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Formulation and Evaluation of Bifonazole Ethosomal Gel for Enhanced Topical Delivery

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Key words:
Ethanol, lecithin, ethosome, Permeation

Abstract:
The goal of this study was to create and test an ethosomal gel formulation of bifonazole. Ethosomes are a new lipid carrier that can be utilised to deliver drugs transdermally. The study's main goal is to improve the permeability of Bifonazole, a broad-spectrum antifungal imidazole medication. It is a class IV medicine with limited permeability and solubility, hence it has been loaded into one of the best vesicular systems, ethosomes, to boost permeability and solubility. Ethosomes are made using hot process by taking varying quantities of ethanol and lecithin. The drug entrapment efficiency, vesicle shape, and size of the produced formulations were all examined. The highest entrapment efficiency (95.99%) was found in F5 ethosomal vesicles containing 3% w/w lecithin and 30% w/w ethanol and were put into three different percentages of Carbopol gels (1%, 1.5%, and 2%). pH, drug content, viscosity, spreadability, and in-vitro diffusion investigations are performed on all gels. EG1 (1%) demonstrated the highest penetration rate and was proven to be stable. The study revealed that bifonazole ethosomal gel can successfully improve drug bioavailability by penetration enhancement, reduce the frequency of administration, and improve patient compliance. The ethosomal gel could be successfully made at a low cost and had better drug release than traditional dosage forms.

Introduction:

Transdermal drug delivery system (TDDS) showed promising results when compared to oral drug delivery system because it eliminates gastrointestinal interferences and first pass metabolism of the drug. However, the main disadvantage of TDDS is that it encounters the barrier properties of the Stratum Corneum, which means that only lipophilic drugs with a molecular weight of 500 Da can pass through it (1,2). Various strategies have been examined to promote medication penetration through the skin, including the use of chemical or physical enhancers such as iontophoresis, sonophoresis, and so on. Liposomes, niosomes, transferosomes, and ethosomes have also been shown to improve medication permeability over the stratum corneum barrier (3,4).

Ethosomes:

"Ethosomes are also known as ethanolic liposomes." Ethosomes are non-invasive drug delivery carriers that allow medications to go deep into the skin layers and/or the systemic circulation. These are soft, pliable vesicles designed for better active drug delivery. Unlike traditional liposomes, which are best recognised for delivering medications to the skin's outer layers, ethosomes can improve permeability through the stratum corneum barrier. Ethosomes penetrate the skin layers faster and have much higher transdermal flow than regular liposomes (5).
Ethosomes are a small variation of the well-known drug carrier liposome. Ethosomes are lipid vesicles that contain phospholipids, rather high concentrations of alcohol (ethanol and isopropyl alcohol), and water.

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Ethosomes are soft vesicles composed of phospholipids, ethanol (in greater quantities), and water. Bifonazole is a broad-spectrum antifungal imidazole medication that is effective against dermophytes, moulds, yeast, dimorphic fungi, and other organisms. It is used to treat topical fungal diseases such as athletes' foot, tinea cruris, ringworm, and tinea corporis, and it is a class IV medication with low permeability and solubility. The goal of this study is to boost drug permeability and solubility by inserting it into an ethosomal gel by hot method with soya lecithin, ethanol, and water (6-8).

Materials And Methods:

Materials:

Bifonazole was obtained as a gift sample from A.R. Life Sciences in Hyderabad. Vital Laboratories pvt. Ltd, supplied Soya lecithin, Ethanol, and Carbopol, and all other chemical reagents utilized were of analytical laboratory grade.

Methodolology:

Preparation Of Ethosomal Vesicles:

Soya lecithin was dispersed in water and dispersion was heated in water bath at 40 °C. In separate vessel, drug dissolved in ethanol and mixed with propylene glycol heated to 40 °C. When both mixtures reach 40 °C then organic phase was added to aqueous phase under magnetic stirrer then subjected to sonication. (9,10)

Formulation Table:

Table No. 1 Formulation of Ethosomal suspension

	DRUG (g)	LECITHIN (%W/W)	ETHANOL (%W/W)	PROPYLENE GLYCOL (%W/W)	WATER
F1	0.01	2	20	10	qs
F2	0.01	3	20	10	qs
F3	0.01	4	20	10	qs
F4	0.01	2	30	10	qs
F5	0.01	3	30	10	qs
F6	0.01	4	30	10	qs
F7	0.01	2	40	10	qs
F8	0.01	3	40	10	qs
F9	0.01	4	40	10	qs

Preparation of Bifonazole ethosomal gel

The best achieved ethosomal vesicles suspension, was incorporated into Carbopol gel (1% w/v, 1.5% w/v, 2%w/v).

Table No. 2 Formulation of Ethosomal gel

FORMULATION CODE	CARBOPOL GEL
EG1	1%
EG2	1.5%
EG3	2%

Analytical Study:

Solubility Studies:

Solubility test of Bifonazole was performed by using varius solvents such as water, ethanol, DMSO and methanol.

Determination of λMax of Bifonazole:

100µg/ml solution was taken to determine absorption maxima. The solution was scanned in a range of 200-400 nm using phosphate buffer pH 5.5 as a blank. The drug showed maximum wavelength at 254 nm.

Calibration procedure for standard curve:

Bifonazole drug solutions were prepared in 2-10 µg/ml concentrations using 5.5 pH phosphate buffer as solvent. Absorbance noted for each concentration using UV Visible spectrophotometer at 254nm.

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Drug Excipient Compatibility Studies:

FTIR spectrophotometer was used to observe any interaction between drug and excipients.

Evaluation Of Ethosomal Vesicles:

The prepared ethosomal suspension was characterized for vesicle morphology, zeta potential and entrapment efficiency.

Vesicle morphology: (11)

Ethosomes are visualized by using SEM and can be seen under optical microscope.

Particle Size, Zeta Potential:

vesicle size, size distribution zeta potential was determined by dynamic light scattering system by Malvern zeta sizer.

Entrapment Efficiency:(12,13)

The ultracentrifugation technique was used to investigate the entrapment effectiveness of ethosomal vesicles. 5 ml of drug-loaded ethosomal solution was poured in tubes and centrifuged for 30 minutes at 400 rpm. The supernatant layer was separated, appropriately diluted with water, and the free drug concentration was measured at 254 nm.

% Entrapment Efficiency=Total drug added - drug detected only in the supernatant layer / Total drug added

Gel Evaluation Parameters:

Physical Examination And Homogeneity:

The prepared ethosomal gel formulations were inspected visually for their color intensity difference. All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were also tested for their appearance and presence of any aggregates.

Skin Irritation Test:

Skin irritation test was conducted on healthy volunteers. 100 mg of gel was applied on area of 2 cm and observed for any lesions or irritation/redness.

Determination Of Ph:

A digital pH meter was used to determine the pH of the gels. One gram of gel was dissolved in 50 ml of Phosphate buffer (5.5), and the electrode was dipped into the gel formulation until a consistent reading was obtained. (14-17)

Viscosity:

The viscosity of all the formulated gels was measured with a Brookfield viscometer (model Lvdv-e) spindle 63 at 40 rpm.

Spreadability:

Spreadability is determined by spreading 0.5 g Gel on a 2 cm diameter circle on a glass plate. A second glass plate is then placed on top. 20g weight placed on the upper glass plate for 5 minutes.

Finally, diameter of circle spreading is measured.

S = ML/T

S= Spreadability

M= weight of an upper side

L= length of circle/ diameter

T= time in minutes

Drug Content:

1g of gel was dissolved in 100ml of pH 5.5 phosphate buffer while being constantly stirred with a magnetic stirrer. Solution was filtered and observed with u.v spectrophotometer at 254 nm. (18)

In-Vitro Drug Release Studies:

The Franz diffusion cell was used to study the in-vitro drug release from ethosomal gel. Permeation effective area was 1cm^2, and diffusion cell receptor volume was 20ml. In the Franz diffusion cell, egg membrane served as a semipermeable barrier between the receptor and donor chambers. As the dissolution medium in the receptor chamber, phosphate buffer (5.5 pH) was employed. The temperature in the diffusion cell was kept at 37 ^{0}C, and the phosphate buffer was agitated with a magnetic stirrer at 100rpm. At specified time points up to 12 hours, 1ml samples were withdrawn through the Franz diffusion cell sampling port and replaced with the equal volume of fresh dissolving medium in the diffusion cell.

In-vitro release kinetics:

- Zero -order kinetic model– Cumulative% drug released versus time.

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- First–order kinetic model–Log cumulative percent drug remaining versus time.
- Higuchi's model–Cumulative percent drug released versus square root of time.
- Korsmeyer equation/Peppa's model–Log cumulative percent drug released versus log time.

Stability Studies:

According to ICH guidelines, an ethosomal gel stability study was performed at room temperature (27±2° C) for three months. To avoid any sort of interaction between the ethosomal gel and the glass of the container, the formulation was stored in a borosilicate container. (19,20)

Results And Discussion:

Solubility Studies:

Table No. 3 solubility studies

SOLVENTS	SOLUBILITY
Water	Slightly soluble
Ethanol	Soluble
DMSO	Soluble
Methanol	soluble

The drug Bifonazole is soluble in solvents like ethanol, DMSO, Methanol and slightly soluble in water.

Determination Of λMax of Bifonazole:

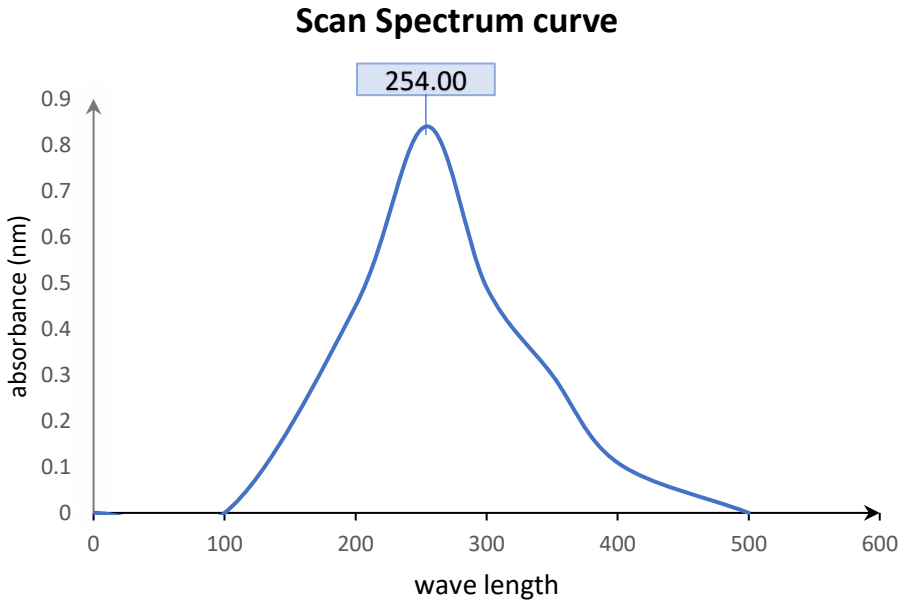

Fig No- 1 λ_{max} of bifonazole

The λ_{max} of bifonazole was found to be 254nm.

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Construction Of Calibration Curve:

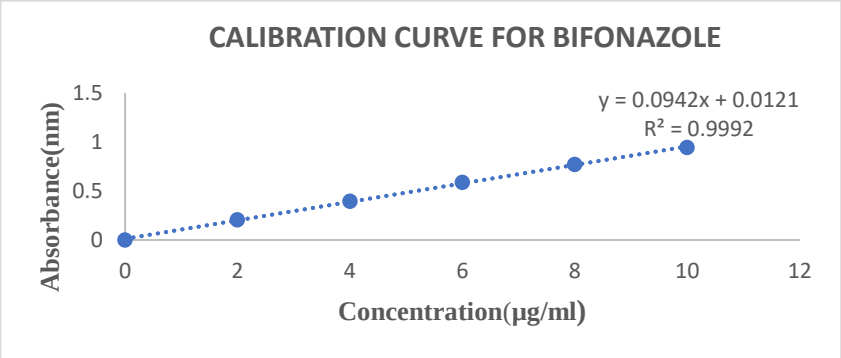

Fig No. 2 calibration curve of bifonazole at 254nm.

Compatibility Studies:

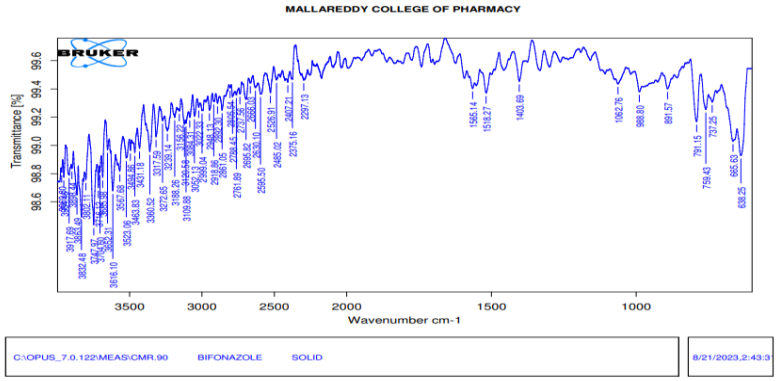

Fig No-3 FTIR Spectrum of Bifonazole

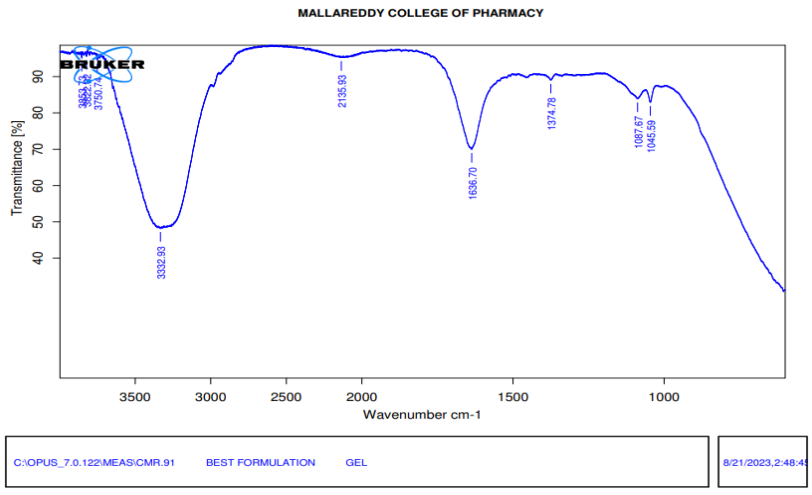

Fig No- 4 FTIR Spectrum of EG1 formulation

The FTIR spectra of ethosomal gel showed peaks for C-H stretching and N-H stretching which is same as the FTIR spectra of pure drug. Spectra analysis revealed that there is no physical and chemical interaction between the bonds of drug and excipients.

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Ethosomal Suspensions:

Fig No- 5 F1 to F5 Ethosomal suspension

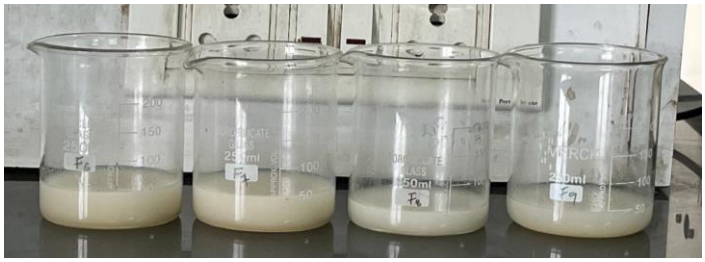

Fig No- 6 F6 to F9 Ethosomal suspension

Nine ethosomal suspension formulations are prepared and evaluated.

Characterization Of Ethosomes:

Table No.- 4 Entrapment efficiency

FORMULA CODE	ENTRAPMENT EFFICIENCY (%)
F1	69.56±0.25
F2	75.45±0.74
F3	79.25±0.65
F4	87.89±0.58
F5	**95.99±0.25**
F6	87.07±0.41
F7	77.67±0.36
F8	75.01±0.23
F9	73.55±0.13

Entrapment Efficiency was performed for all the Nine formulations by centrifugation method and they were in the range of 69% to 95%. F5 formulation showed highest entrapment efficiency i.e 95.99%. And F5 is considered as the optimized formulation.

Characterisation Of Ethosomal Suspension:

Scanning Electron Microscopy:

SEM Studies were performed for F5 Formulation (optimized) and the ethosomes are found to be in spherical shape.

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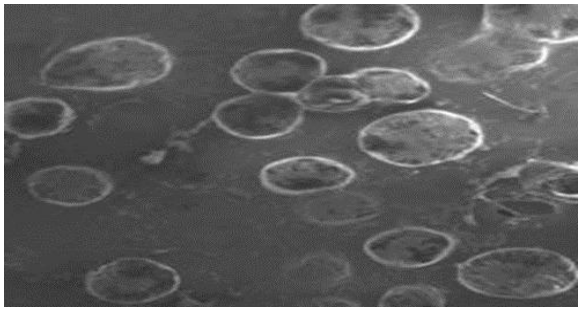

Fig No-7 SEM Image for F5 Formulation

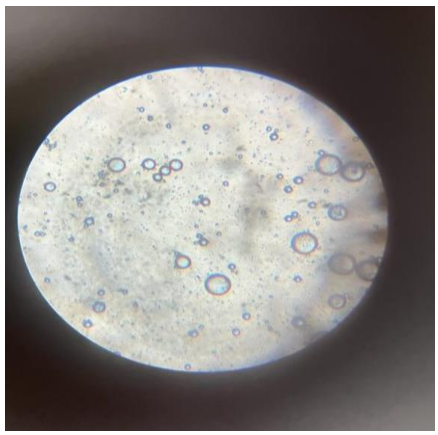

Fig No- 8 Microscopic view of ethosomes

Ethosomes when viewed under optical microscope(10x) are found to be as shown in the above figure.

Zeta Potential:

Zeta potential analysis was done to determine the surface charge of vesicles. The zeta potential of F5 formulation was found to be -42 mV.

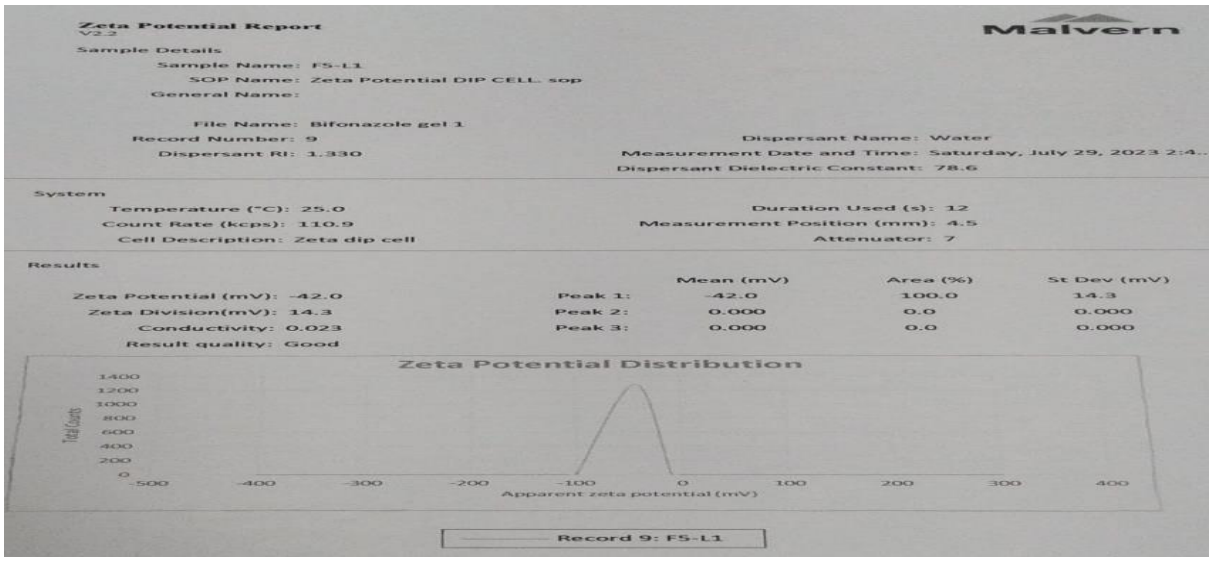

Fig No- 9 Zeta potential

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Vesicle Size Analysis:

The vesicle size for F5 formulation was found to be 191.2 nm.

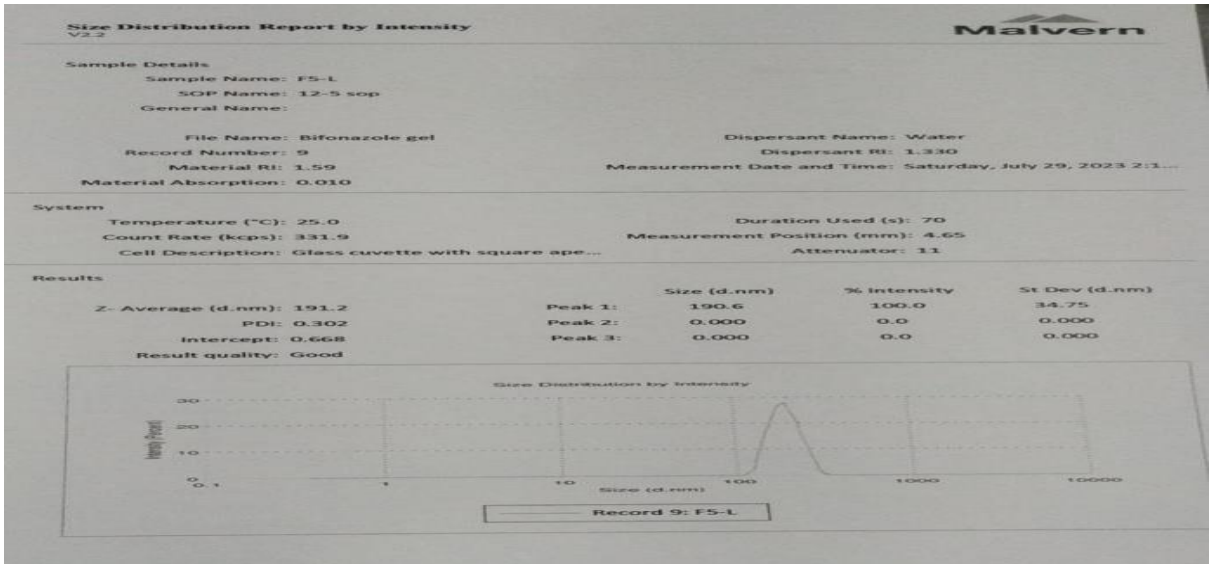

Fig No-10 Vesicle size analysis

Gel Evaluation Parameters:

Table No. 5 Gel evaluation parameters

	Homogeneity and Texture	Skin Irritation test	pH	Spreadability (gm.cm/sec.)	Viscosity (cps)	Drug content (%)
EG1	Smooth	NO	5.5±0.12	12.25±1.25	3345±10	95.72±0.25
EG2	Smooth	NO	5.7±0.08	11.65±1.23	4445±15	95.48±0.23
EG3	Smooth	NO	5.8±0.15	10.95±1.56	5626±23	95.09±0.25

All the three gel formulations were evaluated for different parameters like homogeneity, skin irritation, pH, Spreadability, viscosity and drug content.

In-Vitro Diffusion Studies:

Table No.-6 *IN-VITRO* Diffusion studies

TIME (hr)	EG1	EG2	EG3
0	0	0	0
1	15.67±0.12	10.67±0.24	8.9±0.34
2	22.89±0.29	14.56±0.32	10.89±0.23
3	31.56±0.34	22.9±0.37	15.77±0.45
4	39.55±0.21	33.98±0.25	23.66±0.34
5	47.97±0.12	40.77±0.22	30.45±0.44
6	56.34±0.45	48.76±0.36	43.21±0.32
7	62.88±0.34	56.07±0.65	51.66±0.25
8	75.66±0.13	68.56±0.76	60.56±0.35
10	87.45±0.26	75.33±0.43	68.06±0.22
12	95.45±0.34	85.01±0.32	78.67±0.14

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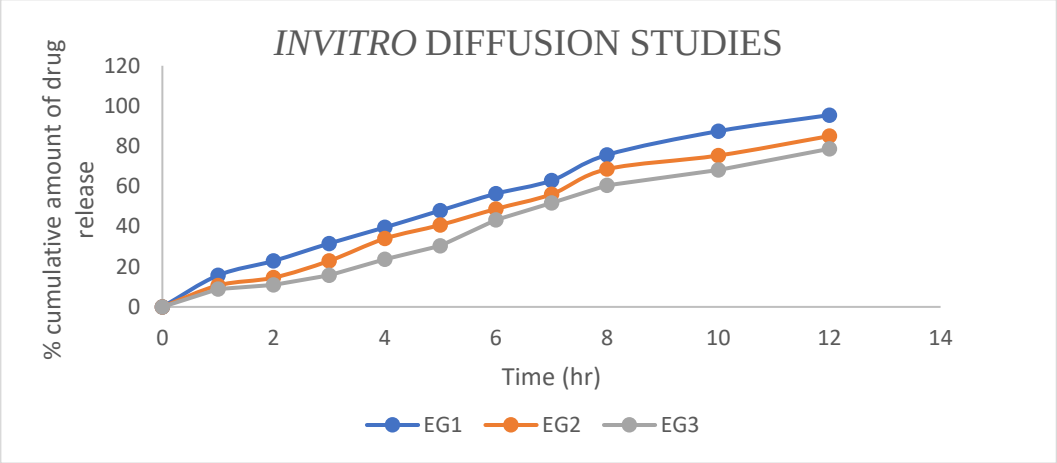

Fig No- 11 *In-vitro* Diffusion studies

EG1 Formulation showed highest drug release when compared to all other formulations.

Kinetic Data of Eg1 Formulation:

Table No-7 Kinetic Data of EG1 Formulation

ORDER OF KINETICS	ZERO ORDER	FIRST ORDER	HIGUCHI PLOT	PEPPAS MODEL
r^2	0.98	0.96	0.95	0.83

The drug release kinetics were studied to know the type of drug mechanism followed.The release kinetics of an optimised formulation were investigated for various kinetic equations (zero order, first order, Higuchi, and Korsemeyerpeppas equations).R^2 values of EG1 formulation was found to be highest for zero order. So it follows zero order kinetics. n value (0.89) of Korsmeyer-Peppas model of the optimized formulation strongly signifies that mechanism of drug release is Case-II transport.

Stability Studies:

Stability studies conducted for optimized formulation (EG1) for 3 months at room temperature (25±2 ^{0} C). By the end of 90 days there is no major change in color, pH, drug content and drug release.

Conclusion:

In the current study, an effort was made to increase the permeation of bifonazole by incorporating it as an ethosomal gel that boosts the drug's therapeutic effectiveness, decreases adverse effects, and increases bioavailability. Bifonazole ethosomal gel was created by a hot process method and various ethanol and lecithin concentrations. F5 (3% lecithin, 30% ethanol) demonstrated the highest entrapment efficiency (95.99%) and Zeta potential (-42.5 mV) among all formulations, and it is made into three different gel percentages (1%, 1.5%, and 2%). Additionally, the evaluation parameters for all three gel compositions were favourable. The EG1 gel formulation outperformed the other two in terms of penetration rate (95.45%) and stability. As a result, it is regarded as the optimal formulation. Thus, it was proven that ethosomes are particularly effective drug carriers for topical delivery.

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