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Optimization and Evaluation of Azithromycin-Loaded Organogels: In- Vitro Characterization and Rheological Behavior Studies

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ABSTRACT

This research aims to formulate azithromycin organogels for the localized treatment of skin infections, such as acne vulgaris and wound infections. By delivering azithromycin topically, the study seeks to minimize systemic side effects like diarrhoea, nausea, and abdominal pain. This approach offers a non-invasive administration method, avoids first-pass metabolism, improves patient compliance, suits nauseated individuals, reduces dosage through direct application to affected areas, and enhances overall acceptance. Additionally, the gel serves as a scaffold biomaterial to support wound healing. Findings indicate that an IPM-Lecithin (300 mM) oleogel with Carbopol 940 as an organogelator is a promising delivery system. The organogels were optimized by varying concentrations of Carbopol 940, Isopropyl Alcohol, and Propylene Glycol, while maintaining a fixed Triethanolamine weight ratio. A pilot study identified the lecithin-based azithromycin oleogel as the most effective formulation, prompting further investigation. Results highlight its ability to accelerate healing of Propionibacterium acnes-related infected acne, alongside demonstrating stability, safety, and cost-effectiveness as key antibacterial attributes...

Keywords: *Organogels, azithromycin, Propionibacterium acnes, lecithin, Carbopol.*

1. INTRODUCTION

Transdermal drug delivery system (TDD) is very convenient for patients. This treatment is painless, easy to apply, avoids the first-pass effect, and has a better pharmacokinetic profile over a longer period of time. There are also fewer side effects associated with TDD. Transdermal delivery is mainly limited by the penetration of drugs across the stratum corneum (SC). Pharmaceuticals are being delivered with several nanocarriers that improve their pharmacokinetics and pharmacodynamics. It is for this reason that vesicular systems have been used in order to increase the absorption of encapsulated drugs, while providing controlled and sustained therapeutic effects as well. [1-3]

Organogels offer a convenient means of avoiding the problem of microbial contamination due to the existence of organic solvent as the continuous phase which itself inhibits any chances of microbial contamination. The formation of these organogels was first reported by Haering and Luisi in 1986. [4-8]

Azithromycin (AZM) is an antibiotic in the macrolide class. It is available in a variety of forms, including oral, parenteral, and ophthalmic solutions.[9-11] As a treatment for upper and lower respiratory tract infections, AZM is commonly prescribed. Among adults and children alike, it has been shown to be effective in treating skin infections. The clinical utility of azithromycin in dermatology extends beyond infectious diseases. Its immuno-modulatory and anti-inflammatory properties make AZM ideal for treating dermatological conditions like persistent rosacea, psoriasis, and acne in addition to its antibacterial properties. In addition, it has a poor bioavailability of 37%, a relatively long half-life of up to 96 h, and is widely distributed throughout the body.[12-17] As a result, increased bioavailability and lower dose of AZM delivery systems will reduce adverse gastrointestinal effects (GI) and improve patient compliance. In the biopharmaceutical classification system (BCS), AZM is classified as class III, which is characterized by low permeability. [18-20]

An attempt was made to develop and evaluate the suitability of a lecithin-based organogel for the topical delivery of azithromycin, with the aim of enhancing its chemical stability and improving in vitro drug release.[21-25] The study.

investigated the solubility of azithromycin in ethyl oleate (EO) and isopropyl myristate (IPM), as well as in EO/IPM-based lecithin reverse micellar systems. The drug's partition coefficient with the organogel matrix were also assessed

2. MATERIAL AND METHODS:

2.1 Material:

Azithromycin was purchased from IPCA Lab. Ltd., Mumbai. HPLC-grade Ammonium acetate and HPLC-grade water were purchased from S.D. Fine chemicals, Mumbai. Cellulose Acetate Membrane (0.45µ), Ethyl Oleate, Iso propyl Myristate, Triethanolamine, Propylene glycol, Methanol (HPLC grade), n-Octanol, Potassium Chloride, Carbopol-940 were purchased from Sigma-Aldrich S.D. Fine chemicals, Mumbai. Soy Lecithin (Epikuron 200) was purchased from De-gussa Bioactive (Germany). Micrococcus luteus (ATCC 9341) was purchased from Indoco Pvt. Ltd., Mumbai.

Methods:

2.2.1 Preparation of gel formulations

Lecithin organogel was prepared by according to the previously reported method. Accurately weighed quantity of lecithin was dissolved in IPM in different test tubes. The mixtures were sonicated for at a frequency of 15000 KHz 10 min at room temperature to obtain a clear homogenous solution of IPM-Lecithin [300 mM].[26-30]

Azithromycin [3% w/w] was incorporated in lecithin solutions and sonicated till the drug gets dissolved in this mixture. Appropriate amount of distilled water was added using a microlitre syringe to induce organogelation [Wo =3 for IPM].[31-35] Hydrogel was prepared by using Carbopol 940 as polymer with reported pH adjustment technique.220 An appropriate amount of Carbopol 940 (1% w/w) was slowly added into beaker containing water under constant stirring. Azithromycin (3%w/w) was dispersed in mixture of isopropyl alcohol and propylene glycol in a separate beaker, this mixture was then added to the beaker containing carbopol in water.[36-41] After the mixture had been kept at ambient temperature for 24 h, a small amount of 0.5% (w/w) triethanolamine was added to adjust pH 7.7 to 8 and mixed well until the gel was formed.[42-44] Mixing was continued for further one h. The speed of the mixer was controlled so that the air entrapment was minimized.[45]

Evaluation of organogels:

Standard curve using HPLC

The standard solutions of azithromycin were prepared in the range of 0.5 -2.5 mg/ml in the mobile phase [Acetonitrile-0.2M Ammonium acetate- Methanol-Water (35: 15: 5: 45). HPLC analysis of azithromycin was performed using a Jasco 2000 series HPLC system consisting of Jasco PU 2080 plus pump set at the flow rate 1 ml/min and a Jasco UV 2075 detector set at 215nm. The samples were injected using Rheodyne injector fitted with 20 µl capacity loop. The column used was Hypersil C-18 (250 mm X 4.6 mm, 5.0µm particle size). Jasco Borwin version 1.5, LC-Net II/ADC software was used for data analysis. The calibration curve was obtained from the area of peak measured.

2.3.2 Partition coefficient study

The study used n-octanol as an oil phase and phosphate buffer as an aqueous phase. The two phases were mixed and saturated, separated by centrifugation, and then added to conical flasks. 100 mg of azithromycin was added, shaken, and separated by centrifugation. HPLC analysis was used to analyze the drug concentration in the buffer phase, while the azithromycin concentration in octanol was calculated by comparing initial and final concentrations. The partition coefficient (P) of drug Ko/w was calculated using the following formula.

$$Ko/w = \frac{\text{Concentrat ion of drug in octanol}}{\text{Concentrat ion of drug in phosphate buffer pH 7.4}}$$

Log P was calculated from P.

Solubility analysis

Solubility of the azithromycin was evaluated in IPM, EO, IPM- lecithin reverse micellar system (RMS-1), and EO- lecithin reverse micellar system (RMS-2). By dissolving an excess amount in 2 ml of each of the selected oils in 5 ml capacity stoppered vials separately. An excess amount of drug was added to each stoppered vial and mixed using a vortex mixer. The vials were then kept at 32°C for 72 h on a mechanical water bath shaker to get to equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 um membrane filter. The filtrate was diluted with ethanol and the concentration of azithromycin was determined by HPLC at 215 nm. The experiment was performed in triplicates.[46-47]

2.3.4 Drug content uniformity

In order to determine drug content uniformity, samples of organogel, hydrogel and marketed cream equivalent to 5 mg of azithromycin was taken from a tube from three different sites - top, middle and bottom. The contents were dissolved in methanol and drug content was analyzed by HPLC. This study was performed in triplicate. [48]

2.3.5 In vitro diffusion study

2.3.5.1 Effect of lecithin concentration on in vitro diffusion of drug from organogels

The study investigated the diffusion of azithromycin from IPM-based organogels using a Keshary-Chien type diffusion cell. The cell, with a capacity of 20 ml and a 3.14 cm^2 effective surface area, was filled with a 0.45µ cellulose acetate membrane. The receptor compartment was filled with saline phosphate buffer, and the drug formulation was placed on the skin surface. Aliquots of the drug were withdrawn and replaced with fresh medium. The samples were diluted and analyzed for azithromycin content using HPLC. [49]

2.3.5.2 In vitro permeation study using excised rat skin

Ethical clearance was obtained from the institutional animal experimentation committee before the study. Full thickness abdominal skin of albino rats [125-150 g] was used. The dermal surface was carefully cleaned to remove sub cutaneous tissues and fats without damaging the epidermal surface. The skin was cut into circular patches and carefully mounted on the donor compartment of Keshary-Chien type diffusion cell facing the stratum corneum towards donor side and dermis towards the receptor compartment. The amount of azithromycin permeated across the skin was estimated by analyzing the drug concentration within receptor medium using the HPLC method. [50]

2.3.5.3 Permeation data analysis

Average values of three readings of in vitro permeation data were calculated and the average cumulative amount of drug permeated per unit surface area of the skin was plotted versus time. The slope of the linear portion of the plot was calculated as flux Jss (ug/cm^2/h) and the permeability coefficient was calculated using Eq.5C.1:

$$K_p = \frac{Jss}{Cv} \qquad (1)$$

Where Kp is permeability coefficient and Cv is total amount of drug. The enhancement of drug penetration due to organogel formulation was noted as enhancement factor (EF), which was calculated using Eq.5C.2:

$$EF = \frac{K_p\ organgel}{K_p\ market\ cream} \qquad (2)$$

The drug fluxes from IPM organogel and hydrogel were compared with the flux of marketed cream by applying independent Student's t-test. A value of $P < 0.05$ was considered significant. [51-52]

2.3.6 Effect of added water on properties of organogel

2.3.6.1 Spreadability

An apparatus suggested by Mutimer *et al.,* to determine spreadability of the formulations was suitably modified in house and was used for the study. It consisted of a wooden block provided by a pulley at one end. A rectangular ground glass plate was fixed on this end. An excess of the gel formulation (3 g) under study was placed plate and another glass plate having a hook to which a pan is attached with the help of on this ground glass plate. The gel formulation was then sandwitched between this a string. The top plate was subjected to a weight of 50 g by putting weight in the pan and the time (in s) required by the top plate to cover a distance of 10 cm was noted. A shorter time interval indicates better spreadability. [53]

2.3.6.2 Viscosity study

Viscosities of both plain and medicated organogel sample were determined at 37°C. For the viscosity measurements, a Cone and Plate viscometer CAP 2000^+ (spindle no 1) was used. All measurements were made on freshly prepared samples except stability samples. A 0.5 ml of sample was used for measurements. The measurements were carried out at different speeds ranging from 100 rpm to 900 rpm with 30 s interval between each two successive speeds. [54]

2.3.6.3 Moisture uptake study

The weighed organogels of azithromycin were filled in a vial (2 ml) and kept in a desiccator at room temperature for 24 h. Then they were taken out and exposed to 84% relative humidity (saturated solution of potassium chloride) until a constant weight for the organogel was obtained. The potassium chloride solution was poured into the reservoir of the desiccator covered by mesh of pore size≈ 1mm, placed above the surface (≈5 mm) of salt solutions. After specific time intervals, vials were withdrawn and reweighed. A graph of percent weight gain with respect to time was plotted. [55]

2.3.7 Evaluation of optimized organogel formulation

2.3.7.1 Effect of organogel on histopathology of rat skin

The rat abdominal skin region measuring approximately 4 cm^2 was mounted on modified Keshary Chien diffusion cell. The optimized batch of IPM organogel containing 3% w/w of azithromycin (3 g) was applied identical to diffusion study and the effects were compared against water as control. The skin was fixed in 10% neutral formalin for 24 h and then cut vertically against the surface at the central region (4mm width). Each section was dehydrated using graded solutions of ethanol and

then embedded in paraffin wax. Tissues were divided into small pieces and stained with haematoxylin and eosin. The sections were observed under 100x magnification and photographed. [56]

2.3.7.2 *Stability evaluation of azithromycin organogel*

The stability of developed organogel, hydrogel and marketed formulation (cream) was established over a period of 3 months as per ICH guidelines. Organogel (3g) containing 3% w/w azithromycin was placed in aluminium tubes. The head space was saturated with nitrogen. Three containers of each containing organogel, hydrogel and marketed formulation (cream) were stored at 40±2°C / 75% RH and 50 ±2°C / 75 % RH respectively. The gel formulations were observed for physical changes and drug content was analyzed (HPLC method) each at 1, 2, and 3 months. The shelf-life of the gel formulations was calculated using Arrhenius Plot.

To determine the degradation kinetics of azithromycin in formulations at 25 °C, Arrhenius plots were constructed by plotting the In (k) values of the observed K_{deg} values, computed from the degradation plots at 40 °C and 50 °C, versus 1000/T. Using least square regression, linear relationships with correlation coefficients were obtained. From Arrhenius plots, the degradation rate constant (K_{deg}) x 10^{-3} was found out to per month at 40 °C and 50 °C for organogel, hydrogel and marketed cream respectively. Shelf life was estimated for organogel, hydrogel and marketed cream by using equation

$$In\,[C/Co] = -kx \quad \text{.....(3)}$$

Where,

C- 90% Concentration of drug, Co - initial concentration of drug,

k -k deg value at 25°C, X - time to drug degradation up to 90% (shelf life).

Arrhenius equation,

Log K Log A-Ea/2.303 RT.....(4)

Where K-rate constant, A-frequency factor, Ea-energy of activation (cal/mol)

R-gas constant (1.987 calories/deg mol) and T-absolute temperature (K)

2.3.8 Microbiological determination of the azithromycin

2.3.8.1 Determination of MIC (Minimum Inhibition Concentration)

A 24 h old culture suspension of *Micrococus luteus* (ATCC 9341), grown on nutrient agar slant was prepared. 0.1ml of this suspension was inoculated into each tubes contained in 10 ml of sterile nutrient broth, with different concentrations of azithromycin in each tube (Range 0.05 to 2 µg/ml). The tubes were incubated at 37°C for 24 h, and observed for no turbidity, as with negative control which is uninoculated sterile nutrient broth. The concentration of tube showing no growth (MIC) for the culture was found to be 0.25µg/ml. [57]

2.3.8.2 Microbiological assay

The microbiological assay was carried out as per the method mentioned in IP. The study was used to assay the azithromycin content in developed organogel. The standard azithromycin solutions were prepared by dissolving a known concentration of azithromycin in methanol / ethanol 90% and then diluted to 50 ml in a phosphate buffer pH 8 (0.5 to 3 g KH_2PO_4 and 16.73 g K_2HPO_4, distilled water to 1 L). Working solutions of 0.25 and 1µg/ml were prepared in a phosphate buffer pH 8. The susceptible organism used was *Micrococus luteus* (ATCC 9341). Extracted azithromycin from an organogel was placed in each well with a control (vehicle free azithromycin). The analysis was carried out by two level factorial assay methods as mentioned in Indian Pharmacopoeia. Zone of inhibition was measured using digital vernier caliper. Mean zone of inhibition (the antibacterial activity) was calculated by using the following equation (2).

Percent potency= Antilog (2.0+ a log I) .....(1)

(The value "a" may be positive or negative & should be used algebraically)

Where, I is ratio of dilution (1:4) log I=0.6021

Where,

$$a = \frac{(U1 + U2) - (S1 + S2)}{(U1 - U2) + (S1 - S2)}$$

Where,

U1 & U2: Unknown sample at lower and higher concentration,

S1 & S2: Standard sample at lower and higher concentration.

Potency Std. Wt. x Std. Potency x Percent potency/ 100 x sample wt. ....(2)

2.3.9 Preparation of test microorganism suspension

To a 24 h old slant of culture strain *Micrococus luteus* (ATCC 9341), 3 ml sterile saline was added, mixed and poured into roux bottle containing sterile 250 ml agar and incubated at 37° C for 24 h. After incubation the growth on agar was washed using 50 ml sterile saline. The suspension-containing microorganism was checked at 530 nm by UV percentage transmission. If percentage transmission was less than 25%, incubation was repeated and if more than 25% then, it was diluted with saline to get 25%. This suspension was used as inoculum. [58]

2.3.10 Petri plate preparation

10 ml of un-inoculated agar base and 5 ml soft agar containing 0.1ml of inoculum was poured by mixing in sterile petriplate of 90 mm diameter. Allowed solidify then, four wells of 6 mm diameter were cut on each petri-plate. The agar plugs were removed and the wells were charged, and marked respectively, with standard low and unknown low concentrations of 0.25 µg /ml and unknown high and standard high concentrations of 1 µg/ml. Similarly, for each assay four sets of plates were made and incubated the charged plates, without inverting, at 37°C for 24 h. [59]

3. RESULT AND DISCUSSION

3.1 Analysis of physical properties of Azithromycin for organogel

3.1.1 Standard curve using HPLC

The standard curve was obtained from the area of peak measured using HPLC with a correlation coefficient of 0.9966 (Figure 1). The standard plot was used for drug estimation during solubility study, drug content and for diffusion samples of in vitro permeation studies.

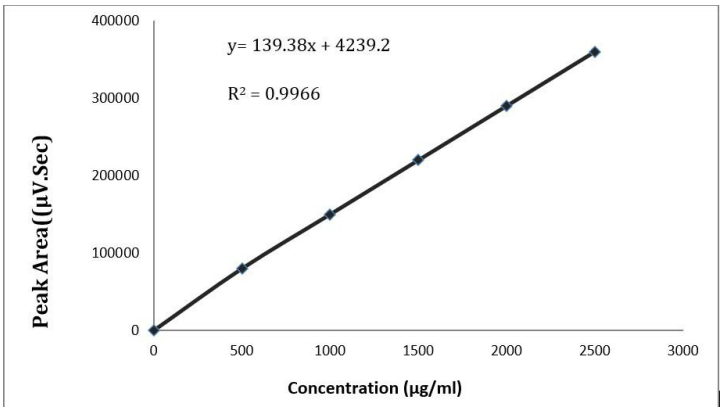

Figure 1. Calibration curve of Azithromycin using HPLC.

3.1.2 Partition coefficient of drug

The partitioning of drug is desired attribute for its effective diffusion through delivery vehicle into the lipid-rich environment of the stratum corneum and thereafter from the stratum corneum into the aqueous environment of the viable tissue. Therefore, the partition coefficient was determined to assess the drug partitioning. The logarithmic value of partition coefficient (log P) was experimentally found to be 0.627±0.019. The results obtained indicate that the drug possesses requisite lipophilicity for formulating it into a topical gel. This also indicates that the drug has ability to penetrate into the lipophilic skin membranes. The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin.

3.1.3 Solubility analysis

The study aimed to determine the solubility of Azithromycin in lecithin organogel and its corresponding mixtures in biocompatible oils. The solubility was highest in reverse micellar systems of IPM (21.63±0.92), followed by Azithromycin by EO, and was almost three times higher than in plain IPM and EO. Organogels, three-dimensional aggregates of gelator molecules, have solubilizing ability for drugs of diverse chemical nature. The molecular dispersion of azithromycin was more in IPM than EO, resulting in a difference in solubility. The results are shown in Table 1, Figure 2.

Table 1: Solubility of Azithromycin in plain oils and their corresponding reverse micellar system

Sr. No.	*Solvent*	*Solubility [mg/ml]*
1	IPM	8.22± 0.17
2	EO	4.97±0.35

3	IPM-lecithin mixture (RMS1)	21.63±0.92
4	EO-lecithin mixture (RMS2)	11.31±2.14

Each value represents mean ± S.D. (n = 3).

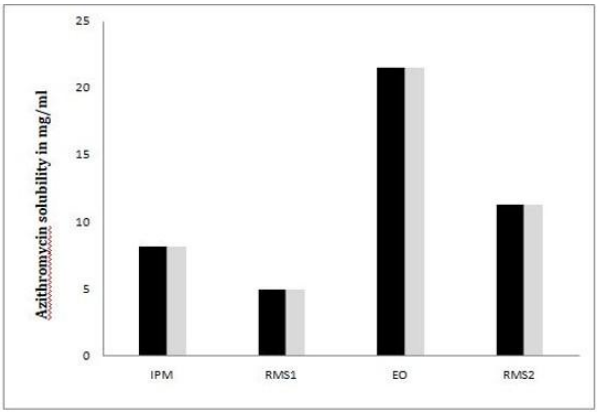

Figure 2: Solubility of azithromycin in plain oils and their corresponding reverse micellar system

3.2 Formulation development

3.2.1 Drug content uniformity

The distribution of azithromycin in conventional preparations is non-uniform and is reported in some studies.[60, 61] The drug has limited solubility and content uniformity in aqueous disperse systems is evident. The hydrogels prepared using polymers require water for its swelling leaving limited scope for drug solubilization and distribution. The non-uniform drug distribution is also responsible for enhanced hydrolytic degradation. The results obtained are shown in Table 2.

The non-uniform drug content was also evident in the prepared hydrogels and creams. The concentration of drug from all the three sites top, middle and bottom was found to be similar in organogel. whereas it was not same in case of hydrogel and marketed cream. This test demonstrated that drug in organogel is dispersed uniformly throughout the gel structure, but the hydrogels and marketed cream suffers from the disadvantage of nonhomogeneity. The organogels are prepared from isotropic solution. The gelation of oil is due to micellar aggregation. It is expected that a. micellar gel has provided uniformity in drug content.

Table 2: Content uniformity of Azithromycin formulations

Sr. No.	*Portion of tube analyzed*	*Organogel (µg/ml)*	*Hydrogel (µg/ml)*	*Cream (µg/ml)*
1	Top	1404.51±2.12	1361.46±3.26	1035.40±2.19
2	Middle	1403.23± 1.64	1398.02±1.52	1404.4± 1.95
3	Bottom	1399.4± 2.89	1404.51± 3.32	1302.96+ 2.43

3.2.2 In vitro diffusion study

3.2.2.1 Effect of lecithin concentration on in vitro diffusion of drug from organogels

The diffusion of Azithromycin containing organogels was carried out across cellulose acetate membrane (0.45µ) in order to investigate the effect of lecithin concentration on drug release. The IPM organogels each containing 15.2%, 22.8%, and 30.4% w/v concentration of lecithin with 3% w/w Azithromycin were subjected to in vitro diffusion study. The results obtained have been summarized in Table 3. The result reveals that the drug release decreased with increasing concentration of lecithin.

Table 3: Effect of lecithin concentration drug diffusion

Sr. No.	***Concentration of lecithin (%w/v)***	***Flux(µg/cm²/h) IPM organogel***	***Relative consistency***
1	15.2	54.74±1.08	Less viscous
2	22.8	36.31±1.31	Viscous
3	30.4	14.6±0.72	Highly viscous

The study compared three formulations of organogels for drug release, with 15.2% w/v organogel showing the highest release rate but low viscosity. The lowest release rate was achieved at 30.4% w/v organogel, which was highly viscous due to the development of lecithin micellar interconnections. The drug's diffusion is controlled by the strength of the gels, which is influenced by the presence of a three-dimensional network in the oils. The gel strength is influenced by the gelator concentration. The 22.8% w/v organogel was found to be the most satisfactory, but higher lecithin concentrations led to more extensive entanglement of long cylindrical micelles, resulting in a network-like structure with high viscosity. Further studies are needed to explore the 22.8% w/v organogels of IPM.

3.2.2.2 In vitro permeation study using excised rat skin

In vitro release profile is an important tool that predicts the drug flux in vivo. Thus, we can reduce number of animal experiments. The permeation studies with rat skin were performed to compare the results in artificial membrane. The mean cumulative drug permeated per unit surface area of the skin was plotted versus time. The IPM organogels, hydrogel, and marketed cream containing 3% w/w azithromycin were subjected to *in vitro* permeation study. The results are shown in Table 4, Figure 3.

Table 4: Results of *in vitro* permeation study using excised rat skin

Sr.No.	***Gel formulations***	***Flux (µg/cm²/h)***	***Permeability coefficient (Kpx10⁻³) (cm²/h)***	***Enhancement factor (EF)***
1	IPM organogel	193.1	6.37	1.81
2	Hydrogel	146.9	4.89	1.39
3	Marketed cream	105.4	3.51	-

The study compared the permeation profiles of Azithromycin loaded topical formulations through excised rat skin. The results showed that the permeation of Azithromycin from organogel formulation was significantly higher than that of hydrogel and marketed cream. The permeation-enhancing effect of reverse micelles in organogel may be attributed to the vectoring effect, wherein the micelles carry entrapped drug molecules. The higher permeation profiles of organogel may be attributed to the presence of lecithin in the organogel, which reversibly disorganizes the structure of skin lipids and increases drug permeation. This may be due to the interaction between skin lipids and the phospholipids in the organogel. The significant difference in Azithromycin permeation between organogel, hydrogel, and marketed cream was likely due to the mean size of internal phase droplets, which are noticeably smaller in organogels. Other factors contributing to the increased permeation of Azithromycin through excised rat skin include the solubilizing effect of the drug elicited by the microemulsion lecithin matrix and the penetration enhancer effect mediated by the lecithin component. Transdermal delivery of indomethacin incorporated in lecithin organogel showed significantly higher permeation compared to pure drug in IPP alone.

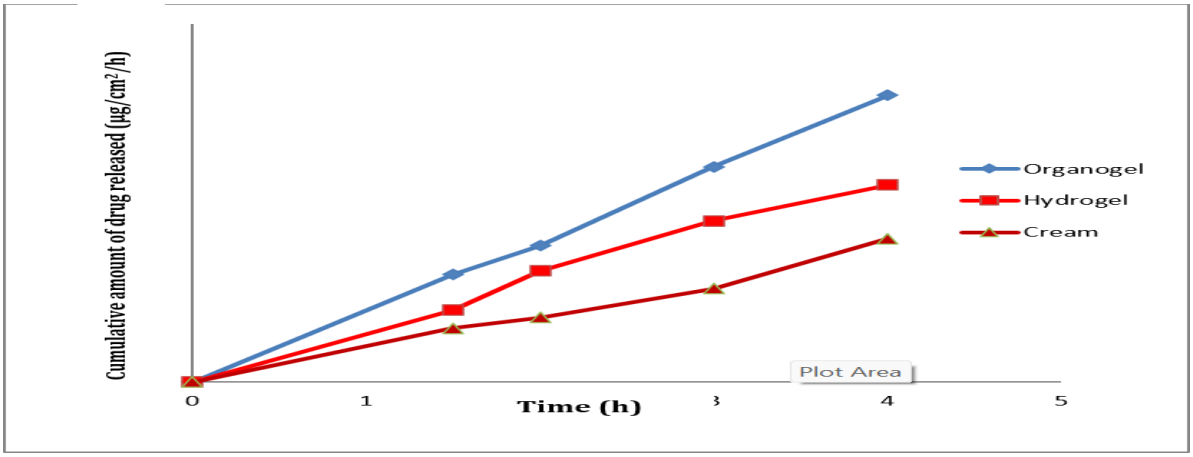

Figure 3: Comparative in vitro skin permeation of Azithromycin from organogel, hydrogel and marketed cream

3.3. Effect of added water on properties of organogel

3.3.1 Spreadability

The rheological properties of topical preparation influence the performance of drug delivery systems. The spredability is important for uniform and ease of application of topical preparations. The spreadability of Azithromycin loaded topical formulations is shown in Table 5. The spredability of these formulations was of the decreasing order of organogel> hydrogel > marketed cream. It is based on the composition and principle of formulation. The marketed cream being an emulsion has less spredability. The organogels are micellar aggregates and hence show desired spredability

.

Table 5. Spreadability of Azithromycin loaded topical formulations

Sr. No.	***Formulation***	***Spreadability (sec)***
1	Organogel	18±0.98
2	Hydrogel	23 ± 1.03
3	Marketed cream	29±1.35

Each value represents mean ± S.D. (n = 3).

3.3.2 Viscosity of IPM-lecithin organogel

In gel systems, consistency depends on the ratio of solid fraction, which produces the structure, to liquid fraction. Differences in concentration and kind of the gelling agents result in changes in the structure consistency. The viscosity data of plain and medicated IPM organogel has been summarized in Table 6, Figure 4. As observed plain IPM organogel has higher viscosity than organogel containing Azithromycin.

Table 6: Viscosity of plain and medicated IPM organogel

Sr. No.	***Speed in rpm***	***Viscosity of plain IPM organogel (cp)***	***Viscosity of medicated IPM organoge 4l(cp)***
1	100	392.4± 0.03	223±2.03
2	500	126±1.52	94± 1.14
3	900	88± 0.43	68.3± 0.63

The physical properties of drugs affect the aggregation of lecithin, weakening it. Plain organogel has higher viscosity than

Azithromycin-containing organogel, as Azithromycin is hydrophobic and partitioned predominantly in the hydrophobic region of the micellar aggregate. This results in a weaker lecithin network and low viscosity. The drug added is only 3%, causing a lower viscosity. Stress alters the gel's structure, with a linear decrease in viscosity with applied stress.[62-65]

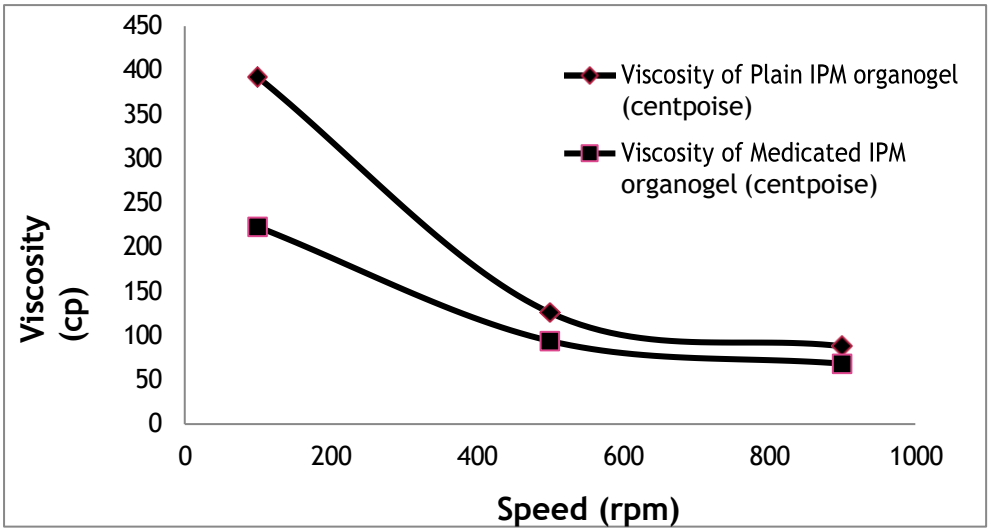

Figure 4: Viscosity of Plain and Medicated IPM organogel

3.3.3 Moisture uptake study

The result of moisture uptake studies for organogel formulation of Azithromycin are shown in Figure 5. the moisture uptake was increased with time up to 2 h.

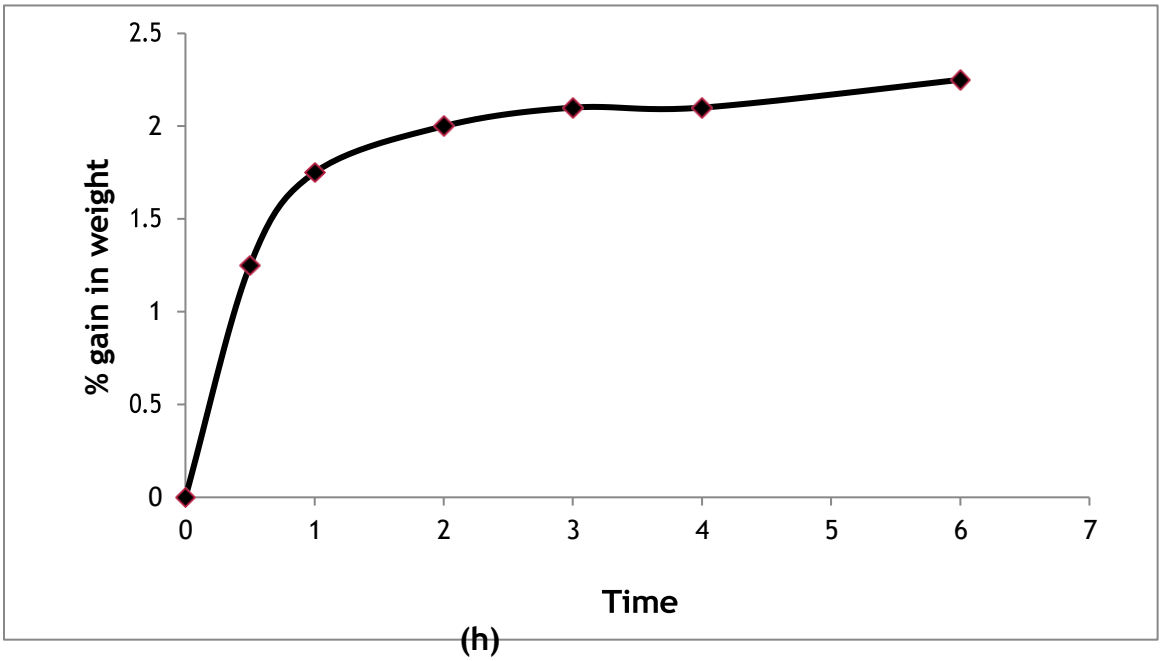

Figure 5: Weight gain of Azithromycin organogel (at 84% RH)

The azithromycin organogel did not show significant moisture absorption at 84% relative humidity. The small moisture content in the formulations helps them to I remain stable and protects the material from microbial contamination. The test demonstrates that lecithin organogel shows no instability during storage and is also a good formulation for moisture sensitive drugs like Azithromycin.

3.4 Evaluation of optimized organogel formulation

3.4.1 Effect of organogel on histopathology of rat skin

Lecithin organogel system is composed of nonirritating pharmaceutically acceptable ingredients. However, the level of surfactant and organic solvent used in formulation is fairly high. Hence, histopathological investigation of rat skin using organogel formulation was determined and compared against water as control. The histology of excised rat skin in control

and treated with organogel formulation after 24 h is shown in Figure 6a, 6b. respectively.

The microscopic observations indicate that the organogel has no significant effect on the microscopic structure of the skin. The surface epithelium lining and the granular cellular structure of the skin were totally intact. No major changes in the ultra-structure of skin morphology could be seen and the epithelial cells appeared mostly unchanged. Willimann and Luisi have also reported that IPM lecithin organogels does not shows any toxic effect on the skin. Lecithin used in the formulation has GRAS status. IPM is widely used in cosmetics and topical formulations and is generally regarded as nontoxic. Hence, it can be safely concluded that the IPM based lecithin organogels are biocompatible and safe for topical applications.

Figure 6a: Histology of test rat skin sample

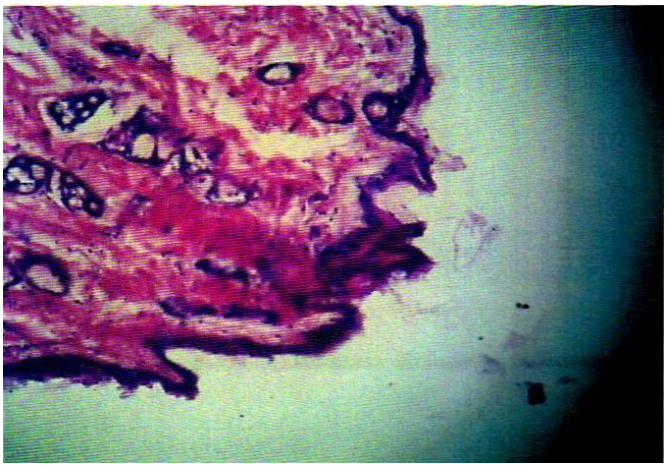

Figure 6b: Histology of control rat skin sample

3.4.2 Stability evaluation

Stability evaluation is crucial for drug quality and efficacy during storage. Physical stability studies were conducted on Azithromycin organogel, hydrogel, and marketed cream at 40°C and 50°C, focusing on macroscopic aspects like phase separation and changes in color or drug precipitation. The formulations were free from phase separation phenomena during the stability period. Chemical stability studies were conducted on Azithromycin loaded organogel, hydrogel, and marketed cream formulations at 40°C and 50°C, revealing specific degradation rate values for each.[66-68] The results are shown in Table7, 8 and figure7a-7b, 8a-8b, 9a-9b, 10a-10c and 11a-11d.

Table 7: Specific degradation rate values of Azithromycin at elevated temperature in different dosage form

Sr. No.	Temperature		K Value (per month) x 10^{-3}		
	(°C)	(°k)	Organogel	Hydrogel	Marketed cream
1	40	313	18.2	24.7	30.2
2	50	323	47.2	60.8	74.2

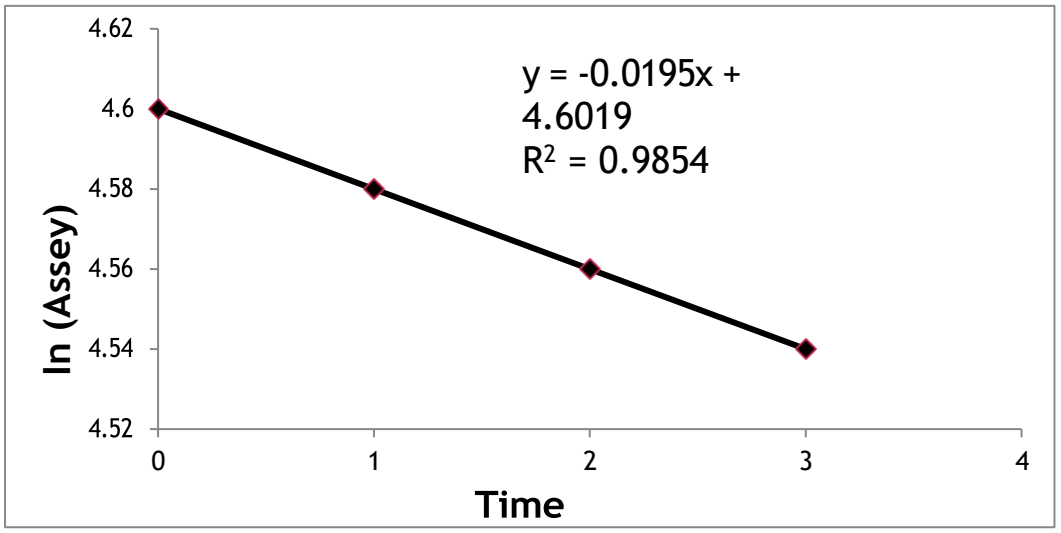

Figure 7a: Degradation kinetics of azithromycin in IPM organogel (40° C)

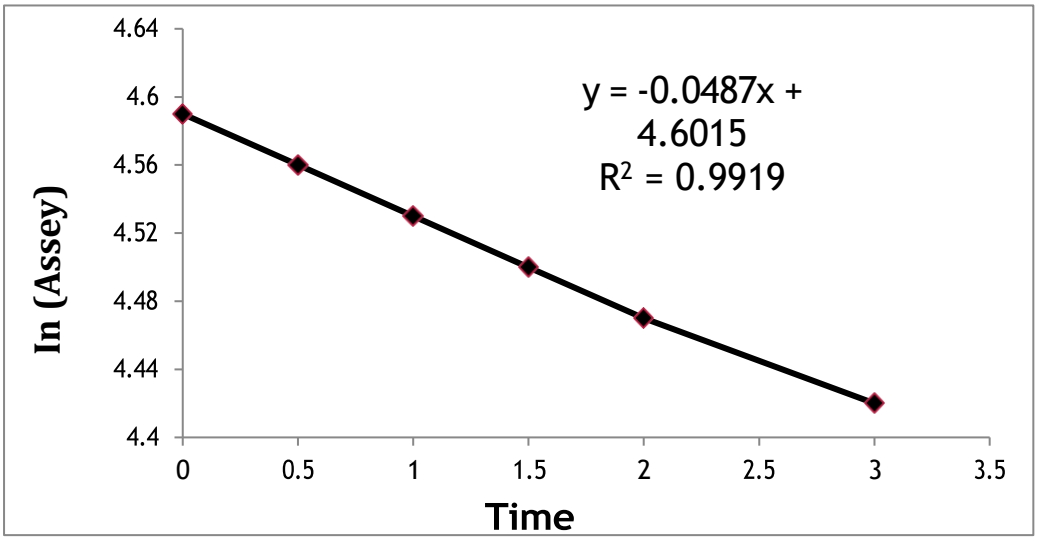

Figure 7b. Degradation kinetics of azithromycin in IPM organogel (50° C)

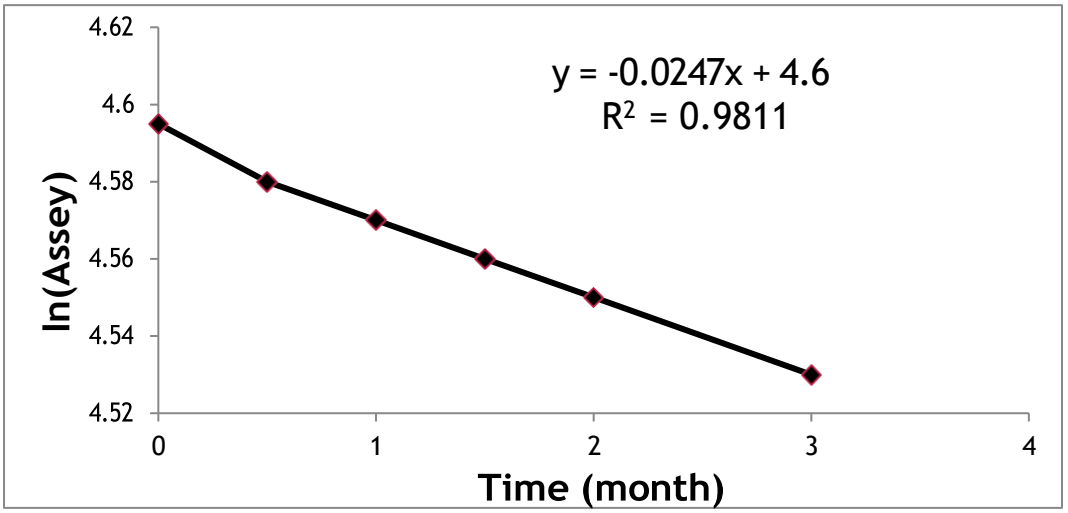

Figure 8a: Degradation kinetics of azithromycin in hydrogel (40° C)

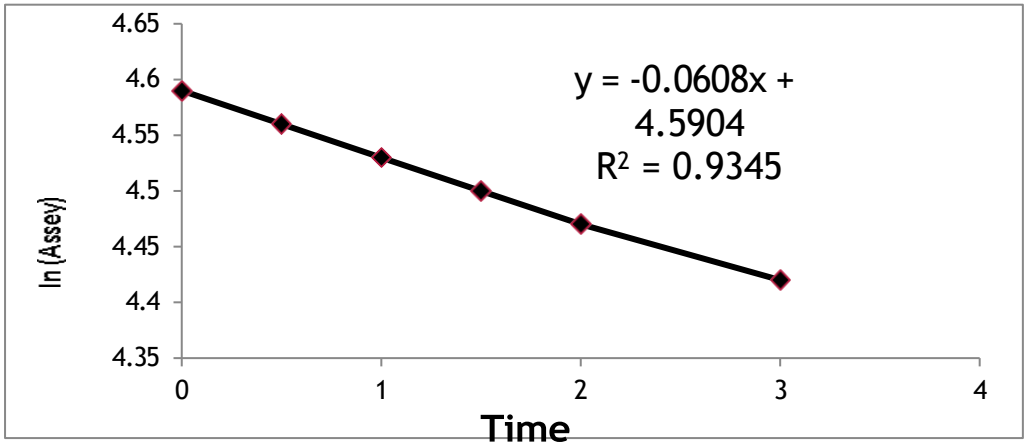

Figure 8b: Degradation kinetics of azithromycin in hydrogel (50° C)

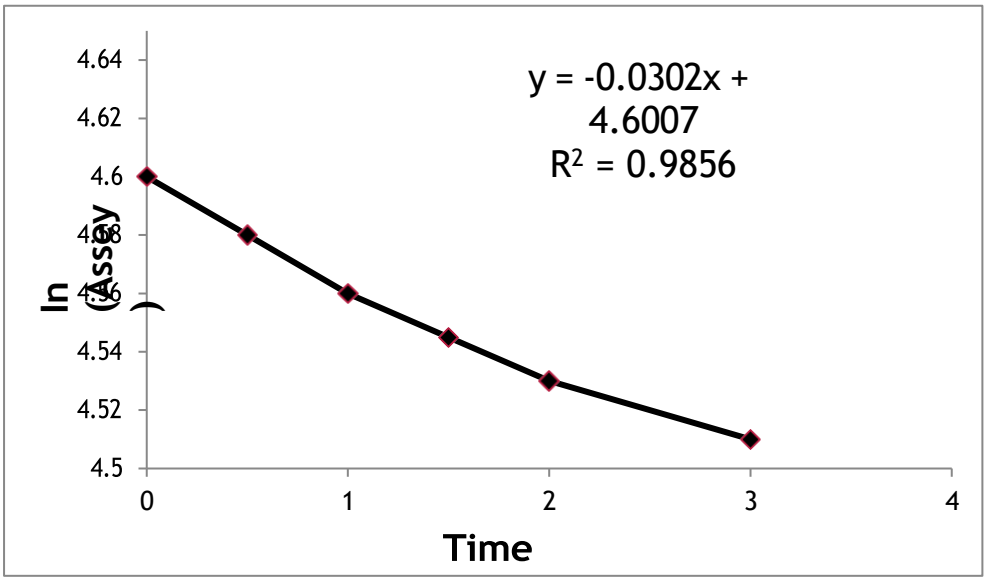

Figure 9a. Degradation kinetics of azithromycin in marketed cream (40° C)

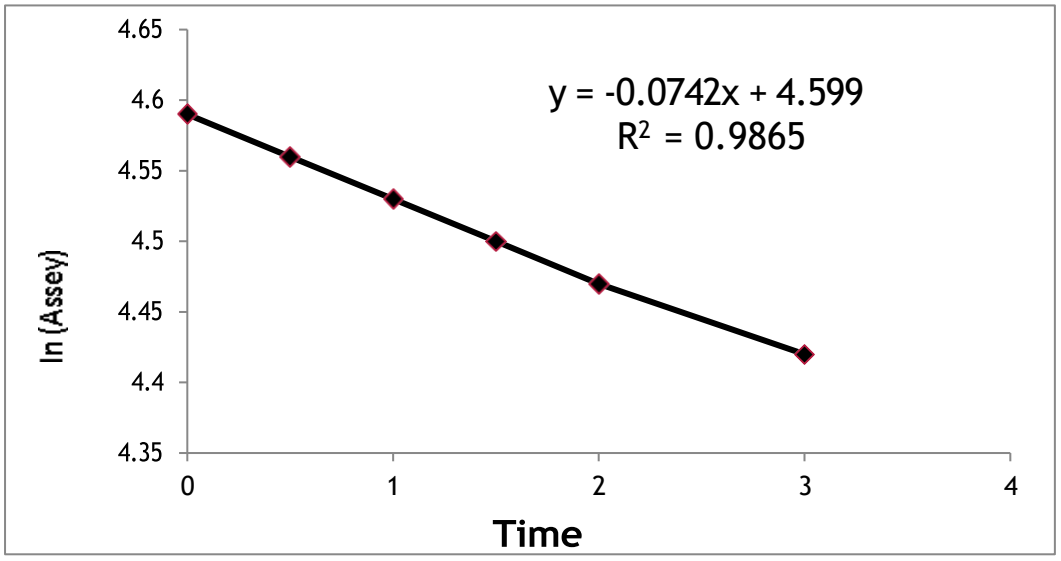

Figure 9b: Degradation kinetics of azithromycin in marketed cream (50° C)

The stability of Azithromycin in organogel is more stable than in hydrogel and marketed cream, as shown by Arrhenius plots. The stability decreases with higher temperature and humidity, leading to increased hydrolytic degradation. The shelf life of Azithromycin in organogels, hydrogel, and cream is 24, 18, and 15 months respectively. Organogels provide an effective barrier for hydrolytic degradation, but they are not sensitive to moisture at low temperatures. The Azithromycin content in the organogel formulation is above 95% up to six months, while hydrogels and creams show 7-9% degradation. The stability of Azithromycin in organogels was confirmed by microbiological assay. [69-70]

Table 8. Equation of Arrhenius plots for azithromycin formulations

Sr. No.	Formulation	Equation of Arrhenius plot
1	Organogel	In k = 25.583-9.2424x (r= 1)
2	Hydrogel	In k=25.336-9.0909x (r = 1)
3	Marketed cream	In k = 25.505 -9.0808x (r= 1)

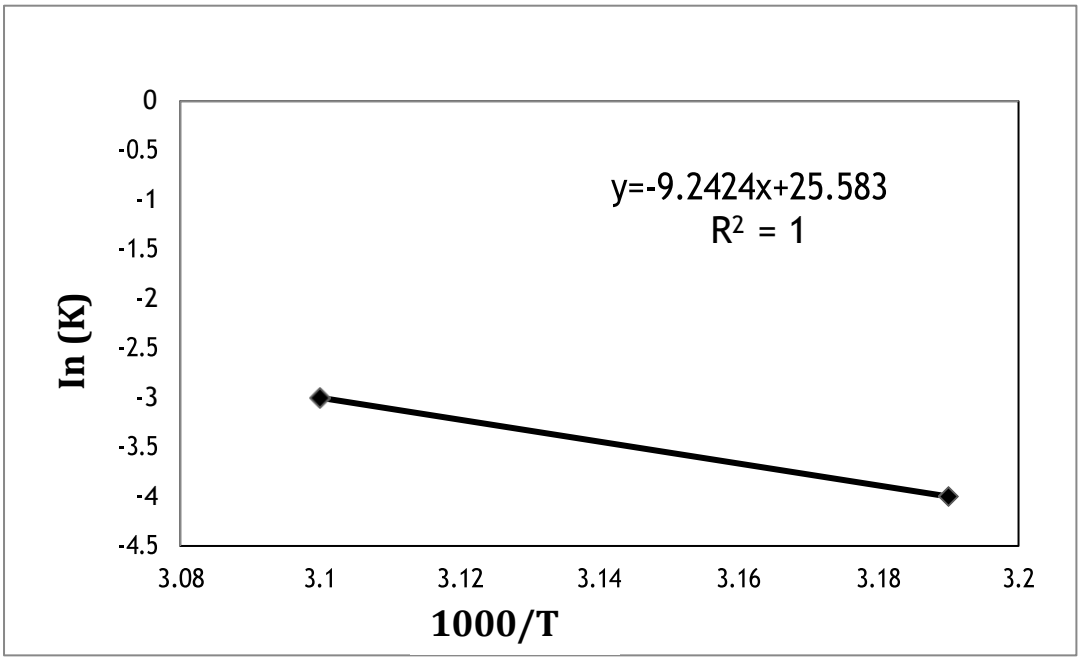

Figure 10a: Arrhenius plots for Azithromycin in IPM organogel.

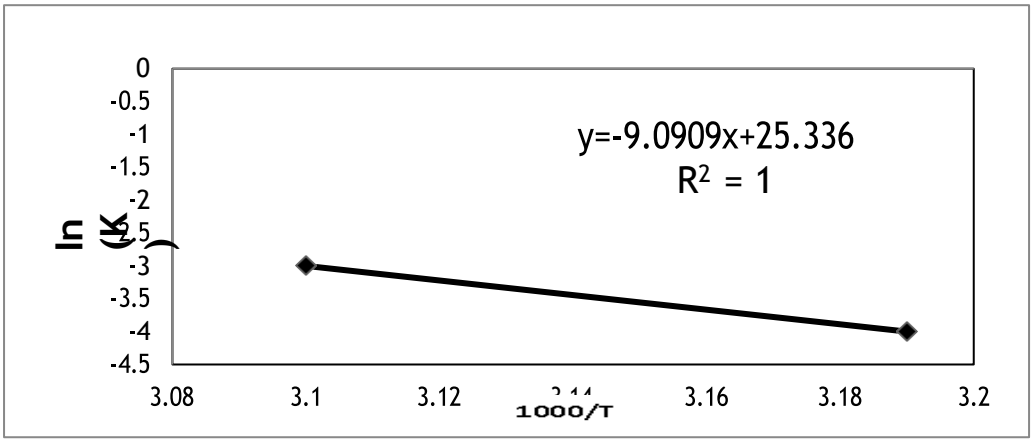

Figure 10b: Arrhenius plots for azithromycin in Hydrogel

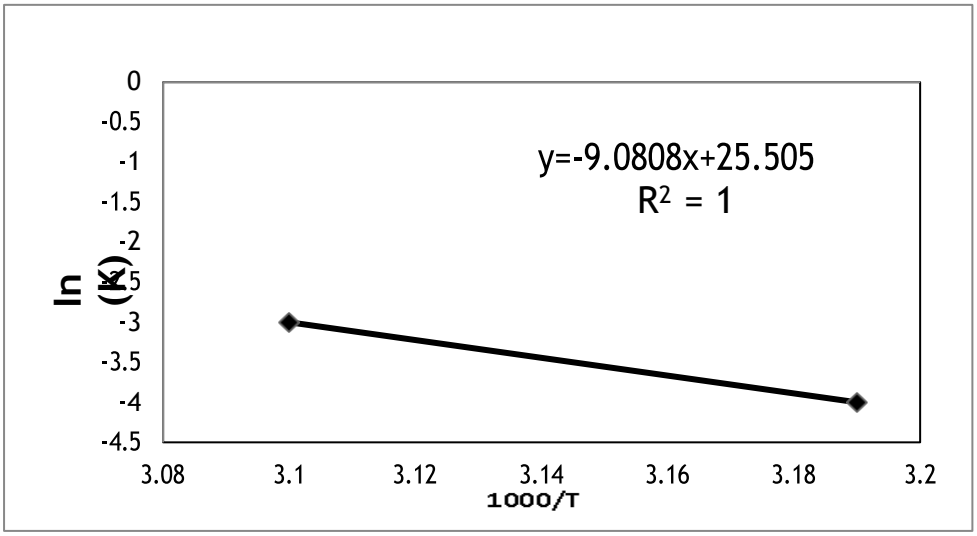

Figure 10c: Arrhenius plots for azithromycin in marketed cream

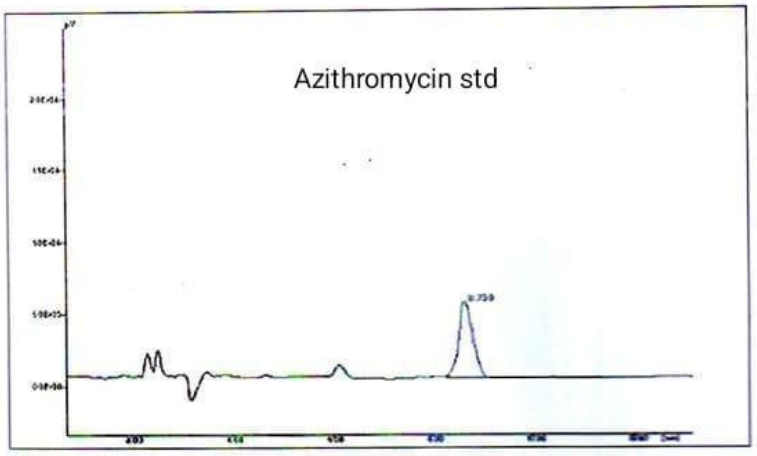

Figure 11a: chromatogram of standard azithromycin base (tR= 8.730 min) in mobile phase (Acetonitrile- 0.2 M Ammonium acetate- Methanol-Water (35:15:5:45)

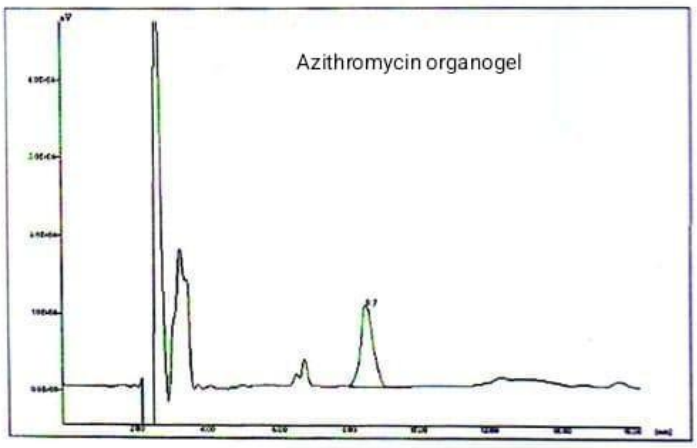

Figure 11b: Representative HPLC chromatogram of azithromycin organogel in mobile phase (Acetonitrile- 0.2 M Ammonium acetate- Methanol-Water (35:15:5:45)

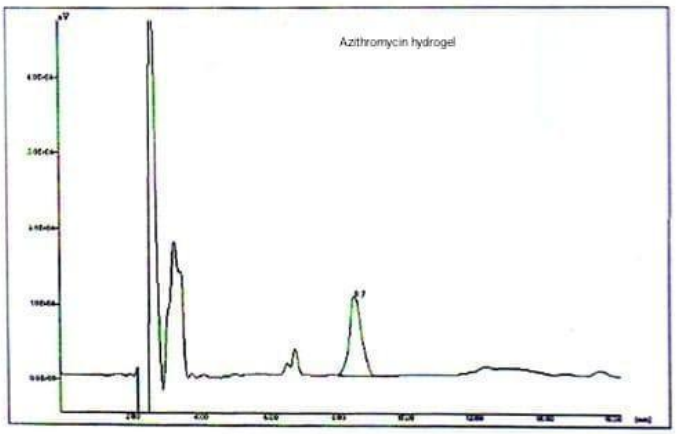

Figure 11c: Representative HPLC chromatogram of azithromycin hydrogel

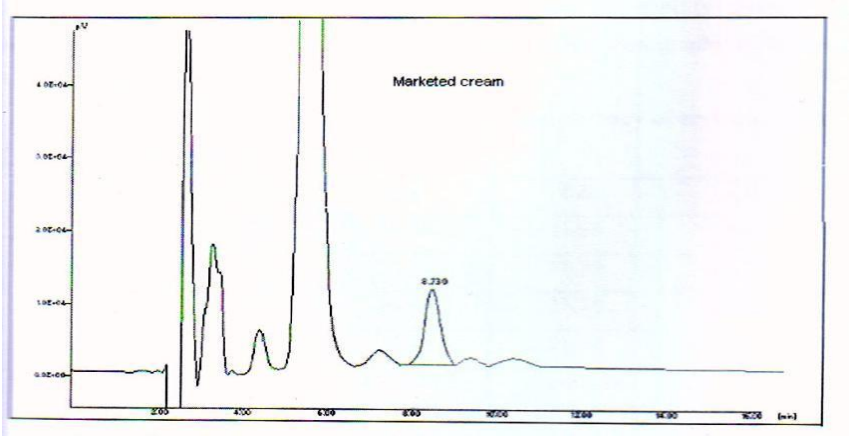

Figure 11d: Representative HPLC chromatogram of azithromycin marketed cream

3.4.3 Microbiological determination of Azithromycin

Microbiological assays are useful for demonstrating the therapeutic efficacy of antibiotics by comparing their antimicrobial activity with a known concentration of a standard antibiotic. They help resolve doubts about possible changes in antibiotic potency. In this study, the antibacterial activity of Azithromycin in organogel against Propionibacterium acnes was determined using a microbiological assay. The stability of the new formulation of Azithromycin was determined using both chemical and microbiological assays, following ICH guidelines for new dosage formulations. The results are shown in Table 9a – 9g, Fig. 12 and Table 10.

Table.9a: Diameter of zones of inhibition and potency of Azithromycin in organogel: Initial

Plate No.	***SL***	***SH***	***UL***	***UH***
1	21.52	26.50	21.58	26.57
2	21.69	26.61	21.70	26.66
3	21.64	26.54	21.74	26.59
4	21.47	26.43	21.62	26.50
SUM	S2=86.32	S1=106.08	U2=86.64	U1=106.32
a=192.96-192.4/19.68+19.76, a =0.01419 Percent potency = Antilog [2.0+ (0.014198 x 0.6021)] = 101.98% Potency = 993.28 units/mg				

Table 9b: Diameter of zones of inhibition and potency of Azithromycin in organogel: 40°C/1st month

Plate No.	*SL*	*SH*	*UL*	*UH*
1	21.56	26.50	21.54	26.48
2	21.48	26.42	21.46	26.41
3	21.52	26.46	21.50	26.40
4	21.60	26.54	21.58	26.47
SUM	S2=86.16	S1=105.92	U2 =86.08	U1=105.76
a= 191.84-192.08/19.68+19.76, a = -0.006085 Percent potency = Antilog [2.0+(-0.006085 x 0.6021)] = 99.15 % Potency = 965.721 units/mg				

Table 9c: Diameter of zones of inhibition and potency of Azithromycin in organogel: 50°C / 1st month

Plate No.	*SL*	*SH*	*UL*	*UH*
1	21.52	26.43	21.45	26.35
2	21.46	26.44	21.42	26.41
3	21.48	26.37	21.39	26.27
4	21.54	26.36	21.42	26.21
SUM	S2=86	S1=105.6	U2 =85.68	U1=105.24
a=190.92-191.6/19.56+19.6, a=-0.01736 Percent potency = Antilog [2.0+(-0.01736x 0.6021)] = 97.62% Potency=950.818 units/mg				

Table 9d: Diameter of zones of inhibition and potency of Azithromycin in organogel : 40°C/2nd month

Plate No.	*SL*	*SH*	*UL*	*UH*
1	20.64	25.57	20.51	25.47
2	20.58	25.51	20.53	25.43
3	20.60	25.59	20.47	25.39
4	20.66	25.65	20.45	25.43
SUM	S2=82.48	S1=102.32	U2 =81.96	U1=101.72
a=183.68-184.8/19.76+19.84, a = -0.02828 Percent potency = Antilog [2.0+(-0.02828x 0.6021)] = 96.15% Potency = 936.50 units/mg				

Table 9e: Diameter of zones of inhibition and potency of Azithromycin in organogel: 50°C/2nd month

Plate No.	*SL*	*SH*	*UL*	*UH*
1	20.55	25.54	20.35	25.31
2	20.52	25.62	20.46	25.42
3	20.61	25.5	20.31	25.42
4	20.64	25.42	20.2	25.12
SUM	S2=82.32	S1=102.08	U2 =81.32	U1=101.08
a=182.4-184.4/19.76+19.76, a=-0.05060 Percent potency = Antilog [2.0+(-0.05060x 0.6021)] = 93.22% Potency=907.96 units/mg				

Table 9f: Diameter of zones of inhibition and potency of Azithromycin in organogel: 40°C/3month

Plate No.	*SL*	*SH*	*UL*	*UH*
1	20.71	25.62	20.55	25.46
2	20.62	25.52	20.41	25.38
3	20.79	25.64	20.47	25.5
4	20.88	25.74	20.61	25.58
SUM	S2=83	S1=102.52	U2=82.04	U1=101.92
a=183.96-185.52/19.88+19.52, a = -0.03959 Percent potency = Antilog [2.0+(-0.03959x 0.6021)] = 94.65% Potency=921.89 units/mg				

Table 9g: Diameter of zones of inhibition and potency of Azithromycin in organogel: 50°C/3rd month

Plate No.	*SL*	*SH*	*UL*	*UH*
1	20.62	25.75	20.54	25.38
2	20.83	25.69	20.48	25.40
3	21.06	25.83	20.3	25.32
4	20.85	25.89	20.36	25.30
SUM	S2=83.36	S1=103.16	U2=81.68	U1=101.4
a=183.08 - 186.52 /19.72+19.80, a = -0.8704				

Percent potency = Antilog [2.0+(-0.8704x 0.6021)]

= 88.63%

Potency = 863.25 units/mg

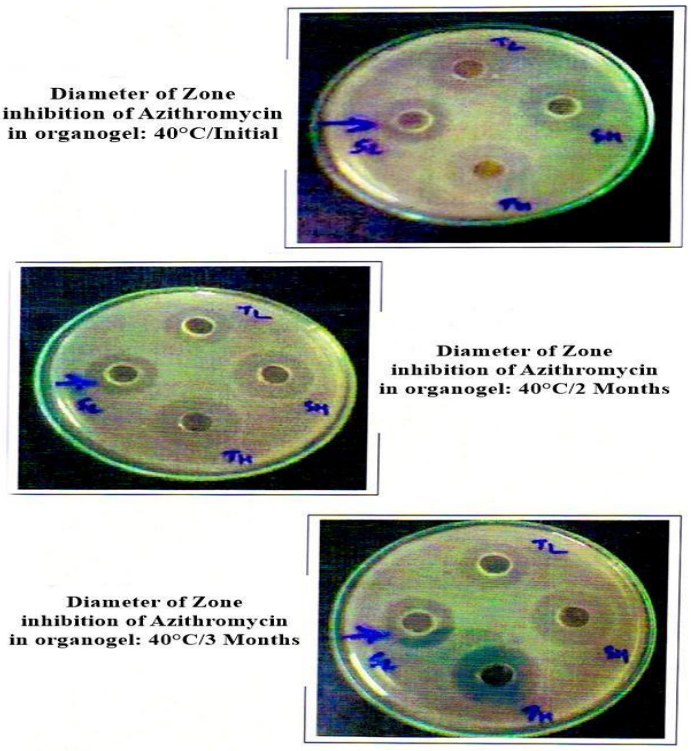

Figure 12: Representative zone of inhibition of azithromycin

Table 10. Stability data for Azithromycin loaded IPM organogel

Tests	***0 month***	***40°C/75% R.H***			***50°C/75% R.H***		
		1st month	***2nd month***	***3rd month***	***1st month***	***2nd month***	***3rd month***
Microbiological assay (%)	101.98	99.15	96.15	94.65	97.62	93.22	88.63
Chemical assay (%)	100	97.5	96.11	94	95.1	90.4	86

The results obtained indicated that temperature and humidity affect the activity of Azithromycin in organogel that reduces to around 6 to 7% for 40°C/75% R.H. and around 11% for 50°C/75% R.H. after 3 months. Thus, by comparing chemical assay with microbiological assay of stability samples (Table 10), it can be concluded that the organogel formulation of Azithromycin will remain stable physically, chemically, and microbiologically for 2 years. There was no variations in drug amount estimated in both assays, which is due to homogeneity of drug in organogel. Thus, the instability drawbacks of Azithromycin in aqueous topical formulations were successfully eliminated by incorporating Azithromycin in IPM lecithin organogel.

4. CONCLUSION:

The study conclusively establishes that isopropyl myristate (IPM) outperforms ethyl oleate (EO) as a gel formation system, offering superior stability and uniform drug distribution. The IPM-lecithin organogel system was selected for enhancing azithromycin stability due to its clarity and consistent drug dispersion. The release of azithromycin from these organogels is

influenced by lecithin concentration, with IPM organogels containing 300 mM lecithin demonstrating optimal release patterns and uniformity. The physical characteristics of the organogels are modulated by water content and gelator concentration, while medicated IPM organogels exhibit reduced viscosity compared to plain organogels, alongside excellent spreadability. Furthermore, the IPM/lecithin organogel shows minimal moisture absorption at 84% relative humidity, rendering it an ideal formulation for moisture-sensitive drugs like azithromycin. Safety assessments on rat skin confirmed the absence of toxic effects, validating the suitability of this organogel for topical delivery. Overall, incorporating azithromycin into the lecithin-based organogel with IPM as the oil phase significantly improves its chemical stability.

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