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FORMULATION AND *IN – VITRO* CHARACTERISATION OF NAIL LACQUER CONATINING FLUCONAZOLE FOR PREUNGUAL DRUG DELIVERY SYSTEM.

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Abstract: Topical route administration for treatment of fungal infections is more effective route as compared to the oral route in treatment of fungal infection of nail the topical therapy is preferred route as it avoids the hepatotoxicity related to anti fungal drugs when taken orally. The purpose of present study was to formulate Fluconazole nail lacquer containing two different penetration enhancers and different concentrations of polymer for treatment of Onychomycosis and to find out which polymer concentration and concentration of penetration enhancers gave better release as well as to carry out the anti fungal testing on the best formulation obtained. The *in – vitro* diffusion studies was carried in using Franz diffusion cell using phosphate buffer pH 7.4 as medium. Whereas the permeation studies were carried by using hooves membrane. The percentage cumulative drug released was determined by UV spectrophotometer. The formulation containing 10%w/v of ethyl cellulose along with 2.5%v/v Thioglycolic acid and 2.5%v/v of Dimethyl sulfoxides showed highest release. The sensitivity of Fluconazole nail lacquer against *Candida albicans* determined by measuring zone of inhibition by comparing with standard drug. The formulation showed release by zero order and Higuchis model for mechanism of release.

Keywords: Nail lacquer, onychomycosis, Fluconazole, Thioglycolic acid, dimethyl sulfoxide, preungual drug delivery.

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INTRODUCTION

The major constrains of the preungual drug delivery (drug delivery through the nail) to nail is lack of understanding about barrier property related to the nail and formulations. Topical drug delivery system owes many advantages in case of anti fungal drugs such as it avoids hepatotoxicity, high tissue concentration which is required for the treatment of fungal infection of nails. Most of topical formulations in form of gels, lotions etc pose limitations such as removal by whipping, rubbing and less adherence of formulation to the affected site of nail.[1]

Conventional nail lacquers are mostly used mainly for the cosmetic purpose. Nail drug delivery can be made as effective route for the treatment of fungal infections of nails. Human nail is a complex structure. It protects the nail bed and the parts which are under the nail plate filled with blood vessels. Medicated nail lacquer is an excellent alternative for the treatment of fungal infection of nails and high efficacy of drug can be achieved. It also provides a optimized and sustained release of drug by formation of an occlusive film which acts as "depot" after the application of lacquer on the nail.[2]

Fluconazole is a broad spectrum anti fungal drug. It is a Triazoles derivative, its chemical formula is 2-(2,4-difluorophenyl)-1,3-*bis*(1*H*-1,2,4-triazol-1-yl)propan-2-ol. Fluconazole acts by inhibiting 14 α- demethylase, a cytochrome P450 enzyme which covert lanosterol into ergosterol. Ergosterol is an essential component of fungal cell membrane, inhibition of it causes increase in the cellular permeability and causes leakage of cellular components.[3 & 4]

The present work investigated the amount of Fluconazole released from different formulations containing different concentration of ethyl cellulose and different proportions of Thioglycolic acid and dimethyl sulfoxides for treatment of onychomycosis. The best formulation was evaluated for anti fungal sensitivity test against the *Candida albicans.* Kinetics release studies as well as stability studies were carried out on the best formulation for evaluation of kinetic model for release of drug through the formulation and to check the stability of formulation.

MATERIALS AND METHODS:

Fluconazole, ethyl cellulose, glycerine was purchased from Yarrow chemical, Mumbai, India. Propylene glycol was obtained from Lobo chemicals, Mumbai, India. Thioglycolic acid, Dimethyl sulfoxide and ethanol was purchased from Hi- media, Mumbai, India.

Fluconazole nail lacquer (1% nail lacquer) was prepared by simple mixing method. Wherein the Fluconazole concentration (1gm) was kept constant. 15 formulations were prepared and given in table 1. Formulations F1, F2, F3, F4 and F5 contained 10%w/v of ethyl cellulose along with the different concentrations of Thioglycolic acid and dimethyl sulfoxide (1%v/v to 2.5%v/v),

whereas formulations F6, F7, F8, F9 and F10 contained 11%w/v of ethyl cellulose with different concentrations of Thioglycolic acid and dimethyl sulfoxide (1%v/v to 2.5%w/v).

Preformulation of studies of Fluconazole:

Spectrum Measurement: The standard solution of Fluconazole was prepared by dissolving 100mg in 100ml of phosphate buffer pH 7.4, further diluted to get 100μg and was scanned between 400-200nm in UV-Visible spectrophotometer (Jasco V-630 UV/Visible spectrophotometer), to obtain λ max.[5]

Construction of calibration curve: A stock solution of Fluconazole was prepared by dissolving 100mg in 100ml of phosphate buffer pH 7.4. From this stock solution, suitable dilutions were prepared using the same solvent in the range of 10, 20, 30, 40, 50, 60, 70 and 80μg/ml. At λ max, the absorbance of all the concentration solutions was measured against phosphate buffer pH 7.4 as blank. Standard curve between concentration and absorbance was plotted and intercept (B) and slope (K) values were noted.[5]

Drug excipients compatibility studies: FTIR can be used to investigate and predict any physiochemical interaction between different excipients. IR spectra matching approach was used for detection of any possible chemical interaction between the drug and polymer. A physical mixture of drug, polymer and other excipients were prepared and mixed with suitable quantity of potassium bromide. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer (F.T.I.R, Shimadzu). The IR spectrum of the physical mixture was compared with those of pure drug and polymer and peak matching was done to detect any appearance or disappearance of peaks.[5]

Evaluation of nail lacquer: [6]

Drug content: Drug content of nail lacquer was determined by dissolving accurately 1ml of nail lacquer in ethanol. After suitable dilution absorbance was recorded by using UV- visible spectrophotometer (UV – 1700, Shimadzu, Japan) at 260 nm. Drug content was determined using slope of standard curve.

Non volatile content: 1gm of sample was taken in a glass Petri dish of about 8cm in diameter. Samples were spread equally. The dish was placed in the oven at 105° C for 1hr the Petri dish was removed, cooled, and weighed. The difference in weight of sample after drying was determined that gives the volatile content present. The amount of volatile content was then subtracted from 1gm weight of nail lacquer

Drying time: A film of sample was applied on a glass Petri dish with the help of brush. The time to form a dry to touch film was noted using a stopwatch.

Smoothness of flow: The sample was poured on a glass slide on an area of 1.5 square inches and spread on a glass plate by making glass slide to rise vertically. And smoothness of flow was determined by comparing with standard marketed nail lacquer.

Gloss: Gloss of the film was visually seen, comparing it with a standard marketed nail lacquer

Water resistance: This is the measure of the resistance towards water permeability of the film. This was done by applying a continuous film on a surface and drying then immersing it in water. The weight before and after immersion was noted and increase in weight was calculated. Higher the increases in weight lower the water resistance.

Diffusion studies across artificial membrane: Diffusion studies were performed using artificial membrane (cellophane).The membrane was soaked for 1hr in solvent system (phosphate buffer, pH 7.4), and the receptor compartment was filled with solvent. Test vehicle equivalent to 10mg was applied evenly on the surface of the membrane. The prepared membrane was mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly was maintained at 37°C, and the speed of stirring was kept constant (600 rpm) for 10hrs. The 5ml aliquot of drug sample was taken after a time interval of 1h and was replaced by the fresh solvent. Each experiment was replicated at least thrice. The drug analysis was done using double-beam UV spectrophotometer (U.V.1700 Shimadzu Corporation).

In vitro **transungual permeation studies:** In Hooves from freshly slaughtered cattle, free of adhering connective and cartilaginous tissue, were soaked in distilled water for 24 h. Membranes of about 1-mm thickness were then cut from the distal part of hooves. In vitro permeation studies were carried out by using Franz diffusion cell (respective volume, 100 ml), the hoof membrane was placed carefully on the cell, and the surface area available for permeation was 1.4 cm². Then the test vehicle equivalent 10mg was applied evenly on the surface of the nail membrane. The receptor compartment was filled with solvent A (phosphate buffer, pH 7.4), and the whole assembly was maintained at 37°C with constant stirring for 30 h. The 5 ml aliquot of drug sample was taken after a time interval of 2 h and was replaced by the fresh solvent A. Each experiment was replicated at least thrice. The drug analysis was done by using double-beam UV spectrophotometer (Jasco Corporation, Japan).

Determination of zone of inhibition: Antifungal activity was checked by cup plate method. In this method a previously liquefied molten sabouraud dextrose agar media was inoculated with 0.2 ml of fungal suspension of *Candida albican* having a uniform turbidity at temperature of 4 to 8°C. 20 ml of culture medium was poured into the sterile petri dish having an internal diameter of8.5 cm. Care was taken for the uniform thickness of the layer of medium in different plates. After complete solidification of liquefied inoculated medium, the wells were made aseptically with cork borer having 6mm diameter. In one plate formulation (nail lacquer) and in

another plate pure drug solution was placed carefully. Plates were kept for pre diffusion for 30 mins. After it normalized to room temperature; the plates were incubated at 22-27°C for 72hrs. After incubation period was over, the zone of inhibition was measured with help of scale.[5]

Stability studies: According to ICH guidelines at $40 \pm 2°C/75 \pm 5\%$ RH sample was stored in stability chamber for one month. The sample was evaluated for non volatile content, drying time, gloss, and smoothness of flow, water resistance and diffusion across artificial membrane.

Kinetic release studies[7, 8]

For determination of drug release kinetics from the buccal tablet, the *in- vitro* release data were analysed by zero order, first order, Higuchi and Kosmeyers and Peppas equations.

Zero order release Kinetic: To study the zero order release kinetics the release data was fitted into the following equation.

$dQ/dt = K_o$

Where 'Q' is the amount of drug release, 'K_o' is the zero order release rate constant and 't' is the release time. The graph is plotted percentage cumulative drug release (%CDR) verses time.

First order Release Kinetic: To study the first order release kinetics the release rate data are fitted into the following equation.

$dQ/dt = K_1 Q$

Where, 'Q' is the fraction of drug release, 'K_1' is the first order release rate constant and 't' is the release time. The graph is plotted log %CDR remaining verses time.

Higuchi Release Model: To study the Higuchi release model the release rate data are fitted into the following equation.

$$Q = K_H t^{1/2}$$

Where, 'Q' is the fraction of drug release, 'K_H' is the release rate constant and 't' is the release time. The graph plotted %CDR verses square root of time.

Kosmeyers and Peppas Kinetics: To study Kosmeyers and Peppas release kinetics the release rate data are fitted into following equation:

$Mt/M^{\infty} = K_{KP} t^n$

Where, M_t/M^∞ is the 'fraction of drug release, 'K_{KP}' is the release rate constant and 't' is the release time and 'n' is the diffusion exponent related to mechanism of drug release. The graph is plotted log %CDR verses time.

RESULTS AND DISCUSSION:

In spectra measurement of Fluconazole λ max was found to be 260 nm (figure 1). The calibration curve of Fluconazole was obtained in range of 10 – 100 µg/ml at the wavelength of 260 nm using phosphate buffer pH 7.4 as medium. It has shown good linearity with a regression coefficient of 0.998(r^2 value) (figure 2). All the characteristic IR peaks related to pure drug, Fluconazole also appeared in the IR spectrum of mixture of Fluconazole with ethyl cellulose, there was no chemical incompatibility between the drug and polymers (figure 3 and figure 4).

Smoothness of flow for formulations F1, F2, F3, F4 and F5 was found to be good whereas for formulations F6, F7, F8, F9 and F10 was showed satisfactory flow property compared to marketed product. Gloss of nail lacquer was evaluated by comparing with the marketed product. It was found to be satisfactory when compared to marketed product.

Non – volatile content results for formulation F1 to F10 is given in table 2. It was seen that as the polymer concentration increases from 10%w/v to 11%w/v the non – volatile content increases. The formulation which had higher concentration of polymer showed higher non – volatile content as the amount of polymer present in the sample for determination of non – volatile content was more as compared to the formulation which contained lower concentrations of polymer. Non – volatile content depends and vary upon the concentration of polymer used. Drying time for formulations F1 to F10 was found between 64 to 74 seconds. It was found that as the polymer concentration increases from 10%w/v to 11%w/v the drying time increases respectively. The time required for the solvent to evaporate from the more viscous solution is more than the less viscous solution.

From the water resistance test, it can be seen as the polymer concentration increases the water resistance increases, as the concentration of polymer decreases the water resistance decreases. Lower the increase in the weight of the nail lacquer film higher is the water resistance capacity (table 3). Formulations F1, F2, F3, F4 and F5 showed lower water resistance as compared to F6, F7, F8, F9 and F10. The drug content for formulations F1 to F10 was found to be in range of 99.89% to 98.70%.

for *in – vitro* diffusion studies for formulation F1 to F10 it can be seen that formulation F4 containing lowest concentration of polymer i.e. 10%w/v and highest concentration of penetration enhancers i.e. 2.50%v/v of Thioglycolic acid and 2.50 %v/v of DMSO showed the highest release of 95.55%. Whereas the formulation F6 containing the highest concentration of

polymer i.e. 11%w/v and lowest concentration of penetration enhancers i.e. 1.00%v/v of Thioglycolic acid and 1.75%v/v of DMSO showed the most sustained release of 85.90% at the end of 10 hours. The percentage cumulative drug released for all 10 formulations ranged between 95.55% to 85.90% (figure 6 and figure 7). It was found that as the polymer concentration decreases and penetration enhancers concentration increases the release of the drug increases. With decrease in the concentration of polymer more sustained release is obtained.

In *in – vitro* permeation studies it was found that formulation F4 showed release of 99.52% at the end of 22 hours, the release data of formulation F1 to F10 is shown in figure 8 and figure 9. From *in – vitro* diffusion studies and *in – vitro* permeation studies it was found that Thioglycolic acid was proved to a better penetration enhancer as compared to dimethyl sulfoxide. The effect of Thioglycolic acid was attributed to its small molecular weight and damage caused on the keratin network and decrease in lipid content in the dorsal nail layer; this act which loosened the nail structure, allowing Fluconazole to penetrate easier.

Form the data obtained by evaluation of nail lacquer, formulation F4 was found to be best formulation among all the 10 formulations.

The zone of inhibition for pure drug was found to be 26mm and for best formulation 25.4mm (figure 8); it was found that best formulation F4 was effective as pure drug as the zone of inhibition of best formulation is closer to that of zone of inhibition for pure drug.

Kinetics release studies revealed that formulations followed zero order release, as the regression value (r^2) is higher as compared to the first order release. Formulations followed Higuchis model for the release mechanism as the regression value is higher as compared to that of Kosmeyers - peppas model. Zero order release kinetics for optimized formulations says that the release of drug is independent of concentration of drug whereas Higuchis model signifies that drug diffusion takes place from matrix system. Regression values are given in table 4.

The stability study data indicated that the medicated nail lacquer, showed good stability for 6 months when it was stored at temperature of 40±2°C / 75±5% RH.

CONCLUSION:

FTIR studies revealed that there is no chemical interaction between the drug and polymer used. The prepared formulations were subjected to different evaluation parameters such as drying time, non – volatile content, water resistance, smoothness of flow, evaluation of gloss, drug content, *in – vitro* diffusion studies, *in – vitro* permeation studies, anti fungal testing, drug release kinetic studies. From the evaluation data it was found that F4 formulation (10%w/v ethyl cellulose, 2.5%v/v Thioglycolic acid, 2.5% dimethyl sulfoxide) was best formulation. It was

found that as the polymer concentration decreases and penetration enhancers concentration increases, percentage drug released also increases. From the *in – vitro* studies it was concluded that Thioglycolic acid was a better penetration enhancer as compared to DMSO. Drug release kinetics revealed that the release from formulations was by zero order and mechanism of release was by Higuchis model. Short-term stability studies of optimized formulations indicate that there were no significant changes in the drying time, drug content and percentage drug release values after 60 days of storage at 40±2 °C with 75±5% RH.
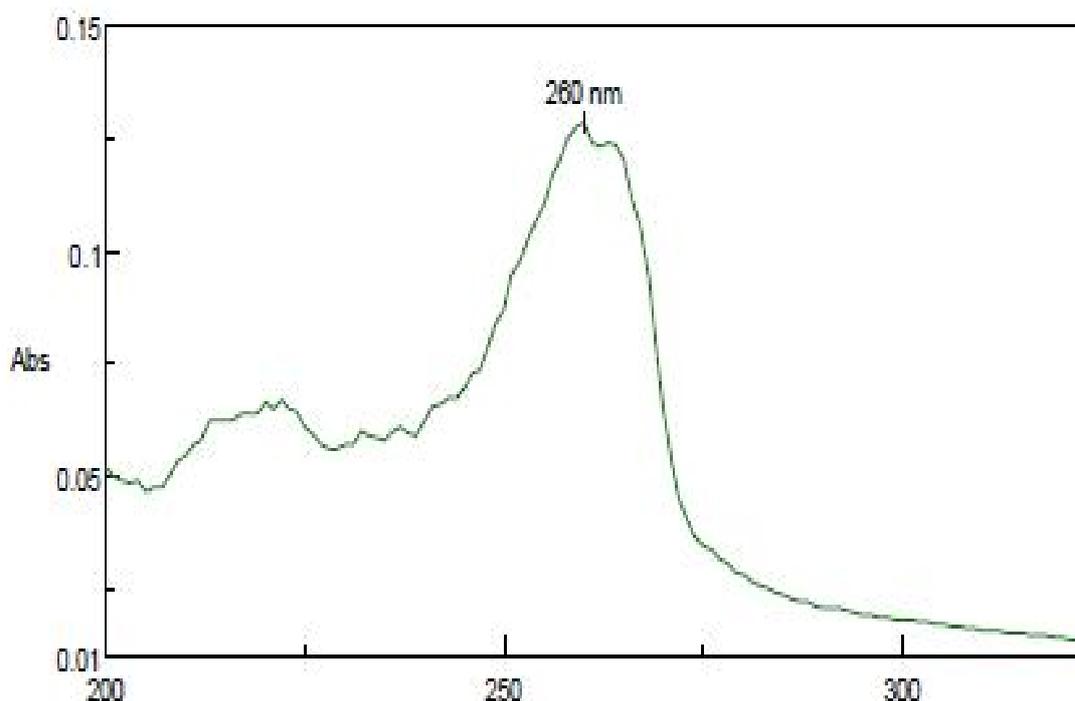

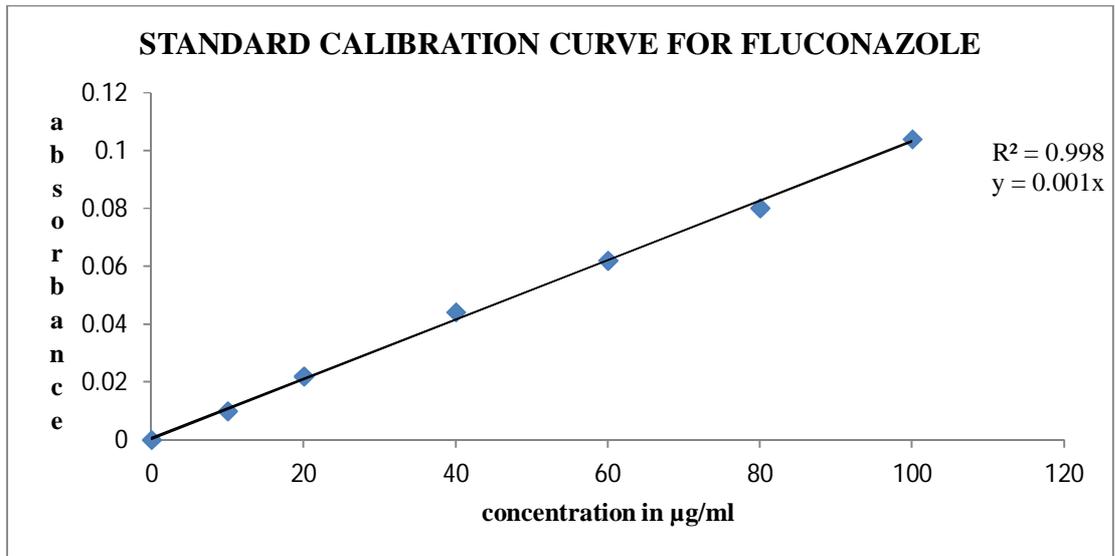**Figure 1 UV spectrum of Fluconazole in phosphate buffer pH 7.4**
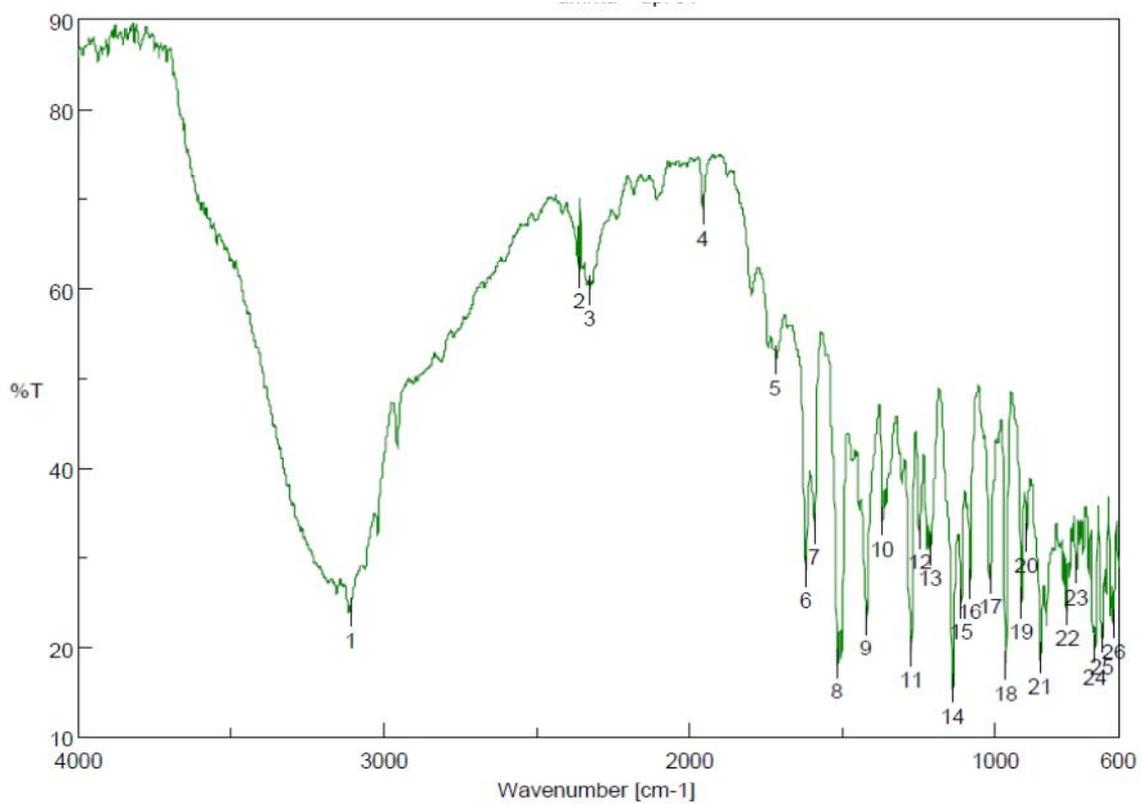
Figure 2 Standard calibration curve for Fluconazole
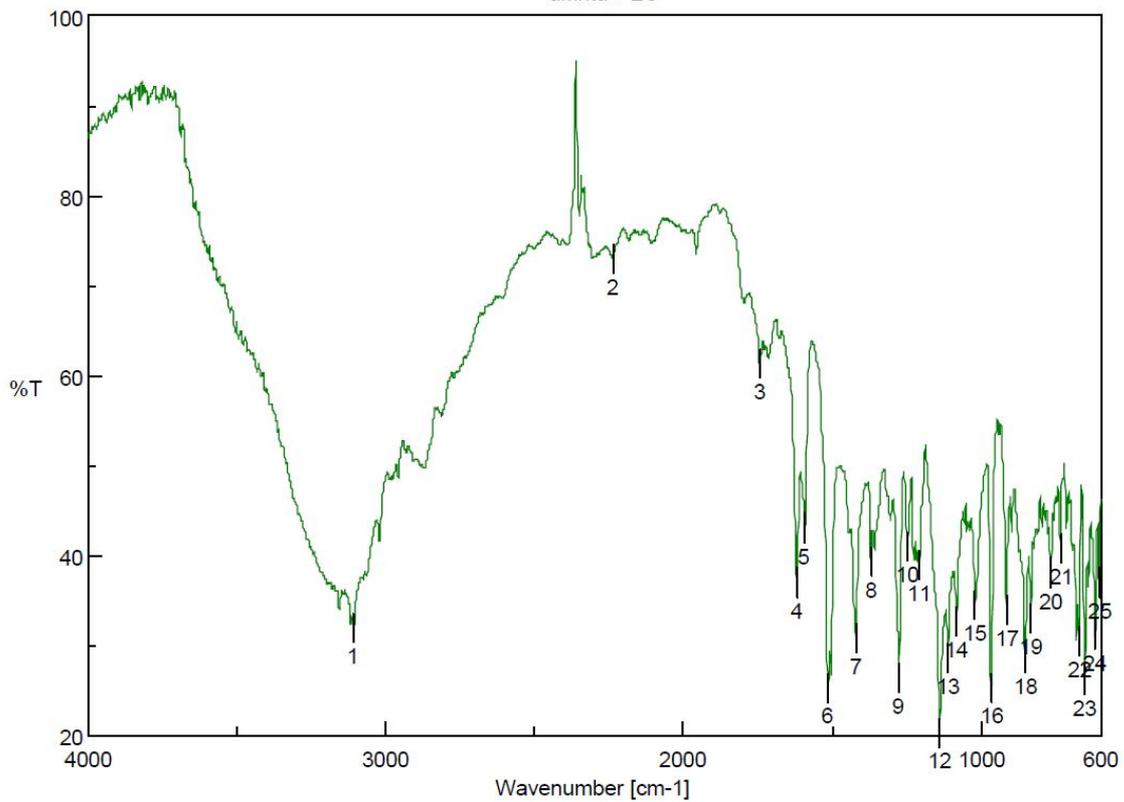

Figure 3 FTIR spectra of Fluconazole (drug)

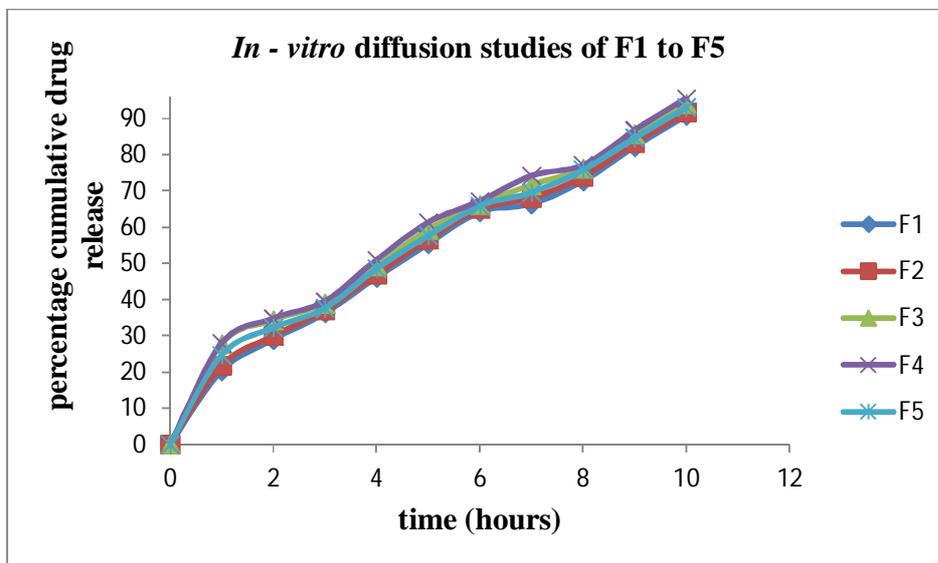**Figure 4 FTIR spectra of Fluconazole + Ethyl cellulose (drug + polymer)**

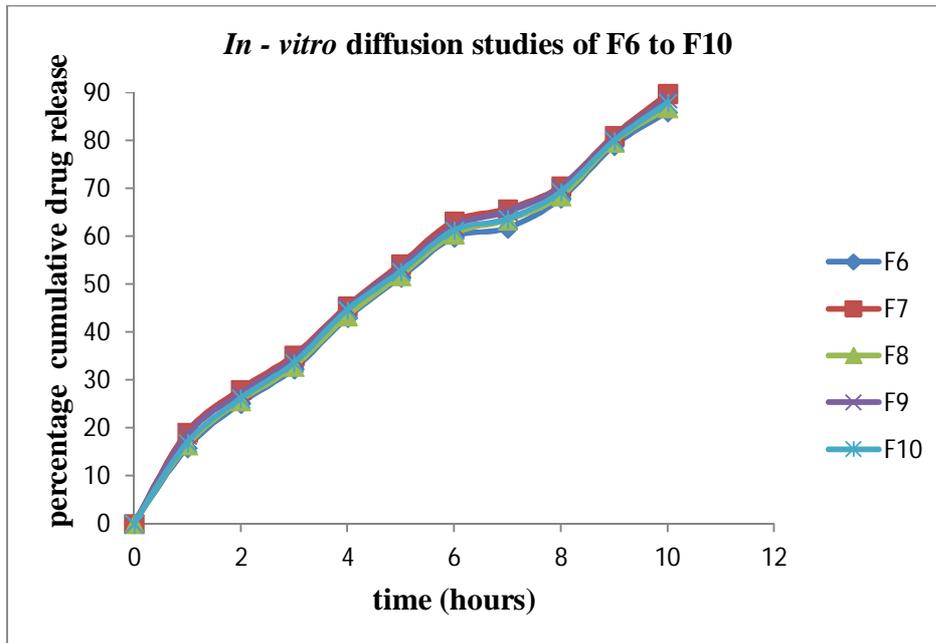**Figure 5 *in – vitro* diffusion studies of formulations F1 to F5**

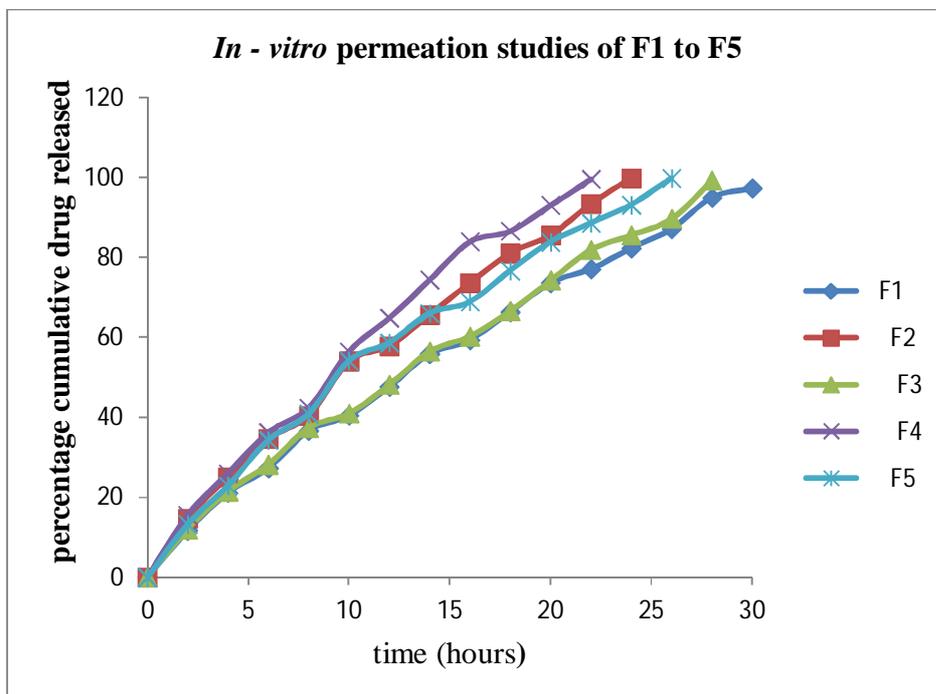**Figure 6** *in – vitro* diffusion studies of formulations F6 to F10

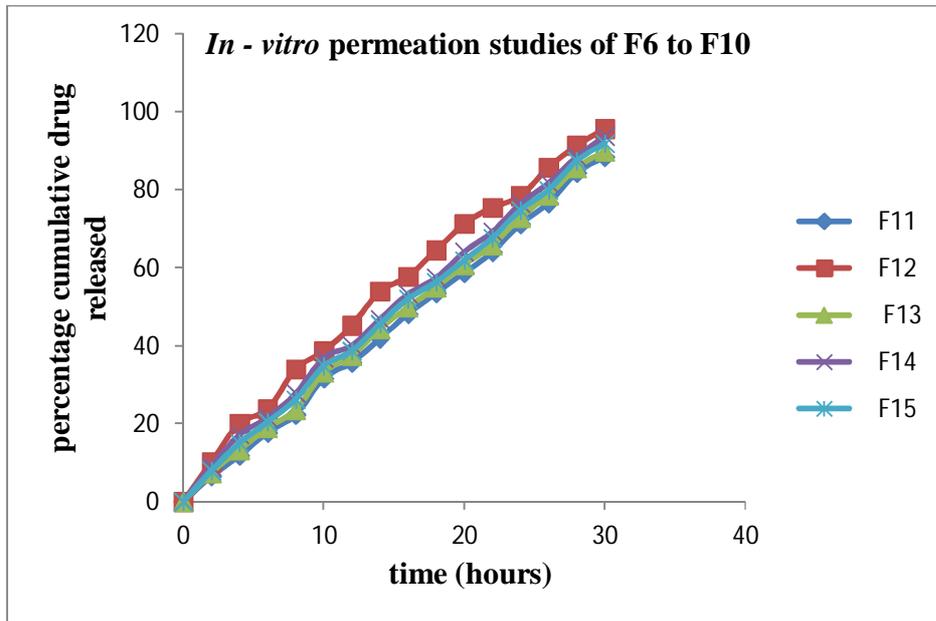**Figure 7** *in – vitro* permeation studies of formulations F1 to F5

Figure 7 *in – vitro* permeation studies of formulations F6 to F10

Figure 8 Zone of inhibition for anti fungal activity

Table1 Formulation details of Nail lacquer containing Fluconazole.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Fluconazole (gms)	1	1	1	1	1	1	1	1	1	1
Ethyl cellulose (gms)	10	10	10	10	10	11	11	11	11	11
Propylene glycol (ml)	10	10	10	10	10	10	10	10	10	10
Glycerine (ml)	10	10	10	10	10	10	10	10	10	10
DMSO (ml)	1	2.5	1	2.5	1.75	1	2.5	1.75	1.75	1.75
Thioglycolic acid (ml)	1	1	2.5	2.5	1.75	1.75	1.75	1	2.5	1.75
Ethanol (ml)	100	100	100	100	100	100	100	100	100	100

Table 2 Non – volatile content of Nail Lacquer

Formulation code	Non – volatile content (%)*	Formulation code	Non – volatile content (%)*
F1	21.3±0.57	F11	25.0±0.50
F2	20.6±0.50	F12	24.4±0.55
F3	20.6±0.76	F13	24.1±0.32
F4	20.3±0.57	F14	23.8±0.26
F5	20.3±0.20	F15	24.8±0.76

*Average of three trials (n=3)

Table 3 Water resistance test for Nail lacquer.

Formulation code	W_1 (g)	W_2 (g)	Difference in weight (g)	Formulation code	W_1 (g)	W_2 (g)	Difference in Weight (g)
F1	7.00	7.24	0.24	F11	7.00	7.16	0.16
F2	7.00	7.22	0.22	F12	7.00	7.16	0.16
F3	7.00	7.22	0.22	F13	7.00	7.15	0.15
F4	7.00	7.22	0.22	F14	7.00	7.15	0.15
F5	7.00	7.22	0.22	F15	7.00	7.14	0.14

W_1 & W_2 are weight of glass slide along with nail lacquer before and after dipping   in water respectively.

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Table 4 Regression analysis (r^2) of *in-vitro* drug release data based on best curve fitting method.

Formulation code	Zero order	First order	Higuchi model	Best fit release mechanism
	r^2	r^2	r^2	
F1	0.9358	0.8712	0.9669	Higuchis
F2	0.9343	0.8690	0.9494	Higuchis
F3	0.9365	0.8757	0.9652	Higuchis
F4	0.9076	0.8441	0.9925	Higuchis
F5	0.9301	0.8316	0.9873	Higuchis
F6	0.9210	0.9195	0.9820	Higuchis
F7	0.9158	0.8872	0.9793	Higuchis
F8	0.9217	0.8345	0.9755	Higuchis
F9	0.9201	0.8604	0.9731	Higuchis
F10	0.9222	0.9213	0.9712	Higuchis

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