic, transfersomes may effectively carry controlled drugs while getting across a number of transport obstacles. Because transdermal delivery is safe and convenient, it is especially beneficial. Benefits include avoiding first-pass metabolism, optimizing therapeutic response, reducing adverse effects, and guaranteeing stable medication concentrations. The penetration of substances through the skin may be improved by a number of chemical and physical techniques, including lipid vesicles (liposomes, proliposomes), nonionic surfactant vesicles (niosomes, proniosomes), iontophoresis, and sonophoresis. The very flexible membranes of phospholipid vesicles, sometimes referred to as transsomes, further promote transdermal medication administration. Transfersomes can effectively permeate the skin thanks to their membranes, which also enable them to adjust to the skin's natural water gradient and mechanical stressors. Transfersome membranes may be optimized for flexibility and stability by varying the mix of surface-active molecules, therefore mitigating the likelihood of vesicle rupture. All things considered, transfersomes are a major breakthrough in drug delivery technology, especially when it comes to improving the effectiveness and distribution of transdermal medications (Cevc et al., 1991).

1. INTRODUCTION

Fungal infections are a common and extensive health issue that affect a significant number of people worldwide. Notably, the most common forms of fungal infections identified are dermatophytosis, candidiasis, and pityriasis versicolor. Ketoconazole, an imidazole derivative, has become a widely used antifungal medication to treat these infections. However, ketoconazole's poor absorption and restricted water solubility limit its therapeutic efficacy. Conventional ketoconazole formulations, such pills, capsules, and creams, have a number of drawbacks, such as poor systemic side effects, insufficient skin penetration, and subpar absorption.[1] A family of deformable vesicles made of phospholipids and surfactants, transfersomes are an inventive way to distribute pharmaceuticals. They can encapsulate both hydrophilic and lipophilic medicinal substances. These vesicles have the unique ability to pass through the stratum corneum, which is the outermost layer of the epidermis, allowing drugs to diffuse into the tissues underneath. Numerous studies have shown that transfersomal formulations significantly increase skin permeability and drug absorption; the improved administration of ketoconazole and other medicinal chemicals is one example of this.[2] administration, which makes them a good choice for the precise administration of medications. Their unique characteristics, including as regulated release, extended duration of action, and improved patient compliance, make them very advantageous. Thus, the development of a ketoconazole-loaded transfersomal gel may provide a viable approach to the effective topical delivery of the medication, leading to improved therapeutic effectiveness.[3] A significant body of research has been conducted in the last several years on the development of transfersomal gels as a medication delivery method. A multitude of studies have provided evidence on the effective encapsulation of various pharmaceuticals in transfersomes, which are then integrated into gels. However, there has been little focus on the creation and evaluation of transfersomal gels that include ketoconazole.[4]

The process of formulation optimization required the methodical adjustment of many factors, such as the drug, surfactant, and phospholipid concentrations, as well as the sonication temperature and time. The resulting improved formulation was characterized by measuring

particle size, zeta potential, entrapment efficiency, and drug release in vitro, among other characteristics. Furthermore, ex vivo skin penetration investigations and in vitro antifungal activity tests were used to evaluate the efficacy of the transfersomal gel.[5, 6] Several studies have clarified the use of transfersomes as a delivery system for a variety of pharmacological agents, including antifungal drugs such as ketoconazole. Elastic liposomes containing ketoconazole were created and their in vitro effectiveness was evaluated in a research carried out by Patel and Vavia in 2007. The results showed that, in comparison to traditional liposomes, the elastic liposomes had a noticeably increased rate of skin penetration and higher drug deposition.[7]

Similar to this, transfersomal ketoconazole gel was made by El-Kamel et al. (2016) to be used topically to treat fungal infections. Compared to the conventional gel, the transfersomal gel exhibited a much better rate of skin penetration and antifungal activity. The enhanced medication penetration and retention via the use of transfersomes was ascribed by the authors as the reason for the increased performance.[8] Cevher et al. (2018) synthesized ketoconazole transfersomes and evaluated their effectiveness in vitro. Comparing the transfersomes to traditional liposomes, the researchers found that the former showed a much higher rate of drug deposition and skin penetration. They added that the ability of the transfersomes to pierce the stratum corneum and aid in medication transport to the underlying tissues was the reason for their improved performance.[9] Additionally, the use of gels to give medications, such as ketoconazole, an antifungal drug, has been the subject of several research. When compared to traditional cream formulations, Patel et al. (2010)'s research shown that ketoconazole gel was more successful in treating fungal infections. The scientists attributed this increased performance to the gel's ability to promote better epidermal penetration and maintain medication release.[10]

There has been a noticeable increase in the scientific community's attention to the development of transfersomal gels as a medication delivery method in recent years. Notably, a research by Kalepu and Nekkanti (2015) showed how to successfully produce a transfersomal gel that contains curcumin and is intended to treat skin cancer. Comparing the transfersomal gel to traditional gel formulations, their results showed a significantly increased rate of drug deposition and skin penetration. This improved performance was ascribed to transfersomes' innate capacity to efficiently pierce skin and deliver medication to underlying tissues.[11]

The goal of the current research is to examine the possibility of a ketoconazole-loaded transfersomal gel formulation as a novel treatment option for fungal infections, providing a workable substitute for traditional dosing forms. By means of optimization, the produced formulation demonstrates the capacity to increase ketoconazole's bioavailability and skin penetration, thereby amplifying its therapeutic effectiveness.[12]

2. MATERIAL AND METHOD

2.1. Pre-formulation Study

The formulation of creams is critical to ensure the stability, effectiveness and manufacturability of the finished product. These investigations involve assessing the physical, chemical and biological properties of the drug components and excipients so that improvements can be made to the formulation and processing parameters. The essential factors involved in the pre-formulation of creams are outlined here.[13]

2.2 Organoleptic evaluation

Organoleptic evaluation involves evaluating the sensory characteristics of a product such as appearance, color, odor, taste, and texture. This form of review is particularly important for goods such as drugs, cosmetics, and foods, as sensory characteristics can have a significant impact on customer acceptance and compliance. This is a complete review of the organoleptic evaluation.[14]

2.3 Solubility Study

To establish the quality of ketoconazole, we tested its solubility in several solvents using USP NF, 2007 criteria. 1 mg of ketoconazole was properly placed into a 10 ml test tube and dissolved in 1 ml of every solvent, the ingredient are chloroform, methanol, ethanol, Dimethylsulfoxide, and water. A visual inspection showed the solubility (mg/mL) in each solvent.[15]

2.4 Determination of Melting Point

To determine the melting point.

Open Capillary Method

A capillary tube of about 1 mm diameter and 10-15 mm length was used, one end of which was sealed and the drug was added to it. The melting point was then accurately measured by heating the sample capillary evenly and gently.[16]

2.5 λ max

Preparation of Ketoconazole standard solution

Make a standard solution of the chemical used, ketoconazole. Ketoconazole standard material, methanol (HPLC grade), and so on. Use an analytical balance to precisely weigh 10 mg of ketoconazole standard material. Transfer the weighed ketoconazole to a clean, dry 10-mL volumetric flask. Add approximately 5 mL of methanol to the volumetric flask containing ketoconazole. Mix the liquid carefully with a glass stirring rod until the ketoconazole is completely dissolved.[17]

Fill the volumetric flask with methanol to the mark, then invert several times to achieve thorough mixing. Mix well to ensure that the ketoconazole is evenly distributed throughout the solvent.

To achieve a desired concentration of 5 g/ml, we diluted 0.3 ml of the stock solution with solvent in a 10 ml volumetric flask for each solution. The wavelength of maximum absorption was determined, and this data was used to create a calibration curve.[18]

2.6 Standard Curve

Aliquots of the 100 g/ml drug working standard solution at concentrations of 2, 4, 6, 8, 10, and 12 g/ml were diluted with medicine and transferred to a series of 5 ml calibrated flasks. The finished solution's absorbance was measured at 239.0 nm in comparison to a solvent blank.[19]

The medication's potency was assessed throughout a range of 2 to 12 ng/ml, resulting in the creation of a six-point calibration curve. The medicine had a linear response within the dose range studied, with a correlation value of 0.977 and a linear regression equation of y = 0.096x + 0.390.[20]

2.7 IR Spectroscopy

The KBr disc was made by drying 100 milligrams of KBr and 1 milligram of ketoconazole under infrared light. The KBr and drug combination was then hydraulically crushed to form a disc, which was then examined in the FTIR chamber. The measured infrared spectrum ranged from 4000-400 cm^{-1}.[21]

2.8 Preparation of Sample

The thin film was created in a rotating evaporator by heating it at 25°C and 100 rpm for 15 minutes. The film was vacuum-dried for one hour. Ketoconazole was dissolved in ten milliliters of pH 7.4 phosphate buffer and heated at 55°C until completely dissolved. The film was then manually swirled in the warm buffer for 30 minutes to hydrate. The components were then mixed using a magnetic stirrer for one to two hours. The resulting transferosomes were examined under a microscope, and the fluid was refrigerated to 4 degrees Celsius. For further details on transferosome composition, see Table 1 in Sultana et al. (2015) and Malakar et al. 2012.[22]

Table No. 1: Formulation of Sample.

S.No	Ingredients	F1	F2	F3	F4	F5
1.	Ketoconazole (mg)	500	500	500	500	500
2.	Soya lecithin(mg)	50	100	150	200	250
3.	Cholesterol(mg)	250	200	150	100	50
4.	Surfactant (ml)	1	1	1	1	1
5.	Chloroform : Ethanol (1:1) (ml)	10	10	10	10	10
5.	Phosphate buffer (ml)	q.s	q.s	q.s	q.s	q.s

2.9 Evaluation of Sample

2.9.1 Partical Size Determination

Particle size is an important characteristic in the characterisation of microspheres. The diameter of the microspheres was measured using the Malvern Zeta sizer from Malvern Instruments. Before measurement, the microspheres were put into the sample cuvette.[23]

2.9.2 Zeta Potantial

Zeta potential measurements were used for quantification. In this work, the microspheres were tenfold diluted with sterile water before being analyzed using Malvern Zetasizer. Prior to measuring the zeta potential, each sample was sonicated for five to fifteen minutes. Table 11 provides thorough information on the zeta potential values.[24]

2.9.3 Quantitative analisys

Ketoconazole was added to the transferosomal solution. We prepared the sample using ultracentrifugation for forty minutes. To examine the content using UV spectrophotometry at 239 nm, 1 ml of supernatant was collected. This approach allows us to calculate the penetration efficiency as a percentage.[25]

2.9.4 Drug Release study

A magnetic stirrer set at 100 rpm was used to keep the beaker at 37 °C during the experiment. Every 2 ml of sample was replaced with pH 7.4 phosphate buffer. Dialysis bags were used to research drug diffusion. The drug-loaded transferosomes were diluted with 100 ml of pH 7.4 phosphate buffer in a dialysis bag. The contents of the beaker were kept at 37 °C using a magnetic stirrer and the experiment was carried out at 100 rpm

throughout. The pH 7.4 phosphate buffer was replaced after every 2 ml of sample.[26]

The findings revealed first-order kinetics, as seen by the log cumulative percentage of drug remaining over time. The constant K1 can be calculated using this data. The straight line indicates that the release follows first-order kinetics. When the data are plotted as the cumulative percentage log of drug remaining versus time, multiplying the slope by 2.303 gives the constant K1.[27]

2.10 Formulation of Transfersomal Cream

Mt is the total amount of drug released at time t, represented as a percentage. The rate constant is represented by k, while the release exponent is represented by n. In spherical matrices, the value of n indicates release rates.[28]

2.11 Evaluation of Cream

2.11.1 Phase separation study

The prepared cream is stored at room temperature and shielded from direct sunlight. It is monitored throughout the day to ensure that room temperature is consistently maintained. As show in the table no. 10.[29]

2.11.2 Physical evaluation

The subject's appearance was examined to determine their present physical state. Color, scent, texture, and general condition are the most important aspects to consider while researching cream formulation. During the physical examination, the cream formulation was tested for color, fragrance, texture, and overall condition.[30]

2.11.3 Study of Irritation

Make a one-centimeter mark on the area of your right dorsal side. Track how long it takes for the cream to start working after application. Then, monitor and record any signs of discomfort for up to 24 hours.[15]

2.11.4 The pH of the Cream

A digitally calibrated electronic pH meter was used to determine the pH level of the sample. The results were obtained by dipping the glass electrode of the meter into the cream solution. To calculate the pH, 0.5 g of cream was dispersed in 50 ml of distilled water. The average pH value was obtained from three separate tests.[20]

2.12 Stability Analyses

During accelerated testing, a cream formulation with transferosomes was packed and stored in a stability chamber for three months. The formulation was tested at 30, 45, 60, and 90-day intervals to assess pH, skin irritation, and phase separation. Throughout this period, the formulation was subjected to accelerated storage conditions to determine its stability. Various assessment criteria, such as phase separation studies, pH, and skin irritation tests, were used to discover changes in the formulation. All results were compared to the reference formulation, which was at 0 days.[30]

3 RESULT

3.1 Physical Appearance

The physical properties of the drug sample, together with the factors given below, were extensively evaluated:
The organoleptic properties of the API were investigated, including its appearance, colour, odour and state. During analysis, ketoconazole was found to be a white to light coloured powder. The studies confirmed that ketoconazole is odourless and exists in the solid state as a powder. It displayed a similar colour, odour and state as compared to the I.P. standards for these properties.

Table No. 2: Physical Appearance of the ketoconazole.

S. No	Physical parameter	Observation
1	Color	white color
2	Odor	Odorless
3	State	Solid
4	Appearance	Dry powder

3.2 Solubility study

The solubility of Ketoconazole was studied in several non-volatile or volatile liquid vehicles such as Dimethyl sulfoxide, methanol, ethanol, chloroform, and water given in the table mention below. The medicine is soluble in dimethyl sulfoxide, ethanol, methanol, chloroform, and water.

Table No. 3: The Solubility Study of the Ketoconazole.

Drug	Solvents	Observation/Inference
Ketoconazole	Water	Moderately Dissolvable
	Ethanol	Easily dissolved
	Methanol	Simple to dissolve
	Chloroform	Moderately Dissolvable
	DMSO	Easily dissolved

3.3 pH Determination

The pH of ketoconazole was measured using a digital pH meter, which showed a pH of 5.9, which falls within the acceptable range for this drug.

Table No. 4: pH Determinaion.

S. No.	Drug	Observed
1	Ketoconazole	5.9

3.4 Melting Point

The capillary technique was employed to determine the melting point of Ketoconazole, revealing it to be 150ºC. This value comfortably falls within the specified limits for this medication.

Table No. 5: Melting Point.

Drugs	Observed	Reference
Miconazole	150^0C	148 to 153^0C

3.5 UV spectroscopy

The calibration curve for ketoconazole was generated in methanol. Initially, 10 mg of accurately weighed drug was dissolved in a 10 ml volumetric flask using the appropriate solvent. This resulted in a stock solution of 1000 µg/ml, which was further diluted to 100 µg/ml. From this secondary stock solution, further dilutions were made to reach a final concentration range of 2-12 µg/ml. Absorbance measurements were performed using UV spectroscopy, and a calibration curve was created by graphing absorbance versus concentration.

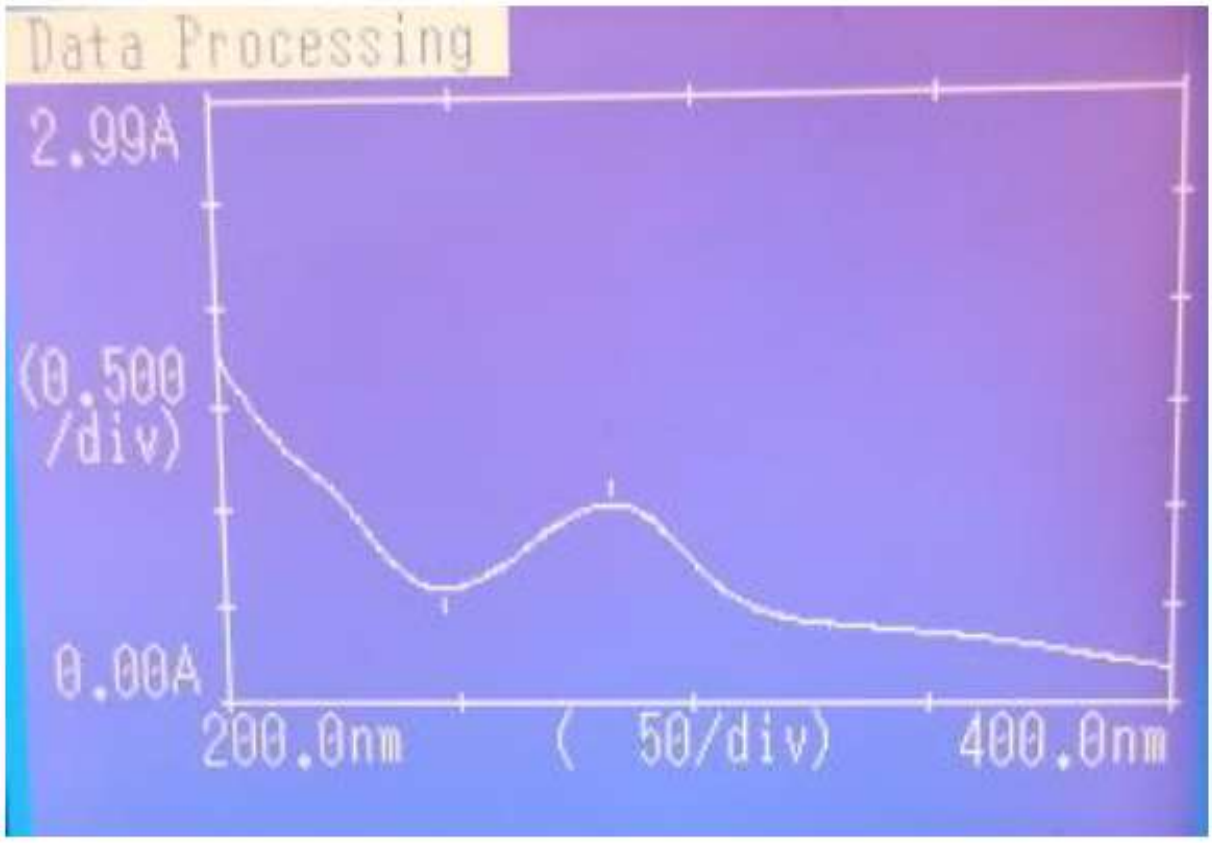


Fig. No. 1: UV Spectroscopy.

3.6 Standard calibration curve

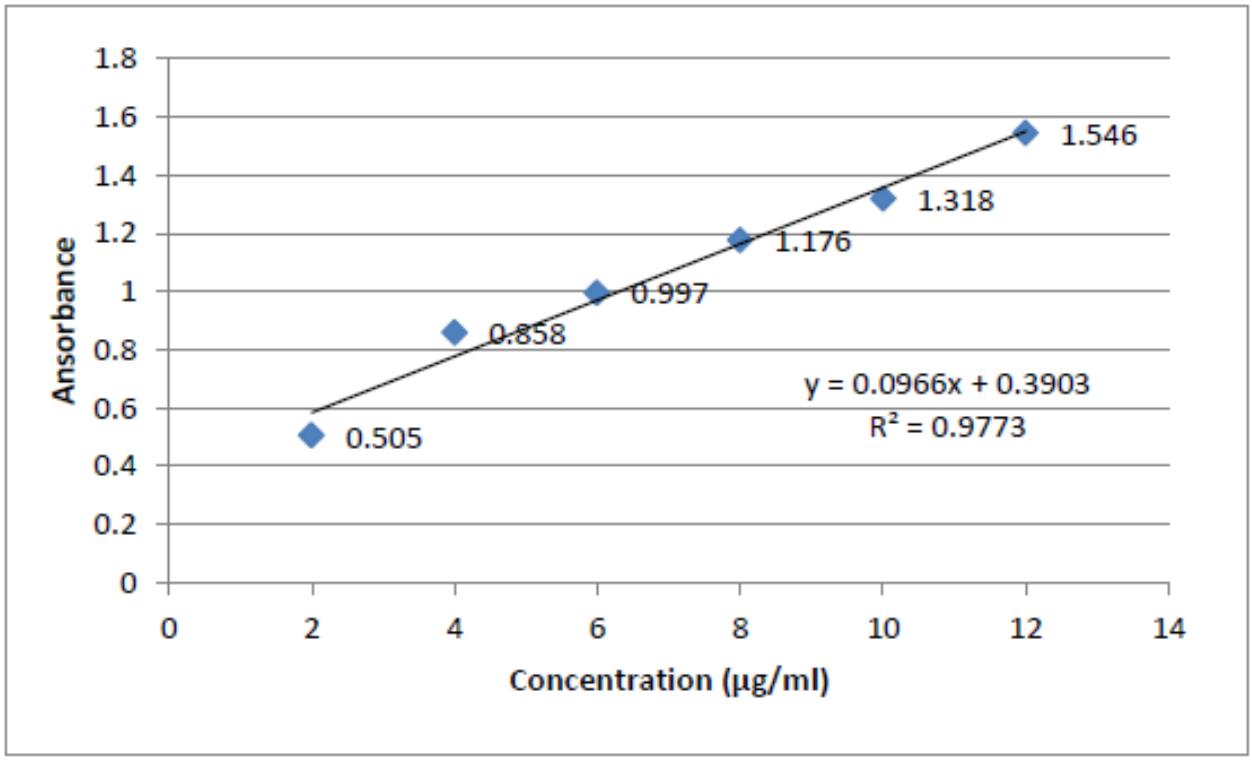


Fig. No. 2: Standard Calibration Curve.

3.7 IR spectroscopy

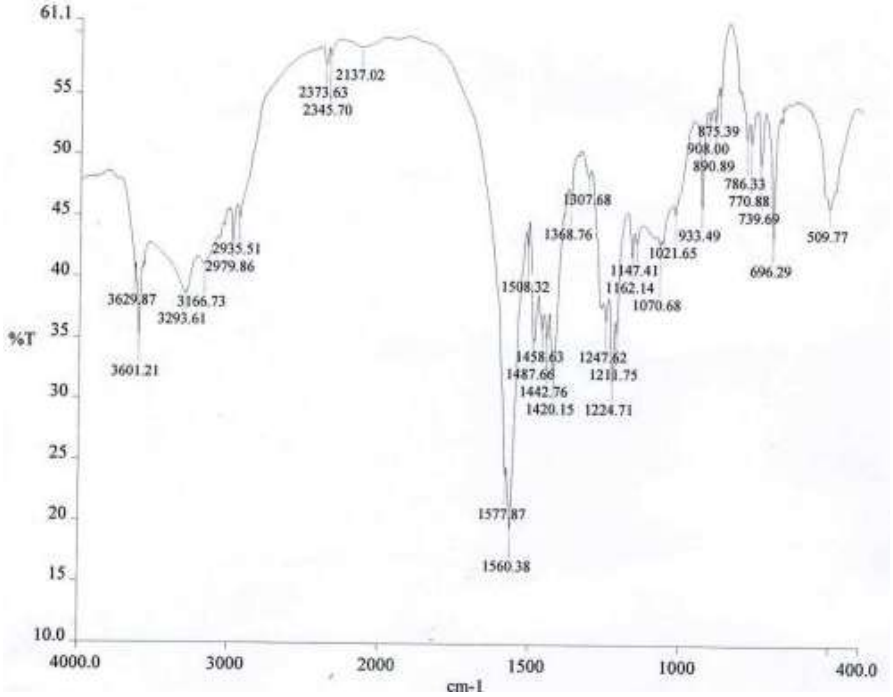
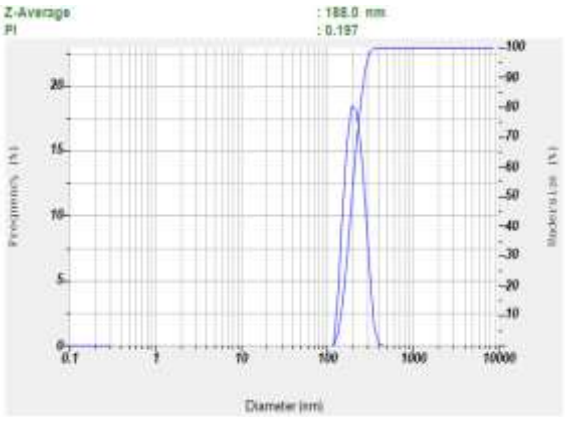


Fig. No. 3: IR Spectroscopy.

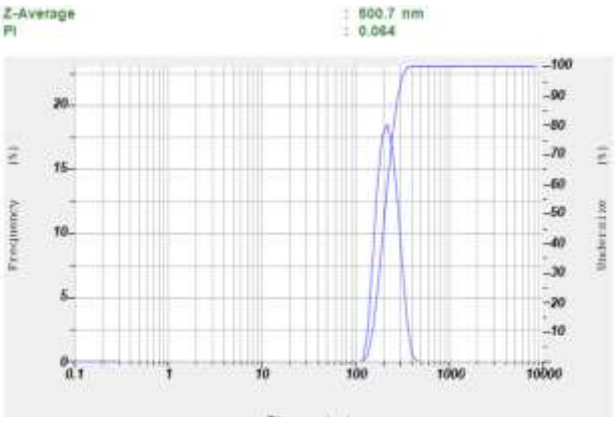
3.8 Transferosomes loaded with drugs

3.8.1 Particle size

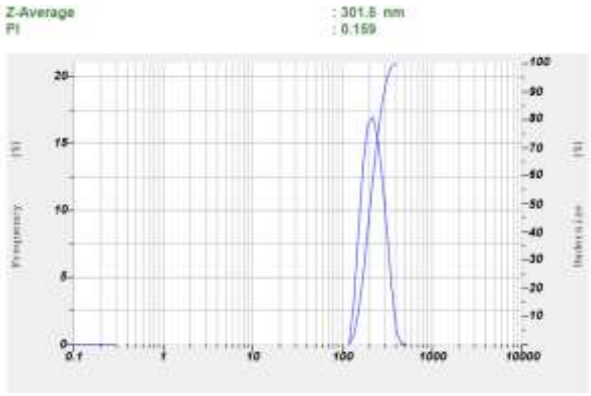
Particle size is an important factor when characterizing transferosome formulations. Malvern Zetasizer was used to assess the general particle size of drug-loaded transferosomes. According to the particle size studies, the average particle size of the formulations ranged from 170.2 to 690.3 nm. Among the transferosome formulations mentioned in Table 10, F4 displayed the lowest particle size.



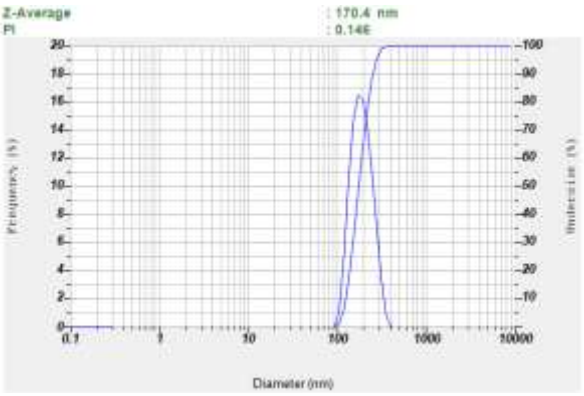
Formulation 1



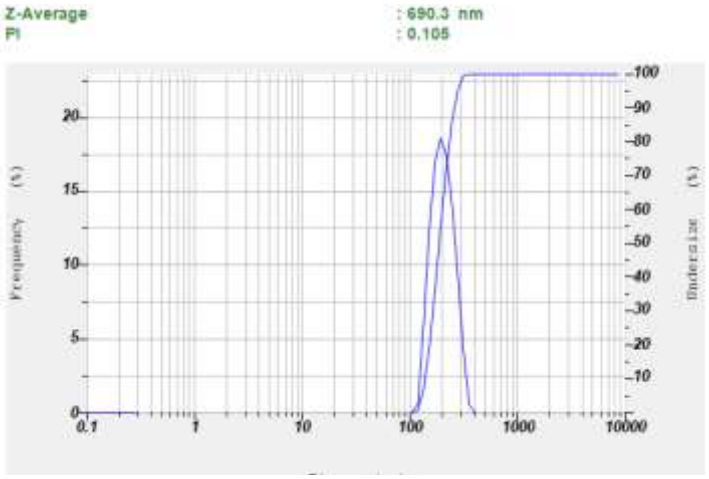
Formulation 2



Formulation 3



Formulation 4



Formulation 5

Fig. No. 4: Particle size of Formulation.

Table No. 6: Particle Size of Formulation.

S. No	Formulations	Particle size (nm)	PI Value
1.	F1	188.0 nm	0.197
2.	F2	500.7 nm	0.064
3.	F3	301.5 nm	0.159
4.	F4	170.4 nm	0.146
5.	F5	690.3 nm	0.105

3.8.2 Zeta potential

The surface charges of the particles were assessed by a zeta potential research, which helps estimate the degree of colloidal stability. All formulations displayed zeta potential values in the range of -8.7 to 0.1 mV, with a peak area of 100 percent intensity. These numbers show that the freshly produced Transferosomes are stable under all circumstances. The findings are given in the table above.

Table No. 7: Zeta Potential.

S.No	Formulation	Zeta potential
1	Transferosomes (F1)	-1.8 mV
2	Transferosomes (F2)	0.1 mV
3	Transferosomes (F3)	-1.9 mV
4	Transferosomes (F4)	-5.9 mV
5	Transferosomes (F5)	-8.7 mV

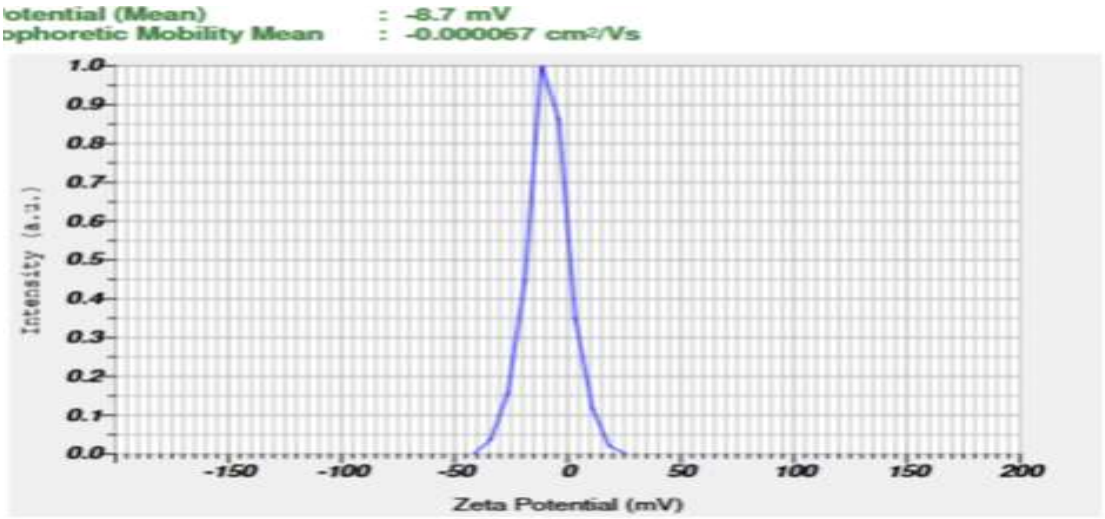


Fig. No. 5: Zeta Potential.

3.8.3 Entrapment efficacy

Possible factors include changes in polymer structure and associated variations in entrapment effectiveness. The percentage of entrapment effectiveness varied from 68.08 percent to 92.63 percent across all formulations, with the F4 formulation exhibiting significantly higher efficiency due to fluctuations in polymer concentration.

Table No. 8: Entrapment Efficacy.

S.No.	Formulations	Entrapment efficacy (%)
1.	Transferosomes (F1)	68.08
2.	Transferosomes (F2)	79.52
3.	Transferosomes (F3)	80.86
4.	**Transferosomes (F4)**	**92.63**
5.	Transferosomes (F5)	89.92

3.8.4 In vitro investigation of formulation drug release

Table No. 9: Drug Release.

S. No	Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time
1.	0	0	100	0.000	2.000	0.000
2.	2	14.12	85.88	1.414	1.934	0.301
3.	4	27.01	72.99	2.000	1.863	0.602
4.	6	34.56	65.44	2.449	1.816	0.778
5.	8	49.16	50.84	2.828	1.706	0.903
6.	10	62.31	37.69	3.162	1.576	1.000

3.9 Evaluation parameter of Transferosome loaded Cream formulation

3.9.1 The composition of the cream's physical properties

Table No. 10: Physical Properties.

S. No	Parameters	Results /Observations
1.	Color	Brownish to creamy color
2.	Odor	Pleasant
3.	Texture	Smooth
4.	State	Semisolid form

3.10 Analysis of the cream formulation's stability

After three months of exposure to accelerated stability conditions (25°C/60% RH and 35°C/70% RH), it was proven that the formulation remained physically and chemically stable. Minimal changes in pH, burning test or phase separation were detected following introduction of transferosomes into the cream formulation. Table 11 presents the test findings and other assessment criteria at various time periods in the stability study.

Table No. 11: Analysis of the cream formulation's stability.

S.No	Time (Days)	$25^0C±2\ ^0C$ and 60 ± 5% RH			$35^0C±2\ ^0C$ and 70 ±5% RH		
		pH	Irritancy test	Phase separation	pH	Irritancy test	Phase separation
1.	0	6.3	Non-irritant	No phase separation	6.3	Non- irritant	No phase separation
2.	30	6.4	Non-irritant	No phase separation	6.5	Non- irritant	No phase separation
3.	45	6.3	Non-irritant	No phase separation	6.6	Non- irritant	No phase separation
3.	60	6.2	Non-irritant	No phase separation	6.4	Non- irritant	No phase separation
4.	90	6.4	Non-irritant	No phase separation	6.3	Non- irritant	No phase separation

4 CONCLUSION

Many different medicinal chemicals can be delivered transdermally thanks to highly-deformable carriers called transfersomes. The osmotic gradient is the main mechanism that allows transfersomes to penetrate the deeper layers of the epidermis. Dialysis bags were used in in vitro drug release research. The correlation coefficient (R2) of the table shows that the transfersome in the F4 formulation has the largest correlation value and is consistent with the Higuchi kinetic model. It is speculated that formulating a transferosomal cream will improve the topical application efficacy of the product.

Due to its many advantages, transdermal administration has long been preferred, and current research is constantly developing new transdermal delivery technologies. Thus, when investigating vesicles as vehicles for transdermal drug administration, it is necessary to synthesize transfersomes, which are extremely deformable vesicles. Greater transdermal flux, sustained release of bioactive molecules, improved site-specific targeting, formulation safety with ingredients approved for pharmaceutical and cosmetic use, and the ability to encapsulate drugs with different solubility profiles are some of the advantages that elastic vesicles offer compared to other transdermal delivery technologies.

Research results indicate that a transfersomal cream including cholesterol, soy lecithin, and Tween 20 can enhance skin delivery of ketoconazole. Furthermore,

there have been no reports of skin irritation after using this cream formulation.

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