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DENDRIGEL: PREPARATION, OPTIMIZATION AND CHARACTERIZATION

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ABSTRACT

The preparation of dendrigel, using dendrimers as drug carrier. Dendrimers were prepared by divergent method and was loaded in varying proportion in carbapol 940. To this mixture itraconazole drug was added and the formulation of dendrigel was evaluated. Dendrimers was prepared by divergent growth method. Evaluation parameter such as colour test, UV spectroscopy, FT-IR. Evaluation parameters for dendrigel were pH, spreadability, viscosity, drug content, antifungal activity and in vitro release studies. The prepared dendrigel having better antifungal activities than other conventional delivery system because dendrimers were enhance the solubility of drug which results in increase in penetration of drug in the skin.In the present study attempt was made to develop dendrimers containing itraconazole as a drug so as to increase the solubility and penetration of the drug through the skin using novel delivery system for better antifungal activity.This will help to increase the solubility and penetration of drug. It increases the antifungal activity of formulation.

KEYWORDS: PAMAM, dendrimer, dendrigel, nanoparticles, FT-IR, UV spectroscopy.

1 INTRODUCTION

Mycosis is a common fungal infection affecting human skin and causes different dermatophyte infections. Fungal infection of skin can be treated with topical creams, gels as well as prescription drugs. There are three types of fungal infections, i.e. superficial, cutaneous and Systemic fungal infection[1, 2]. Fungal infection is a common infection which affects the maximum of population among the world[3]. Many antifungal drugs are available for treatment of fungal infection like Miconazole, Ketoconazole etc. Itraconazole is topically

active antifungal agent with a broad spectrum of activity and effective against several fungal strains such as Candida topicalis, C. albicans which are responsible for topical candidiasis[4].

Itraconazole1-(butan-2-yl)-4-{4-[4-(4-{[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]phenyl}-4,5dihydro-1H-1,2,4-triazol-5-one is an azole-type antifungal agent (Fig. 1) that is known to have topical activity against pathogenic dermatophytes and yeasts[5,6]. Itraconazole having very low water solubility and having log P value 6.5 which indicate high permeability through membrane and it is beneficial for topical delivery. General side effects can be overcome by its topical delivery. For topical delivery semisolid preparations are widely established over liquid and solid dosage forms[7].

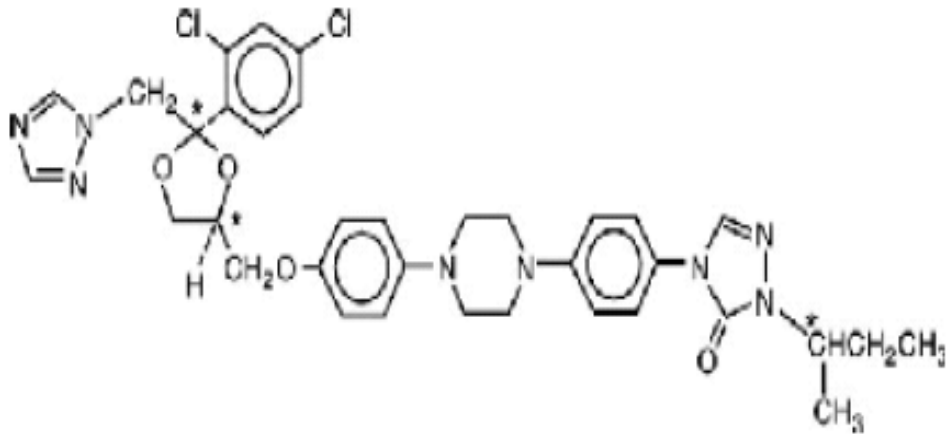


Figure 1: Chemical Structure of Itraconazole

PAMAM dendrimers ideal carriers for enhanced solubility of poorly water-soluble drugs[8]. The dendrimers has received much consideration for their ability to solubilise water insoluble drugs and their ability to promote the transport of drugs across biomembranes[9-11]. The dendrimers of poly (amidoamine) (PAMAM) are nanoparticles which have proven succeed in transporting the drugs due to their high solubility, low toxicity and ability to control drugs release. PAMAM interact with many drugs and plays a vital role as drug nanocarriers in transdermal routes of administration. Dendritic polymers comprising of dendrigrafts, dendrimers, and dendrons, are providing new directions in nanomaterial based drug delivery due to they remain highly branched, mono disperse macromolecules. These unique features have made their application in nanotechnology, pharmaceutical and medical chemistry. As a significance of their exclusive performance, dendrimers are appropriate for a wide range of industrial and biomedical applications[12–15]. Some of dendrimers themselves show

pharmaceutical activity (anticancer, anti-inflammatory, antimicrobial), providing the opportunity for combination therapy in which the dendrimers serve as the drug carrier and simultaneously as an active part of the therapy[16].

2 MATERIALS AND METHODS

The following materials were used: Itraconazole, Egg membrane, Methyl acrylate form Loba Chemical Pvt.Ltd.Mumbai,India., Methanol and Ethylenediamine form Loba Chemical Pvt.Ltd.Mumbai,India., Carbopol 940 from Qualikems fine chem,New delhi,India., Triethanoamine from S.D Fine chem laboratory,Ambala,India., Glycerine from Avarice laboratory reagent,Ghaziabad,India., Tween80, Benzyl alcohol from Loba Chemical Pvt.Ltd.Mumbai,India. All reagents used were of analytical grade.

Intstruments: UV/VIS spectrophotometer (UV-1800, Shimadzu Corporation, Japan), FTIR-spectrometer (Alpha, Bruker Optics, Germany), pH meter (Hanna instruments,Italy), Brookfield viscometer(Brookfield engineering laboratories, USA), Digital Weighing Machine (Ohaus Instruments, Switzerland).

Synthesis of PAMAM Dendrimer[17]

PAMAM dendrimers were synthesized by the divergent growth method. Ethylene diamine (EDA) acts as originator core starting the synthesis of dendrimer by attaching four acrylate moieties on each amino group of EDA. The resultant compound denoted to as generation -0.5 PAMAM tetra ester. Then 0G, 0.5G, 1G, 1.5G, 2G PAMAM dendrimers were synthesized by this method. 2G was used in preparation of dendrigel formulations.

Synthetic procedure

1. **Synthesis of 2G PAMAM Dendrimer: 2g** of 1.5 G + EDA (42.7ml) were dissolved in appropriate amount of methanol in amber colored round bottom flask which was corked tightly and then kept for 144 hours. Finally residual solvent is evaporated on the water bath. Prepared dendrimers are analyzed by UltraViolet Spectroscopy and Fourier Transform InfraRed.

Table 1: Theoretical details of the reactants and product for synthesis of PAMAM dendrimers

S. No.	Dendrimers generation	Theoretical Molecular weight	Free NH_2 / COO^- groups
1.	-0.5 G	405	4
2.	0.0G	517	4
3	0.5 G	1,205	8
4.	1.0G	1,430	8
5.	1.5 G	2,807	16
6.	2.0G	3,256	16

Evaluation of PAMAM dendrimers

- **Color Test**

PAMAM dendrimers and acetylated derivatives were treated with aqueous solution of copper sulphate (1%w/v) [18].

- **Ultraviolet Spectroscopy**

0.01%w/v concentration of PAMAM dendrimers was scanned in the range of 200nm to 500nm against distilled water. The changes in λ_{max} values were analysed [19-20].

- **Infra-red spectroscopy**

IR analysis, dendrimer was scanned in the wave number 4000-600 cm^{-1} by using Bruker FT-IR by ATR technique [21].

Preparation of gel base [22]

Carbopol 940 and purified water were taken in a beaker and allowed to soak for 24 hours. carbopol 940 was than neutralized with sufficient quantity of triethanolamine. Glycerine as a moistening agent and Tween 80 as a penetration enhancer and benzyl alcohol as a preservative added with continuous stirring until the homogenous gel was formed.

Formula and Method

Table no.2 formulation of gel base

S.no.	Ingredients	Quantity
1.	Carbopol 940(gm)	10
2.	Benzyl alcohol (ml)	2
3.	Tween 80(ml)	2
4.	Glycerine (ml)	20
5.	Triethanolamine (ml)	3
6.	Water(ml) q.s.	100

Preparation of dendrigel

1% of dendrimer(1g of generation 2 dendrimers were diluted to 10 ml with distilled water in order to make 1% of dendrimer) in varying proportion (1-5 ml) was mixed with 10mg itraconazole drug and kept for 24 hours to make the Dendrimer drug complex. Then it is loaded into the gel for the dendrigel formulation as per the table no. 3.

Table no. 3 Formulation Design for dendrigel

Formulation code	Dendrimer (ml)	Drug polymer (mg)	Gel base (g)
DG1	1	10	10
DG2	2	10	10
DG3	3	10	10
DG4	4	10	10
DG5	5	10	10

Evaluation of dendrigel

The prepared Itraconazole dendrigel formulations were inspected visually for their colour, homogeneity, consistency, spreadability, viscosity and pH [23].

pH: The pH values of 1% aqueous solutions of the prepared dendrigel were measured by a pH meter (Digital pH meter, Hanna instruments,Italy).

Spreadability: Spreadability is expressed in terms of time in seconds taken by two slides to slip off from dendrigel and placed in between the slides under the direction of certain load[24].

Viscosity: The viscosity of gel formulation was determined at 37ºC using a brook field viscometer (Brookfield DV-E viscometer, Brookfield engineering laboratories, USA). 6 no spindle was used and rpm set at 10.

Drug Content Determination: Itroconazole content in gel was measured by dissolving known (10g) quantity of dendrigel in solvent (water) by Sonication. Absorbance was measured after suitable dilution at 269 nm in UV/VIS spectrophotometer (UV-1800, Shimadzu Corporation, Japan)[25].

Drug Loading and In Vitro Release[26]

Franz Diffusion cell was used for the drug release studies. Dendrigel (1gm) was applied onto the surface of Egg membrane evenly. The Egg membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly

prepared Acetate buffer solutions (pH 5.5) solution to solubilize the drug. The samples were collected at suitable time intervals of 15min. Samples were analyzed for drug content by UV visible spectrophotometer at 269 nm after appropriate dilutions.

Antifungal activity of dendrigel[27]

The antifungal activity of dendrigel formulation was checked by using agar medium plates. 5g of nutrient agar powder Suspended in 200ml distilled water. Heat this mixture while stirring to fully dissolve all components. Autoclave the dissolved mixture at 121ºC for 15min. Once the nutrient agar has been autoclaved, allow it cool but not solidify. Pour nutrient agar into each plate and leave plates on sterile surface until agar has solidified. Then four wells were made in each petri plate, and in first well dendrigel, second well formulation of clotrimazole, third well gel base were filled in these wells and the remaining one well was blank. Place the plates into incubator for 7 days.

Stability study

The dendrigel formulation was kept in tightly closed glass vials. The sample were kept in dark (in amber colours vials) and light (in colourless vials) at 0°c, Room temperature (25°-30°c) and 45°c for a periods of seven weeks. The samples were analysed initially and periodically after every week for up to seven weeks for change in viscosity, pH, colour and consistency. The data obtained was used for the analysis of any physical or chemical degradation, the required storage condition and the precaution required for storage.

3 RESULTS AND DISCUSSION

Physical State and organoleptic properties of dendrimers

The dendrimers were evaluated for their physical characteristic such as appearance, viscosity and odour.

Table 4: Physical characteristic of PAMAM dendrimers generation

S. No.	Generation of Dendrimers	Colour as Concentrated	Physical state	Identification by $CuSo_4$ test
1.	2 G	Light Reddish Yellow	Very viscous oily	Violet

- **Identification of dendrimers**

The dendrimers were distinguished as half generation or full generation by reaction with freshly prepared 1% w/v aqueous solution of copper sulphate with 0.1% w/v dendrimers solution.

- **Copper Sulphate Test**

10 drops of 10% Copper sulphate [$CuSO_4$] reagent was taken in a small test tube and 2 drops of dendrimers was added to it.

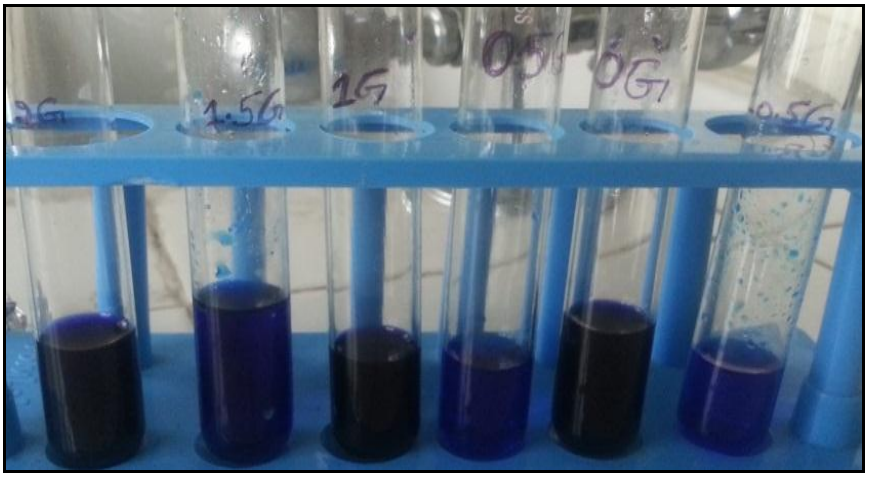


Figure 2: PAMAM dendrimers generations Copper Sulphate Test

- **Identification of Dendrimers by UV-Spectroscopy**

1. UV spectroscopy of 2 G PAMAM dendrimer

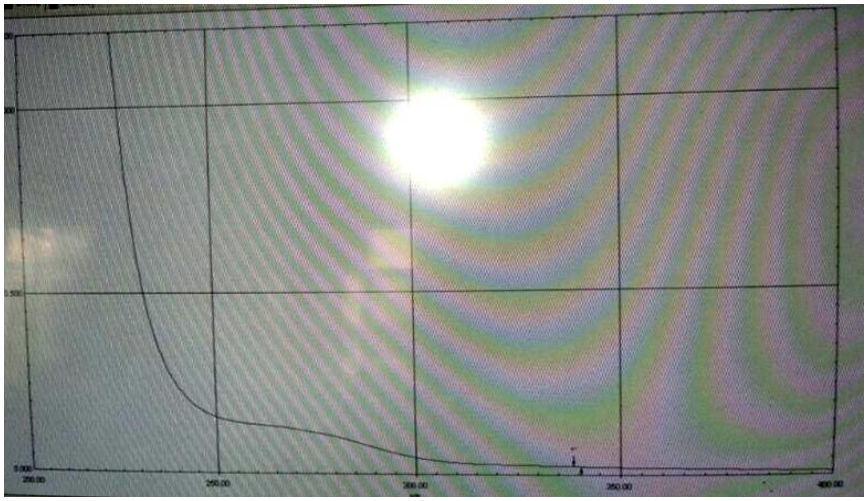


Figure 3: UV Spectra of PAMAM Dendrimers 2G

FT-IR Spectroscopy

1. FT-IR spectroscopy of 2 G

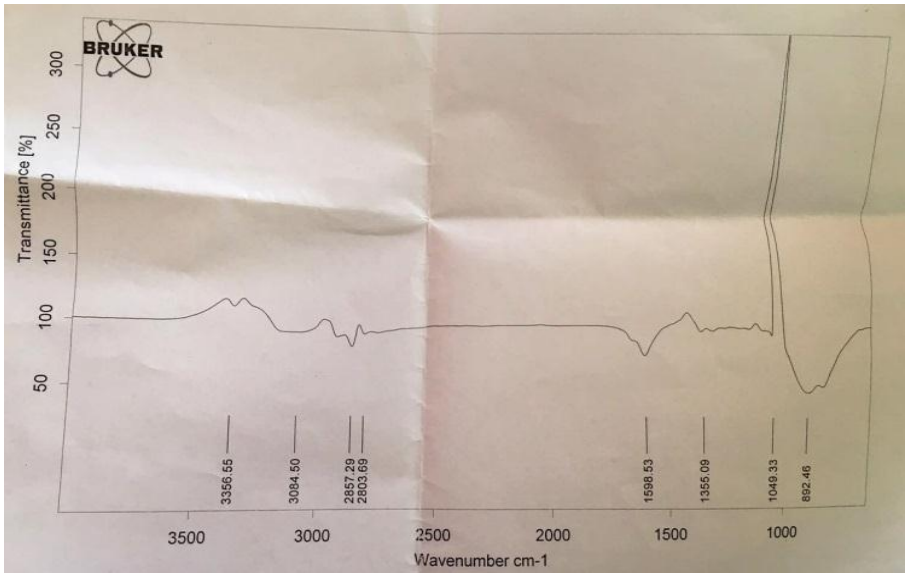


Figure 4: FT-IR spectra of PAMAM Dendrimers 2 G

Table no 5: FTIR Spectrum of 2 G PAMAM Dendrimers showing different wave number with Assignment

Sr.No	IR Absorption Band (cm-1) (Experimental)	IR Absorption Band (cm -1) (Literature)	Functional Groups
1.	3358	3366	(O-H stretching),
2.	2857	2942	Aliphatic C-H stretching
3.	1598	1575	(Aromatic C=N stretching)
4.	1043	1031	C-O stretching

Pharmaceutical evaluation of dendrigel

Various formulations DG1-DG5 was prepared using different formulation variables based on factorial design. Dendrimer in varying proportion is mixed with 10mg itraconazole drug and kept for 24 hours to make the Dendrimer drug complex. Then it is loaded into the gel for the dendrigel formulation. The result showed that the formulation DG-5 provide better release by using 5.5 pH acetate buffer and antifungal activity. Dendrigel formulation F5 of drug itraconazole was capable of exhibiting sustained release properties for a period of 90min.

Table no 6: Evaluation of formulated dendrigel

dendrigel code	Appearance	viscosity	pH	Drug content	% commulative Release
DG1	Light yellow	11235	5.6	78.56	86.67±0.49
DG2	Light yellow	8623	5.4	82.74	89.55±0.71
DG3	Light yellow	10250	5.2	87.56	91.58±0.16
DG4	Light yellow	9500	5.3	90.11	92.74±0.19
DG5	Light yellow	13200	5.5	94.30	94.55±0.81

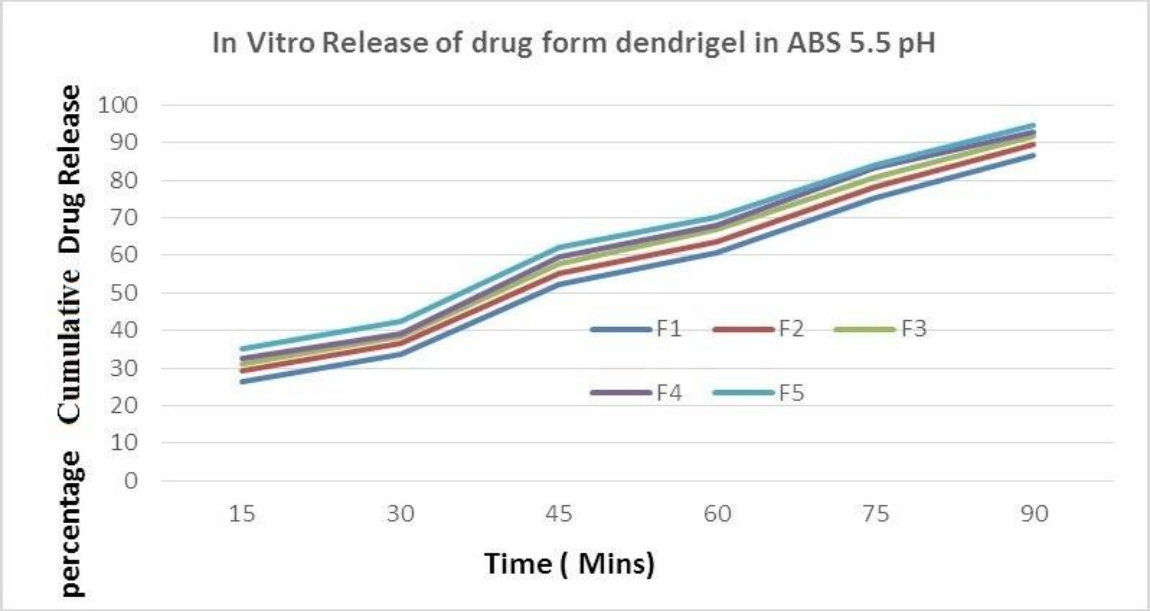
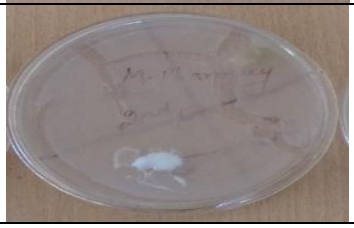
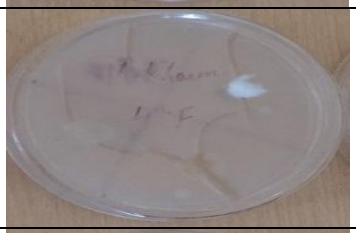


Figure 5: In *Vitro* Release of drug form dendrigel in ABS 5.5 pH

Antifungal Activity of Dendrigel

Table no 7: Antifungal activity (zone of inhibition) of itraconazole in dendrigel and gel base compared with standard drug clotrimazole

Formulation	Agar plates	dendrigel	Standard	Gel base	Blank
F1		0.9±0.81cm	0.8±0.21cm	0.7±0.08cm	0.7±0.05cm
F2		1±0.45cm	0.8±0.21cm	0.7±0.08cm	0.6±0.35cm

F3		1.1±0.36cm	0.8±0.21cm	0.6±0.04cm	0.4±0.15cm
F4		1.2±0.19cm	0.8±0.21cm	0.6±0.05cm	0.5±0.12cm
F5		1.4±0.54cm	0.8±0.21cm	0.7±0.08cm	0.5±0.12cm

Stability Studies

Table no 8: in-*vitro* release studies of F5 batch for 3 Months period.

Time (hours)	% Cumulative drug release from different batches±SD				
	% cumulative release before stability studies	% cumulative release after stability studies			
		0	1	2	3
15	35.22±0.05	35.22±0.05	35.99±0.08	36.11±0.12	36.28±0.78
30	42.44±0.19	42.44±0.19	43.21±0.23	43.32±0.54	43.51±0.32
45	62.33±0.87	62.33±0.87	63.10±0.66	63.21±0.96	63.39±0.45
60	70.05±0.54	70.05±0.54	70.82±0.85	70.93±0.75	71.11±0.15
75	83.99±0.36	83.99±0.36	84.76±0.45	84.87±0.65	85.05±0.36
90	94.55±0.21	94.55±0.21	95.32±1.03	95.43±0.98	95.61±0.87

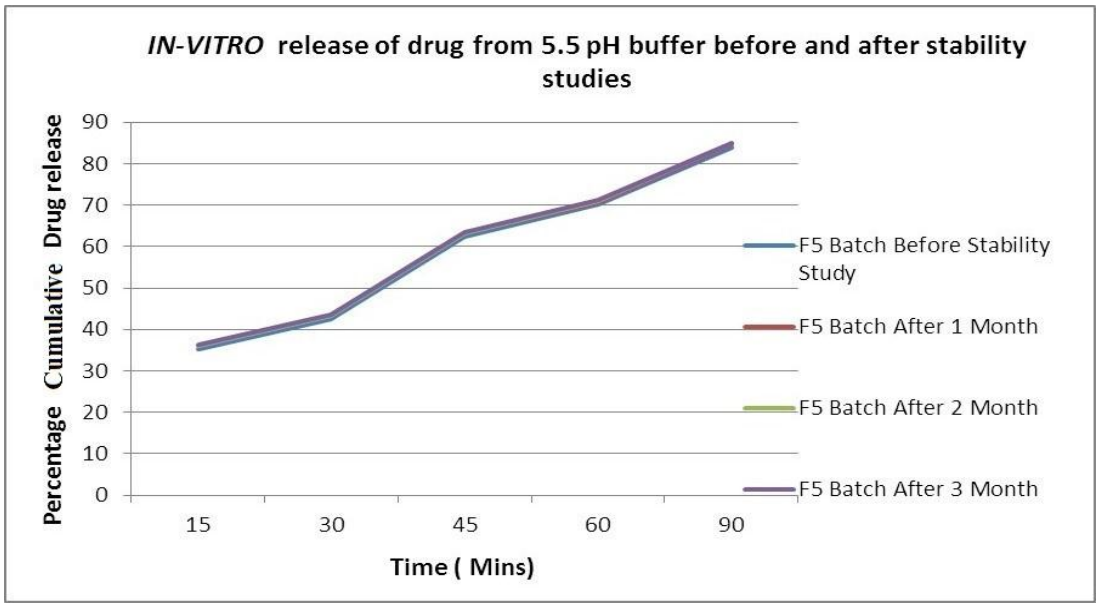


Figure 6: In-*vitro* release of drug from 5.5 pH buffer before and after stability study

DISCUSSION

Poor water dissolvability of itraconazole presents a block for the nearby accessibility of itraconazole and limits the successful antifungal treatment[28]. Topical use of the drug at the affected site offers potential preference of delivering the drug directly to the site of action. PAMAM dendrimers with uniform particle size and shape having well-known interest in biomedical applications because of their capacity to cross cell membranes. Non-polar cavities in PAMAM dendrimers in combination with their hydrophilic exterior surface make them accomplished of encapsulating hydrophobic drug molecules. These noncovalent incorporations offer a alteration of physicochemical advantages over the free drug molecules including the possibility of enhanced water solubility and drug stability. Dendrimers enhance the solvency of hydrophobes due to hydrophobic interactions, electrostatic co-operation between terminal practical gatherings of the dendrimers and hydrophobes[29-31]. The present work deals with the formulation and evaluation of Itraconazole topical dendrigel using gelling agent like carbapol 940.the dendrimers were used as drug carrier in the formulation. Optimized batch F5 shows yellow colour in appearance. pH of all five batches was found between 5 - 5.6. pH of optimized batch F5 was found 5.5 which lies in normal PH of skin. Viscosity is important parameter for characterizing the gels as it affect spreadability, extrudability and release of the drug, all the formulated batches should increase viscosity as the concentration of gelling agent increased optimized batch F5 show ideal viscosity.

All the prepared groups indicate consistency in drug content. Optimized batch F5 shows 94.3 % drug content which show uniform drug dispersion in dendrigel. In vitro realease studies were carried out by using acetate buffer PH 5.5 realease of Itraconazole from all prepared dendrigel formulations was found to be satisfactory. In optimized batch F5 drug diffusion occurs through the fluid phase and hence they offer little resistance to drug diffusion and realese.

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