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Formulation and Optimization of Novel Controlled Release of Topical Thyme Oil-Loaded Organogel with Better Stability and Longer Activity

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Abstract

Despite being effective against a wide range of bacteria and fungi, thyme oil's volatility prevented simple standard gel formulations from being tested in clinical settings. The main issues with formulation and development employing thyme oil as the active component include microbial growth in the formulation, phase separation, and incompatibility with aqueous components. A key component of essential oils, thymol is a phenolic molecule. Due to the prolonged therapy and repeated doses of azole antifungals, there has been an increase in both microbiological and clinical resistance. This is actually due to genomic changes in strains of Rhizopus oryazae that are frequently brought on by gene mutations that change the molecular mechanisms at the micron scale. Topical drug delivery methods include ointments, creams, liquid preparations, powders, aerosols, and gels. To forecast the compositions of gelling solutions, a ternary plot showing the ratios of water, thyme oil, and surfactant mixture with gelator was developed. Antifungal susceptibility testing of Thyme oil was done in order to identify the zone of the ternary plot fulfilling MIC. A study on in vitro diffusion was conducted, and the % release was measured in terms of thymol. Comparing the optimized organogel formulation to the traditional Thyme oil gel formulation, it was discovered that the optimised organogel was more physically and biologically stable. The current study emphasises the value of the method chosen to analyse formulation issues to enhance the physical stability of oily active components.

Keywords: Thyme oil, Rhizopus, Active compound

Introduction

Current clinical regimen available for the treatment of superficial fungal infections includes azole antifungals like sertaconazole, luliconazole, butoconazole, etc.[1] The development of resistance against the antifungal agents used for superficial fungal infections has been a major issue since years and is yet a concern to be addressed. [2] Owing to the long duration of therapy, repeated dose of azole antifungals has led to the microbiological as well as clinical resistance, which is actually attributed to the genomic changes in the strains of Rhizopus oryazae widely occurring due to mutation in genes altering the molecular mechanisms at the micron scale.[3] The other major concern that has led to a reduction in patient compliance of current therapeutic regimens are adverse events (burning, itching, stinging, redness, skin rash, and contact dermatitis) associated with them which propagates a need to explore the agents that can overcome the issue

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of resistance and provide the therapeutic benefit at an equivalent or better efficacy.[4]

Extensive research has been carried out for a past decade on the chemical constituents of plants, which serves as an active moiety for the treatment of diseases. Different parts of plants like seeds, bulbs, roots, leaves, stem, bark, resins, fruits, buds, rhizomes, and essential oils (E. oils) are studied and explored as a source of medicinal agents. E. oils derived from parts of numerous phytomes have reported inheriting antifungal activity without any side effects reported in humans and animals. [5] One such plant essential oil-based formulation is thyme oil derived from the leaves of thymus vulgaris. In literature there are various enlisted medicinal benefits of thyme oil such as its antispasmodic action provides relief in rheumatoid arthritis, also serves as carminative, diuretic, expectorant, emmenagogue stimulant, relieves anxiety, and provides soothing action and hence, is extensively used in aromatherapy.[6] Thyme oil is also reported to have excellent anti-microbial properties against a variety of bacteria and fungus.

Various workers have tried extract of Thymus vulgaris against some well known fungal etiological agents as Rhizopus oryazae. Mucormycosis is a very common disease not only in human but also in animals and therefore always has been a challenge to scientist.

The present work aimed to the development of thyme oil-based microemulsion for treatment of fungal infections due to candida and rhizopus species. From various microbiological studies, it was found that thyme oil containing around 10–50% of thymol which, is a powerful antifungal agent. Yet, its functionality is not explored into a patient-friendly dosage form.[7] Thymus Vulgaris constituted 48.9% of thymol and 19.0% p-cymene while (thymus tosevii) constituted 12.8% carvacol and 10.4% thymol, whereas, both the species inherited a strong antifungal activity against all the species under test.

Topical delivery can be defined as the application of the drug containing formulation to skin to directly treat coetaneous disorders (psoriasis and acne etc) with the intent of containing the pharmacological or there effect of the drug to surface of skin or within. There are two basic types of drug delivery products, external topical and internal topical. The external topical are spread, sprayed or otherwise dispersed on the tissue to cover the diseased area, while the internal topical are applied to mucous membrane orally, vaginally, or on the rectal tissues for local activity.

Advantages of Topical Drug Delivery Systems are avoidance of first pass metabolism, convenient and easy to apply, Achievement of efficacy with lower total daily dosage of drug by continuous drug input. [8, 9] The delivery of drug via transdermal route has been recognized as one of the potential routes for both local and systemic delivery of drugs, due to several advantages. Topical delivery of bioactive substances is indeed a powerful strategy to reduce their systemic toxicity and at the same time restricts the therapeutic effect to specific tissues targeting to a specific site. [10]

Biological factors like skin conditions, skin sage, blood flow, regional skin sites, skin Metabolism and physicochemical Factors like skin Hydration, Temperature and pH, diffusion coefficient, drug concentration, partition coefficient, molecular size affects Absorption of Drug through Skin. Various Topical Drug Delivery Systems are ointments, creams, liquid preparation, powders, aerosols, gels. [11]

Gel is defined as semisolid preparation; consist of dispersion of small & large molecule in aqueous vehicle rendered jellylike through the addition of gelling agent.

Classification of Gel

A. Primary Types
   - Hydrogel
   - Organogel

B. Based on Nature of Bond
   - Chemical Gel
   - Physical Gel

C. Depend on phase System
   - Two Phase System
   - Single Phase System

Organogel

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Organogels are highly self-structured system, which are isotopic, thermo reversible, semi rigid system formed by peculiar kinds of small organic molecules. Organogel consist of macromolecules existing as twisted strands therefore do not form semisolid on standing. The molecules are bound together by stronger types of vanderwaals forces so as to form crystalline amorphous region throughout the entire system.[12] Advantages are not necessary to add penetration enhancer hence less harmful, not produce irritation, Moisture insensitive, less resistance to the microbial contamination. While disadvantages are less stable to temperature, Organogel naturally shrink when kept for longer time, Drug having high liphophilicity and partition coefficient can be used, Stability problem.

Classification of Organogel

1. Lecithin Organogel
2. Microemulsion Based Organogel
3. Gelatin stabilized microemulsion based organogel
4. Premium Lecithin Organogel.
5. Pluronic Lecithin Organogel. [13-15]

Factors Affecting Organogel is polar solvent, non-aqueous solvent, phase transition temperature (PTT), salt addition, temperature, surfactants. Applications are parentral delivery, oral delivery, topical/transdermal delivery.

Thus, from the present literature review the major point to be highlighted is unavailability of stable dosage form of Thyme oil in terms of physical and chemical stability. The aim of present research work would be to focus on development of organogel formulation of thyme oil with improved stability and applications in turn.

Experimental

Characterization of Thyme Oil

Boiling Point

The boiling point of Thyme oil was determined by using Thiele's tube method. The substance under test was filled into sodium fusion tube. Liquid paraffin bath was heated and sodium fusion tube tied to thermometer. The temperature at which the substance boiled was recorded as a boiling point of the substance. This study was performed in triplicate. Results are discussed in result and discussion section.

Construction of Calibration Curves

Thymol marker sample was used to construct calibration curve.

Determination of Wavelength Maximum

Determination of Wavelength Maximum in Methanol and Phosphate Buffer (pH 7.4)

The standard solution of Thymol marker was prepared in methanol and Phosphate Buffer (pH 7.4). The prepared solution was scanned between in the range of 400-200 nm by UV- visible spectrophotometer (Jasco V-630, Japan).

Content Estimation of Thymol from Thyme Oil.

From reported literature Thymol content of Thyme oil is 32%. [16]

- For 32 gm of Thymol required 100 gm of oil therefore 1gm of Thymol required 3.125 gm of oil. For 10 mg Thymol required 0.03125 gm of Thyme oil.
- From considering this content prepared serial dilution of Thyme oil (i.e. 0.2, 0.4, 0.6, 0.8, 1.0 µg/ml) in methanol.
- The absorbances of resulting solutions were measured at 293 nm using UV-visible spectrophotometer (Jasco V-630, Japan) against respective solvent blank.
- Using calibration curve of Thymol in methanol calculate the drug content.

FTIR Spectroscopy

FTIR Spectroscopy of Thyme Marker Sample and Thyme oil

The IR spectra of was recorded using Fourier transform infra-red spectrophotometer (SHIMADZU, Japan) with diffuse reflectance principle. Sample preparation kept between potassium bromide (KBr) cells, finally placing in the sample holder. The

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spectrum was scanned over a frequency range 4000 – 400 cm-1.

In Vitro Antifungal Susceptibility Testing of Thyme Oil

Determination of Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) of Thymol (Thyme Oil)

The broth microdilution assay with some modifications as adapted by Dannaoui et al. [17] and Espinel-Ingroff et al. [18] was performed to determine the MIC of thymol, against R. oryzae. On the day of the test, sterile 96-U-shaped-well microplates were used and each well of the plates contained 100 µL of Sabouraud dextrose broth (SDB) (Difco Lab.). Afterwards, 100 µL of the products (1,024 µg/mL) were added to the first wells. Next, serial two fold dilutions in culture medium were prepared to obtain concentrations ranging from 0.25 to 1,024 µg/mL. Finally, 10 µL of fungal inoculum were added to all wells. The microplates were incubated at 28 °C and MICs were determined visually after 48 h incubation. The MIC was determined from three independent experiments and was defined as the lowest drug concentration that showed absence of growth or complete fungal growth inhibition (100% inhibition). Negative control (without drugs) was performed to confirm the viability of the sporangio spores. The MFC was determined for the drugs that showed strong antifungal activity. After determining the MIC, 10 µL were subcultured from each well that showed complete inhibition (100% or an optically clear well) on SDA plates. The plates were incubated at 28 °C for 24 h, and the MFC was the lowest thymol concentration that showed either no growth or fewer than three colonies to obtain approximately 99 to 99.5% killing activity. The MFC was determined from three independent experiments on different occasions.

Preformulation Study Compatibility Study Based on Turbidity

The samples of oil and surfactants with drug were kept in 1:1 ratio to check compatibility of each ingredient with each other and compatibility of each ingredient with drug for one month. Samples were initially clear. The compatibility was checked on the basis of any turbidity occurred.

Selection of Gelling Agents

Various gelling agents with different concentration were screened for formation of gel.

Formulation Development of Organogel

Table No. 1 : Formulation Development of Organogel

Sr. No	Ingredients %w/w	F1	F2	F3	F4
1.	Thyme oil	1	1	1	1
2.	Surfactant	2.5	2.5	2.79	2.79
	Span 60	1.25	1.25	1.39	1.39
	Tween 20	1.25	1.25	1.39	1.39
3..	Sodium alginate	0.025	0.025	0.025	0.025

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4.	Water	0	1.33	0	1.33
5.	Methyl paraben	0.0015	0.0015	0.0015	0.0015
6.	Propyl paraben	0.0005	0.0005	0.0005	0.0005
7.	NaOH (0.1N)	q.s	q.s	q.s	q.s

Procedure:

1. Preparation of Gelator: Mixture of Span 60: Tween 20 (1:1) and Sodium alginate in Distilled water.
2. With continuous stirring, Thyme oil was added into gelator.
3. Drop by drop water added in mixture of Gelator and Thyme oil.
4. After that, remaining excipients Methyl paraben, Propyl paraben and NaoH added in that mixture.

Evaluation of Optimized Organogel Formulation

Characterization of Organogel by Infra-red Spectrophotometer (FTIR)

The IR spectra of was recorded using fourier transform infra-red spectrophotometer (SHIMADZU, Japan) with diffuse reflectance principle. Sample preparation kept between potassium bromide (KBr) cells, finally placing in the sample holder. The spectrum was scanned over a frequency range 4000 – 400 cm-1.

Physical Appearance

The prepared Thyme oil organogel formulations were inspected visually for their colour, appearance and consistency. [19, 20]

pH

pH of all formulations were determined by using pH meter (DIGITAL pH METER MK VI). The pH meter was calibrated before used with standard pH 4 and 7 buffer solutions. Electrode of pH meter dip in beaker containing formulation and pH of the formulation was measured using pH meter in triplicate. [21, 22]

Rheological Study

The viscosity of different organogel formulation was determined at 370C using a Brook field viscometer. The viscosity was measured by using spindle 64. [23]

Spreadability

- One gm of formulation was placed between the glass slides. 250 gm weight was allowed to rest on the upper slide for 1 to 2 minutes to expel the two entrapped air between the slides and to provide a uniform film of the formulation.
- The weight was removed and the top slide was subjected to a pull obtained by attaching 35 gm weight over the pulley.
- The time required for moving slide to travel premarked distance i.e. 10 cm was noted.
- The readings obtained were indications of relative spreadability of different formulations. [24]

Drug Content Determination

Drug concentration in organogel was measured by UV spectrophotometer (Jasco V-630, Japan). Thymol content in organogel was measured by dissolving known quantity of organogel in solvent (methanol) by sonication. Absorbance was measured after suitable dilution at 281 nm in UV spectrophotometer (Jasco V-630, Japan) and % drug content was calculated. [25]

In Vitro diffusion Study

In vitro diffusion was carried out by diffusion cell. A glass cylinder with both ends open, 10 cm height, 3.7

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cm outer diameter and 3.1 cm inner diameter was used as diffusion cell.[26]

Stability Study

The prepared Tulsi oil organogel and hydrogel formulations were stored away from light in collapsible tube at a) 40°C and 75% RH, b) room temperture and c) 4^0C for 3 months. After storage the samples were tested for their physical appearance, pH, and drug content and drug release. [27]

Result and Discussion

Characterization of Thyme Oil

Characterization by FTIR Spectroscopy

Characterization of Thymol by FTIR Spectroscopy

The IR spectra of Thymol was recorded and analysed for the functional groups. –OH group is responsisble for antifungal activity.

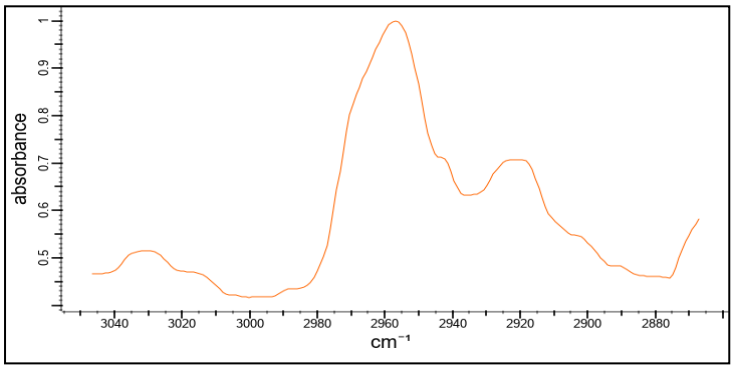

Figure No. 1: FTIR of Thymol

Characterization of Thyme Oil by FTIR Spectroscopy

The IR spectra of Thyme oil was recorded and analysed for the functional groups. –OH group is responsible for antifungal activity. The functional group of Thymol interpretated in IR spectra of Thyme oil from that concluded that Thymol may be present in Thyme oil.

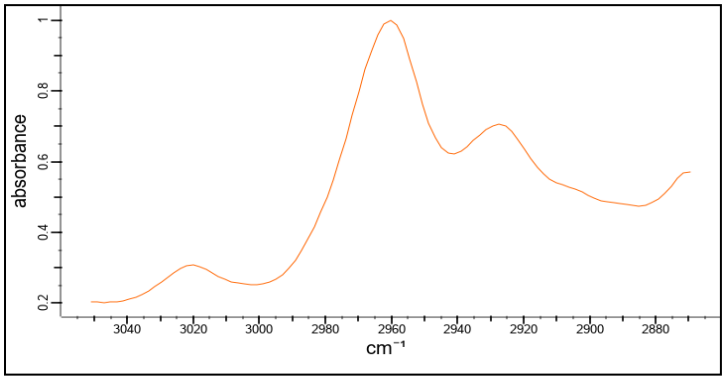

Figure No. 2: FTIR of Thyme Oil

Boiling Point

The boiling point of Thyme oil was found to be in range of 230-233 ºC (Reported 232 °C)

Construction of Calibration Curves

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Determination of Maximum Wavelength

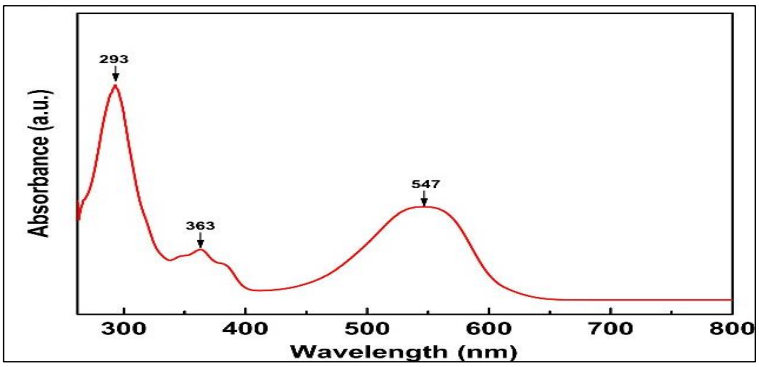

Figure No. 3: UV spectrum of Thyme Oil

Determination of Maximum Wavelength in Methanol

Thymol solution was examined in the range of 400-200 nm and 0.1 ppm solution of Thymol in methanol showed absorption maxima at 293 nm.

Table No. 2: The Absorbance of Thyme Oil in Methanol

Conc(ppm)	Absorbance (293 nm)
0.2	0.1744
0.4	0.2870
0.6	0.3685
0.8	0.4483
1	0.5799

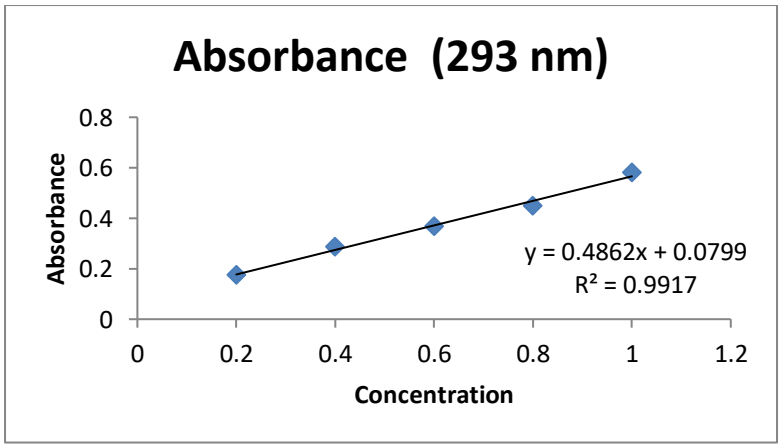

Figure No. 4: Calibration Curve of Thyme Oil in Methanol

Content Estimation of Thymol from Thyme Oil

Using calibration curve of Thymol in methanol calculated the drug content. Drug content calculalated

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from straight line equation obtained from calibration curve of Thymol in methanol. The concentration of Thymol in Thyme oil was found to be 0.2310 µg/ml.

In Vitro Antifungal Susceptibility Testing of Thyme Oil

Determination of Minimum Inhibitory Concentration (MIC) of Thymol (Thyme Oil)

After performing MIC study, concluded that 128 µg/ml Thymol is minimum inhibitory concentration against R. oryzae.

Preformulation Study

Compatibility Study

Compatibility Study Based on Turbidity

After performing compatibility study, it was observed that all ingredients showed clear solution when mixed with each other and doesn't show any turbidity thereafter. Thus, it may be concluded that all ingredients are compatible with each other.

Table No. 3: Compatibility study

Ingredient	Ratio	Observation
Thyme oil: Tween 20	1:1	Clear solution
Thyme oil: Span 60	1:1	Clear solution
Tween 20 : Span 60	1:1	Clear solution
Thyme Oil : Sodium alginate	1:1	Homogeneous White dispersion
Tween 20 : Sodium alginate	1:1	Homogeneous White dispersion
Span 60 : Sodium alginate	1:1	Homogeneous White dispersion

Selection of Gelling Agents

On the basis of stability of Sodium alginate was screened for organogel formation.

Table no. 4: Screening Study of Gelling agent

Sr. No.	Gelling Agents	Observation
1	Gelatin (5%)	Gel formed with rubbery texture
2	Gelatin (7%)	Oil separation
3	Carbopol 934	Oil separation
4	Sodium alginate (1%)	Stable gel formed

Evaluation of Organogel Physical Appearance

Colour, appearance, consistency of all organogel formulation: All the developed organogel was creamy light yellowish, non-transparent and showed good homogeneity.

Determination of pH

Since topical systems are directly applied on the skin, their pH should be compatible with the skin pH. An acidic or basic pH causes skin irritation or disruption of the skin structure. pH of all formulations were found to be between 6-6.8 which is acceptable for skin preparations.

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Table No. 5: pH Study

Formulation	pH
F1	6.53 ± 0.057
F2	6.43 ± 0.057
F3	6.56 ± 0.11
F4	6.7 ± 0.1

Rheological Study

Rheological behavior of the organogel indicated that the systems were shear thinning in nature showing decrease in viscosity at the increasing shear rates. All formulations exhibited shear thinning properties.

Table No. 6: Rheological Study

RMP	F1	F2	F3	F4
0.3	74420	61460	74900	66770
0.5	71520	57460	72560	65310
0.6	66260	54880	65780	62240
1	61540	52010	63340	57750
1.5	57420	49570	57780	52240
2	52220	44730	52430	48750
2.5	46750	42830	44680	46680
3	42440	39570	36830	42240
4	36630	32580	26430	35580
5	31235	26680	19740	33460
6	28740	22780	13880	27530
10	25560	21880	76000	26420

Spreadability Study

Table No. 7: Spreadability Study

Formulation	Spreadability (gm.cm/sec)
F1	14.8 ± 0.6
F2	23.2 ± 0.3
F3	32.5 ± 0.1
F4	20.6 ± 0.4

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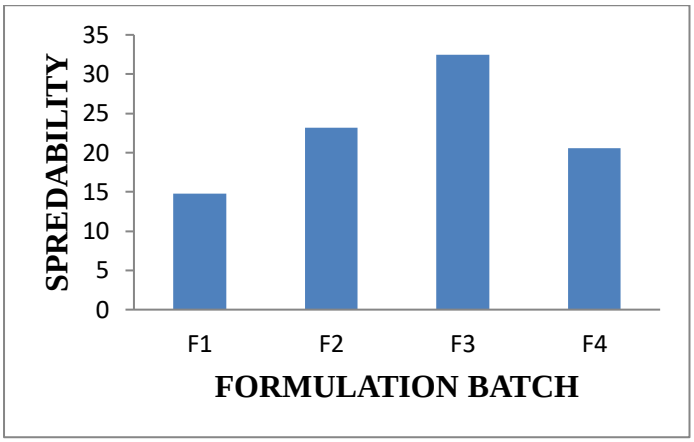

Figure No. 5: Spreadability study

From the result obtained it was observed that the batch F3 formulation shows the more spreading coefficient as compared to other formulations. Batch F3 formulation gives the spreading coefficient 32.5 ± 0.1 gm. Cm/sec which may be due to presence of optimum concentration of gelling agent. Batch F2 and batch F4 gives same the spreading coefficient which may be due to equal concentration of surfactants.

Drug Content Determination

The drug content of all formulations was found to be in the range of 85 % to 100 %. From reported literature the drug content of Thymol formulation is more than 80%. [23]. Hence, uniformity of drug content was found to be satisfactory.

Table No. 8: Drug Content

Formulation	Drug Content (%)
F1	90.03
F2	94.23
F3	98.07
F4	92.41

In Vitro Diffusion Study

Table No. 9 In-Vitro diffusion study

Time	F1	F2	F3	F4
0	0	0	**0**	0
1	22.01 ± 3.58	24.13 ± 27	31.24 ± 3.89	26.11 ± 4.97
2	33.08 ± 4.20	35.02 ± 3.30	44.09 ± 3.64	41.24 ± 3.66
4	45.11 ± 4.22	49.05 ± 4.11	59.11 ± 3.84	55.09 ± 3.84
6	59.23 ± 3.75	63.06 ± 4.63	78.34 ± 4.33	67.98 ± 5.25
8	66.21 ± 3.57	74.43 ± 4.98	90.22 ± 3.87	80.30 ± 4.98
10	77.05 ± 4.51	85.54 ± 4.87	98.54 ± 3.55	97.11 ± 4.81

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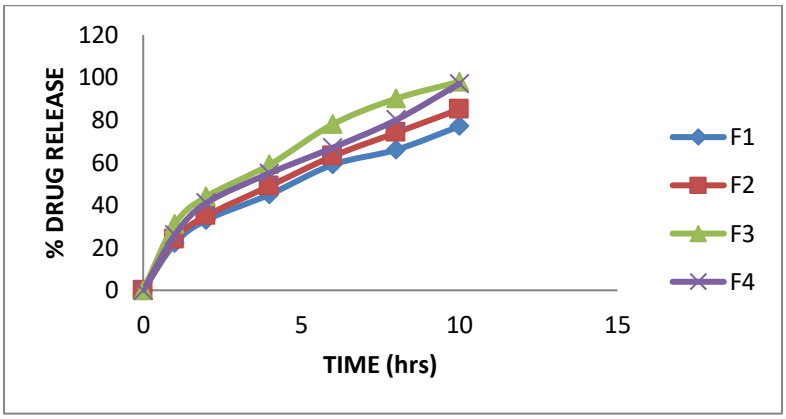

Figure No. 6: In Vitro Drug Release Study

It was observed that all formulations were found to be swelled at the end of experiment due to penetration of diffusing media into gel matrix which cause breaking of gel matrix, thus release of drug. Additionally three dimentional structure system of organogel contains oil provide enhanced solubility, thus increase in permeation of oil from gel formulation. The higher drug release was observed with formulations F3 and F4. This may be due to presence of maximum amount of water (30%). The formulations F3 and F4 showed 98.54 ± 3.55 and 97.11 ± 4.81cumulative drug release at the end of 10 hrs.

It was observed that formulation swelled at the end of experiment due to penetration of diffusing media into gel matrix which cause breaking of gel matrix, thus release of drug.

Diffusion Kinetic Models for Optimized Formulation

The dissolution kinetics of formulations was applied to various dissolution models such as Zero order, First order, Higuchi, Korsemayer-peppas. The mechanism of drug release of optimized F3 batch was found to be Higuchi's model as indicated by highest R^2 value. It indicates purely Higuchi's release pattern with diffusion and erosion of drug through swelling polymer[24].

Table No. 10: Release Kinetics

Formulation code	Zero order R^2	First order R^2	Higuchi R^2	Korsmeyer R^2
F1	0.935	0.988	0.997	0.576
F2	0.945	0.984	0.996	0.575
F3	0.923	0.927	0.997	0.544
F4	0.944	0.853	0.991	0.566

Stability Study

The stability of Thyme oil selected formula (F3) was studied at three different temperatures room temperature, 4° C and 40° C for three months. A sample of the organogel was taken at one month interval and was studied for drug content. After the stability study, formulation F3 doesn't show significant difference for physical properties, pH, drug content and viscosity.

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