# FORMULATION DEVELOPMENT AND EVALUATION OF ITRACONAZOLE LOADED INVASOMES HYDROGEL


## Abstract

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. There are various skin infections caused by fungus. An antifungal medication is a pharmaceutical fungicide used to treat mycoses such as athlete's foot ringworm, candidasis. Antifungal works by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effect on host. Itraconazole (ITZ) is commonly used in the treatment of fungal infections. It has low bioavailability (55%) because of low aques solubility and first pass effect. Hence we attempted to develop Itraconazole-loaded invasomes hydrogel. ITZ-loaded invasomes were prepared by conventional thin layer evaporation technique using Phospholipon 90H, terpene (Limonene) and ethanol. The optimized ITZ-loaded invasomes was incorporated into carbopol 934p (0.5 to 2%) solution to get a hydrogel for improving convenience in superficial application. FT-IR studies revealed no interaction between the drug and excipients. The formulated hydrogel formulation was evaluated with parameter pH, viscosity, gel strength, drug content, spreadability, *in-vitro* release test, washability, extrudability study and stability studies. The formulation OIGF4 showed a drug content of 99.12% and drug release of 99.78% in 72 hrs, which contains carbopol 934p concentration 2%w/w. The present work also focuses on making the formulation more pharmaceutically acceptable.



**Keywords:** Topical drug administration, Antifungal, Itraconazole, Invasomes, Hydrogel, Carbopol.


**Comment [D1]:** Not all antifungal agents are fungicidal

**Comment [D2]:** Spell check

**Comment [D3]:** Better to write complete word - aqueous

## Introduction

Stratum corneum is the outermost layer of the skin, functioning as a primary barrier to protect the skin from potentially harmful environmental agents. In addition, it prevents the loss of moisture to the outside environment, the intercellular lipids in the stratum corneum helps in maintaining homeostasis of the skin. The trasdermal delivery of drugs is rapidly increasing in the formulation development in enhancing the bioavailability of many drugs. When drugs are administered via transdermal route skin barrier is harmfully affected [1, 2]. In recent years, vesicular systems have been intensively studied as drug carrier systems for the dermal and transdermal administration of drugs. Traditional liposomal formulations, compared to

**Comment [D4]:** spelling

conventional dosage forms, have shown *in vitro* an enhanced cutaneous drug accumulation allowing a reduction of the dose applied onto the skin [3]. In the last two decades, new classes of lipid vesicles were introduced by different researchers. Cevc and Blume (1992) [4] introduced the first generation of the highly deformable, elastic liposomes, referred as Transfersomes. They consist of phospholipids and a surfactant molecule, the so called edge activator, which destabilizes lipid bilayer and increases its deformability. Subsequently developed ethosomes, new soft vesicular carriers mainly consisting of phospholipids, ethanol and water [5]. More recently, researchers investigated the novel vesicular systems called as invasomes [6-8]. Briefly, invasomes contain not only phospholipids but also ethanol and terpenes, which make the vesicles deformable, and also serve as penetration enhancers. This system has shown to improve skin penetration of hydrophilic and lipophilic drugs [9]. Ethanol is a good penetration enhancer while terpenes have also shown potential to increase the penetration of many drugs by disrupting the tight lipid packing of the stratum corneum [10, 11]. Hydrogels are 3-dimensional networks consisting of hydrophilic polymers that swell in aqueous solution retaining large amount of water without dissolving. Hydrogels formulated with cellulose have biodegradable properties, high permeation of active materials with high degree of swelling and no associated toxicity or irritation makes them as ideal polymers for delivery of drugs through transdermal route as delivery vehicles [12-14]. Fungal diseases are increasing each year due to the ease of transmission from person to person [15]. Effective treatment options are necessary to avoid the spreading of the disease to peripheral organs leading to potential death [15, 17]. Superficial infections are caused by many species like *Aspergillus*, *Candida*, *Tinea*, *Pneumocystis* and *Histoplasma*. These species causes fungal infection conditions like athlete's foot, finger and toe nail infections, yeast infections, oral thrush and ringworm. Some systemic and opportunistic fungal infections can enter the bloodstream and result in more serious disease in those with compromised immune system [18]. Itraconazole (ITZ) is commonly used in the treatment of fungal infections. Though oral and parenteral route are commonly used for the treatment of fungal infections because of wide bio-distribution of drugs to other tissues the actual amount reaching the site of action is less [19]. Hence, high drug dosing is required for proper treatment which increases the toxicity and cost of the treatment. More efficient way of delivering the drug to combat fungal infections and reducing the cost of the treatment is by using transdermal route of delivery. The efficacy of topical administration of antifungal drugs depends on the penetration through the skin. Use of hydrogels for treatment of infection limits the penetration of drug through intact skin (especially class IV drug) thereby creating an urge to develop formulations for

**Comment [D5]:** How

transdermal route with a combination approach. One such approach is preparing invasomes formulation and incorporating the same in the hydrogels which enhances increase permeability of the drug for treating chronic conditions. The concept of invasomes hydrogels may potentially increase the permeability of drug through the stratum corneum. Hence, the aim of our present study is to develop invasomes hydrogels of itraconazole and evaluate for their enhanced transdermal permeation.

## Experimental

### Materials

Itraconazole was obtained as a gift sample from Yashica Pharmaceuticals Private Limited, Maharashtra, India. Phospholipon 90H, terpene, Carbopol 934 was purchased from Sigma-Aldrich Chem, Germany. High purity 99.9% Ethanol were obtained from SD Fine chemicals, Mumbai, India. All other chemical and materials were of analytical grade. Triple distilled water was generated in house.

### Preparation of Itraconazole loaded invasomes

Itraconazole invasomes formulations were prepared by conventional thin layer evaporation technique [9]. Briefly, Itraconazole, Phospholipon 80H, Phospholipon 90H and terpene (Limonene) were taken in a clean, dry, round bottom flask, and dissolved in chloroform, methanol, 2:1 (v/v). The organic solvent was removed by rotary evaporation. Final traces of solvent were removed under vacuum overnight. The deposited lipid film was hydrated with phosphate buffer saline (pH 7.4) mixture by rotation at 60 rpm for 1 h at room temperature. The resulting vesicles were swollen for 2 h at room temperature to get large multilamellar vesicles. To prepare smaller particles, large particles were probe sonicated at 4°C at 40% output frequency (at 40 W).

### Formulation development of itraconazole Loaded invasomes hydrogel

Polymer carbopol 934p (0.5 to 2%) and purified water were taken in a beaker and allowed to soak for 24 hr. To this required amount of ITZ loaded invasomes (equivalent to 1%) was dispersed in water and then carbopol 934p was then neutralized with sufficient quantity of triethanolamine. Glycerine as a moistening agent, methylparaben and propylparaben as preservatives was added slowly with continuous gently stirring until the homogenous gel was formed. Hydrogel formulations of ITZ were prepared using different concentrations of carbopol 934p Table 1.

**Table 1 Optimized formulae of ITZ loaded invasomes hydrogel**

| Formulation code | Invasomes (eq. to %) | Carbopol (%) | Water (ml) | Alcohol (ml) | Methylparaben (%) | Propylparaben (%) | Glycerine (ml) | Triethanolamine (ml) |
|---|---|---|---|---|---|---|---|---|
| OIGF1 | 1 | 0.5 | 100 | 4 | 0.1 | 0.05 | 10 | 0.5 |
| OIGF2 | 1 | 1 | 100 | 4 | 0.1 | 0.05 | 10 | 0.5 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **OIGF3** | 1 | 1.5 | 100 | 4 | 0.1 | 0.05 | 10 | 0.5 |
| **OIGF4** | 1 | 2.0 | 100 | 4 | 0.1 | 0.05 | 10 | 0.5 |

**Characterization of invasomes hydrogel**

**pH measurements**

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted.

**Measurement of viscosity**

Viscosity measurements of prepared topical hydrogel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.

**Determination of gel strength**

The method by which the properties of polymeric system may be conveniently determined is texture profile analysis. A TA-XT2 Texture analyzer (The experiments were conducted at Digital Scientific Equipments, RK Puram, New Delhi). The experiment was done by placing the gels in standard beaker below the probe. In this an analytical probe is then immersed into the sample. The Texture Analyzer was set to the 'gelling strength test' mode or compression mode with a test-speed of 1.0 mm/s. An acquisition rate of 50 points per seconds and a trigger force of 5 g were selected. An aluminum probe of 7.6 cm diameter was used for all the samples. The study was carried out at room temperature. The force required to penetrate the gel was measured as gel strength in terms of gm.

**Drug content**

Accurately weighed amount of hydrogel formulation equivalent to 20mg of topical hydrogel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using 0.45µ membrane filter. Then 0.1ml of filtered solution was taken in 10 ml capacity of volumetric flask and volume was made upto 10 ml with 7.4 pH phosphate buffer, this solution was analyzed using HPLC method.

**Extrudability study**

Extrudability was based upon the quantity of the hydrogel extruded from collapsible tube on application of certain load. More the quantity of hydrogel extruded shows better extrudability. It was determine by applying the weight on hydrogel filled collapsible tube and recorded the weight on which hydrogel was extruded from tube.

**Spreadability**

Spreadability of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadability, placing 2-5gm of hydrogel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80gm of weight was noted. Good spreadability show lesser time to spread.

$$Spreadibility\,(g.cm/\sec) = \frac{Weight\ tide\ to Upper\ Slide \times Lenth\ moved\ on\ the\ glass\ slide}{Time\ take nto\ slide}$$

***In Vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion. The franz diffusion cell has receptor compartment with an effective volume approximately 60 ml and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two $cm^2$ size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of 428nm [20, 21].

**Release kinetics**

In-vitro diffusion has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of bioequivalence. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where ft is the function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. To compare dissolution profiles between two drug products model dependent (curve fitting), statistic analysis and model independent methods can be used.

In order to elucidate mode and mechanism of drug release, the *invitro* data was transformed and interpreted at graphical interface constructed using various kinetic models. The zero order release Eq. (1) describes the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems, matrix tablets with low

soluble drugs, coated forms, osmotic systems etc., where the drug release is independent of concentration.

$$Qt = Qo + Kot \quad (1)$$

Where, Qt is the amount of drug released in time t, Qo is the initial amount of the drug in the solution and Ko is the zero order release constant

The first order Eq. (2) describes the release from the system where release is concentration dependent e.g. pharmaceutical dosage forms containing water soluble drugs in porous matrices.

$$\log Qt = \log Qo + K1\ t /2.303 \quad (2)$$

Where Qt is the amount of drug released in time t, Q is the initial amount of drug in the solution and K1 is the first order release constant.

Higuchi described the release of drug from insoluble matrix as a square root of time as given in Eq. (3)

$$Qt = KH \sqrt{t} \quad (3)$$

Where, Qt is the amount of drug released in time t, KH is Higuchi's dissolution constant.

The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model).

**Korsemeyer-Peppas**

The curves plotted may have different slopes, and hence it becomes difficult to exactly pin-point which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, data were also analyzed using Korsemeyer's equation.

$$Q_t/Q_\infty = k_{KP}.t^n$$

Where $Q_t/ Q_\infty$ is the fraction of drug released at time t, $k_{KP}$a constant compromising the structural and geometric characteristics of the device and n is the release exponent.

The slope of the linear curve gives the 'n' value. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When n = 1, the release rate is independent of time (typical zero order release / case II transport); n = 0.5 for Fickian release (diffusion/ case I transport); and when $0.5 < n < 1$, anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when $n > 1.0$ super case II transport is apparent. 'n' is the slope value of log $M_t/M_\infty$ versus log time curve [22].

**Stability studies**

Optimized formulations of hydrogel were subjected to accelerated stability testing under storage condition at 4±0.5°C and at room temperature (28±0.5°C). Formulations were stored in screw capped, amber colored small glass bottles at 4±0.5°C and 28±0.5°C. Analysis of the samples were characterized for vesicle size and drug content after a period of 0, 15, 30, 60 and 90 days.

**Results and Discussions**

Results of evaluation of Invasome loaded hydrogel formulation (OIGF1-OIGF4) of optimized formulation were incorporated into four different carbopol 934p concentration 0.5, 1, 1.5 and2.0 % w/w respectively. Formulation OIGF4was found to be good Table 2. The formulation OIGF4 showed a drug content, viscosity, spreadability, extrudability, gel strength of 99.12%, 6285cps, 7.85cm, 1.72gm, 7.4 g/s and drug release of % in 72 hrs, which contains carbopol 934p concentration 2%w/w. Results of *In-vitro* drug release from optimized formulation (OIGF4) are given in table 3 was found 99.78 after 72 hrs. The *in vitro* drug release data of the formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation and Korsmeyer's - pappas models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of formulation was maximum i.e 0.975hence indicating drug release from formulations was found to follow first order model of drug release kinetics Table 4 and Fig. 1-4. Stability studies for optimized formulations were carried out at 4.0 ± 0.5°C and 28 ± 0.5ºC for a period of 3 months. There was no significant variation found in physical appearance, % entrapment efficiency and viscosity of the hydrogel formulation OIGF4 as shown in Table 5.

**Table 2: Results of evaluation of invasomes loaded hydrogel**

| Formulation | Gel strength (g/s) | pH | Extrudability (gm) | Viscosity (cps) | Spreadability (cm) | Drug Content (%) |
|---|---|---|---|---|---|---|
| **OIGF1** | 6.8 | 6.82 | 192 | 6585 | 6.98 | 98.12 |
| **OIGF2** | 7.2 | 6.75 | 198 | 6374 | 7.21 | 98.78 |
| **OIGF3** | 8.5 | 7.12 | 185 | 6545 | 7.45 | 98.45 |
| **OIGF4** | 7.4 | 6.87 | 172 | 6285 | 7.85 | 99.12 |

**Table 3: *In-vitro* drug release data for OIGF4**

| Time (h) | Square Root of Time(h)$^{1/2}$ | Log Time | Cumulative* % Drug Release | Log Cumulative % Drug Release | Cumulative % Drug Remaining | Log Cumulative % Drug Remaining |
|---|---|---|---|---|---|---|
| 0.5 | 0.707 | -0.301 | 6.65 | 0.823 | 93.35 | 1.970 |
| 1 | 1.000 | 0.000 | 8.95 | 0.952 | 91.05 | 1.959 |
| 2 | 1.414 | 0.301 | 15.65 | 1.195 | 84.35 | 1.926 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 1.732 | 0.477 | 26.65 | 1.426 | 73.35 | 1.865 |
| 4 | 2.000 | 0.602 | 36.69 | 1.565 | 63.31 | 1.801 |
| 6 | 2.449 | 0.778 | 48.85 | 1.689 | 51.15 | 1.709 |
| 8 | 2.828 | 0.903 | 56.65 | 1.753 | 43.35 | 1.637 |
| 10 | 3.162 | 1.000 | 68.87 | 1.838 | 31.13 | 1.493 |
| 12 | 3.464 | 1.079 | 75.65 | 1.879 | 24.35 | 1.386 |
| 16 | 4.000 | 1.204 | 80.95 | 1.908 | 19.05 | 1.280 |
| 20 | 4.472 | 1.301 | 85.65 | 1.933 | 14.35 | 1.157 |
| 24 | 4.899 | 1.380 | 92.25 | 1.965 | 7.75 | 0.889 |
| 48 | 6.928 | 1.681 | 96.65 | 1.985 | 3.35 | 0.525 |
| 72 | 8.485 | 1.857 | 99.78 | 1.999 | 0.22 | -0.658 |

*Average of three readings

Comment [D6]: is it possible to give mean ±SD since its done in triplicate?

**Table 4: Regression analysis data of invasomes loaded hydrogel formulation**

| Batch | Zero Order | First Order | Higuchi's Model | Korsmeyers Peppas Equation |
|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | $R^2$ |
| **OIGF4** | 0.573 | 0.975 | 0.810 | 0.907 |

**Table 5: Effect of storage temperature on the % entrapment efficiency, viscosity of drug loaded hydrogel formulation OIGF4**

| Time (Days) | Entrapment efficiency (%) | | Viscosity (cps) | |
|---|---|---|---|---|
| | 4.0 ± 0.5°C | 28 ± 0.5°C | 4.0 ± 0.5°C | 28 ± 0.5°C |
| 0 | 74.45 | 73.25 | 6280 | 6145 |
| 15 | 74.12 | 68.85 | 6245 | 6078 |
| 30 | 73.15 | 65.74 | 6215 | 5895 |
| 60 | 73.05 | 63.12 | 6185 | 5756 |
| 90 | 72.45 | 60.74 | 6175 | 5745 |

*Average of 03 readings

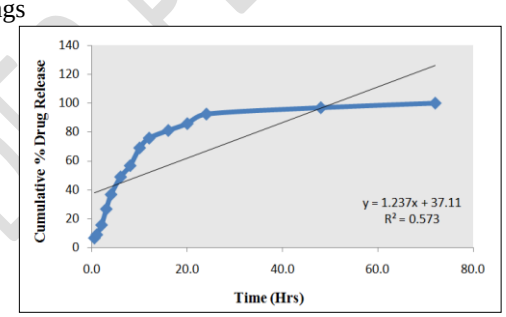


**Figure 1**Cumulative %t drug released Vs Time

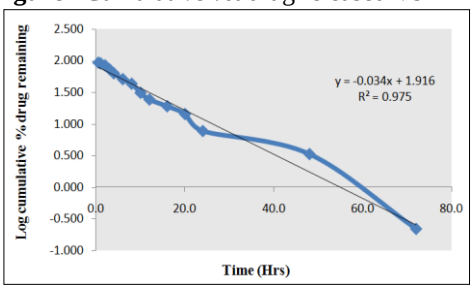


**Figure 2** Log cumulative % drug remaining Vs Time

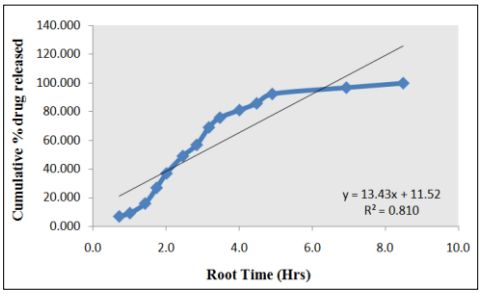


**Figure 3** Cumulative % drug released Vs Square root of Time

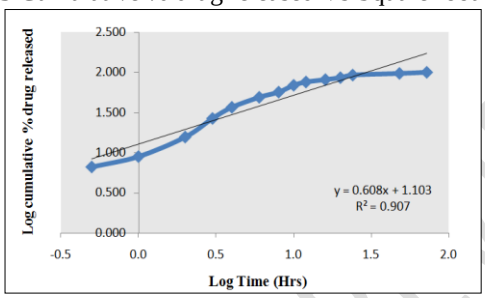


**Figure 4** Log cumulative % drug released Vs log Time

**Conclusion**

ITZ loaded invasomes formulation was successfully prepared by conventional thin layer evaporation technique using phospholipon 90H, terpene (limonene), ethanol and invasomes hydrogel based formulations were prepared with hydrophilic polymer carbopol 934p. It can serve as a useful vehicle for the delivery of ITZ through the affected part of the skin for extended period of time. This study also revealed that invasomes hydrogel (OIGF4) resides at targeted site for a relatively longer period of time with a first order model of drug release kinetics profile. It signifies the improved patient compliance.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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