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FORMULATION DEVELOPMENT AND EVALUATION OF POLY AMIDOAMINE (PAMAM) DENDRIMERS AS POTENTIAL CARRIERS FOR ENHANCED TRANSDERMAL DELIVERY OF ITRACONAZOLE GEL FORMULATION

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Keywords:

Dendrimers, Transdermal drug delivery, Dendrigel, Antifungal, Penetration Enhancer, etc

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ABSTRACT: The drug of dendrigel, uses dendrimers as medicine carriers. Dendrimers were prepared by divergent growth techniques and were loaded in varying quantities in carbapol 934. To this mixture, itraconazole drug was added and the expression of dendrigel was estimated. Dendrimers were prepared by divergent growth techniques. Estimation parameters like color test, UV spectroscopy, and FTIR. Evaluation parameters for dendrigel were pH, spreadability, thickness, drug content, antifungal exertion, and in vitro release studies. The set dendrigel has better antifungal exertion than another transdermal medicine delivery system because dendrimers enhance the drug's solubility, which increases the penetration of the drug in the skin. In the present study, an attempt was made to develop dendrimers containing itraconazole as a drug to increase the solubility and penetration of the drug through the skin using the new delivery system for better antifungal application. This will help to increase the solubility and penetration of the drug. It increases the antifungal application of the formulation.

INTRODUCTION: The word dendrimer originated from the Greek word "Dendron" which means "Tree or Branches" and "Meros" which means "Parts" i.e., Tree-like branched structure was obtained. In the year 1978 dendrimer was proposed by Sir Vogtle and a co-worker, it was the first time that an overall complete synthesis of dendrimer was obtained [1, 2]. This was followed by the independent development of the divergent macromolecule synthesis of dendrimer by Tomalia in the years 1984-1985. It was also the first time that PAMAM dendrimers were obtained completely.

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Then in the year 1990, the convergent synthesis method was proposed by Frechet. Dendrimer in drug delivery system has attracted more attention in recent years [3, 4]. A dendrimer is made of polymer containing an empty inner cavity, which is being used for drug encapsulation of hydrophobic drug molecules. The outermost shell is responsible for the reactivity and thus dendrimer is modified or conjugated by a guest molecule.

Due to these specific properties of dendrimer, it is suitable for drug delivery systems. Dendrimers are new generation highly branch polymers with radially symmetric molecules, Nano-size widely used in drug delivery systems. Dendrimers are three-dimensional, monodisperse, globular macromolecules having a high number of functional groups on the functional group on the surface. The high surface function of highly branch polymeric dendrimer enhances the solubility, stability, higher density, and lesser viscosity of

many drugs [5, 6, 7]. Dendrimer-based drug delivery systems, gene delivery, solubility enhancer, and transdermal drug delivery nanomaterials are some applications of dendrimers [8, 9].

The Structure of a Dendrimer Consists of three Different Components they are:

Central Core: The central core should contain a relative functional group

Repeated Branches: The repeated branch should be organized in a series of generation

Surface Functional Groups: The surface functional group should determine the physical properties and location of molecules [10].

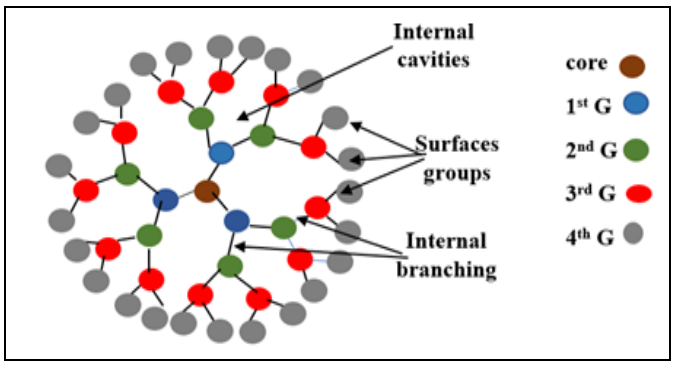


FIG. 1: STRUCTURE OF DENDRIMER

Therefore, dendrimers are a substitute class of three-dimensional regular structural macromolecules produced by color. Synthetic routes. Dendritic polymers are nanometer-sized (10m) globular type particles as shown in Dendrimers are formed by an iterative sequence of different response pathways, each fresh replication leads to a high-generation dendrimer. These unique structures can be employed as a tool for asked functions like internal voids, well-defined shapes, and variable face functionality. Dendrimers retain numerous other names similar as' starburst dendrimer', 'waterfall particles',' arborols',' molecular platforms', and' dendritic polymers' Dendrimers are a class of artificial polymeric macromolecules that play a meaningful part in arising nanotechnology. It is described as a largely fanned macromolecule, which provides a high degree of face functionality and versatility [11].

Disease: Fungal infections affect billions of people every time, ranging from common superficial mycosis to serious systemic infections. The type and severity of the infection depend on the causative agent, the portal and method of entry, the host, and the immunological status of the case. The frequency of circumstances of fungal infections is varied and is related to the degree of exposure to unproductive fungi. Other factors that have a bearing are living conditions, cultural habits, rest exertion, and geographic region. With regard to this last factor, it was noticed that infections caused by dimorphic fungi occur mainly in the Americas, whereas subcutaneous mycoses analogous to mycetoma or chromoblastomycosis occur primarily in the tropics and subtropics Superficial mycoses, analogous to skin, hair, nail and mucous membranes infections, are the most common of all fungal complications and are claimed to affect about 20- 25 of the mortal population worldwide. The superficial infections are caused mainly by Dermatophytes, which is a group of nearly affiliated mold fungi classified in the division Trichophyton, Microsporum or Epidermophyton, and Candida spp., especially. Albicans and lipophilic yeasts Malassezia spp [12-14].

These are medications applied for superficial and deep (systemic) fungal infections. Multitudinous topical antifungals have been available since the antiseptic period. Two important antibiotics *viz*. amphotericin 8- to deal with systemic mycosis and griseofulvin- to supplement suddenly dermatophytes were introduced around 1960. The antifungal property of Aucytosine was noted in 1970, but it could serve only as a companion medicament to amphotericin.

The progress of imidazoles in themid-1970s and tria = oles in the 1980s supplied safer and more accessible preferences to amphotericin B and griseofulvin. An ideal medication remedy achieves effective attention of medication at the target location for a specific time to minimize general and original side effects. To gain a remedial response, the correct quantity of cure is delivered to the point of action with a controlled input rate. The distribution of medications to other tissues seems the necessary and implicit cause of poisons. Targeted medication delivery is to receptors and organs. Effective Targeted medication delivery systems have been for a long time. Targeting of medication delivery system to the right place in the body, controlling and releasing of medication for

the prevention of overdose. The development of new particles in nanosponges has the possibility to answer the problem [15-16].

Drug: Itraconazole is an antifungal medication used to treat a number of fungal infections. This includes aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis. It may be given by mouth or intravenously. Common side effects include nausea, diarrhea, abdominal pain, rash, and headache. Severe side effects may include liver problems, heart failure, Stevens-Johnson syndrome, and allergic reactions including anaphylaxis. It is unclear if use during pregnancy or breastfeeding is safe. It is in the triazole family of medications. It stops fungal growth by affecting the cell membrane or affecting their metabolism.

Itraconazole was patented in 1978 and approved for medical use in the United States in 1992.

It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system [17].

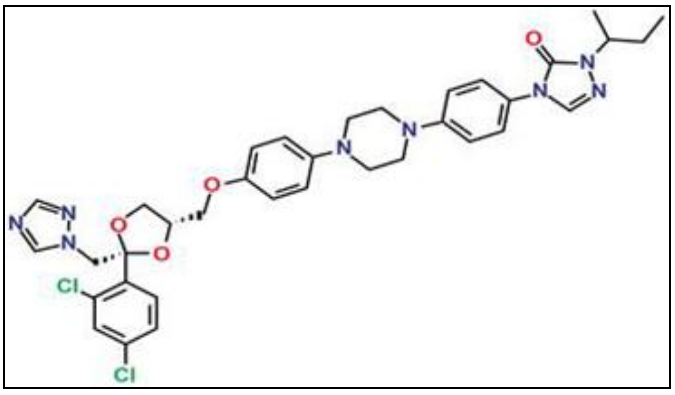


FIG. 2: CHEMICAL STRUCTURE OF ITRACONAZOLE

Itraconazole is an orally or topically active antifungal agent with a broad spectrum of activity. In addition, the drug has an interesting tissue distribution, which has made possible effective and rapid treatments of candidiasis, when the drug is administered topically [18, 16]. A topical itraconazole-containing formulation may be of use for several reasons including the opportunity to generate high local tissue levels and lower systemic exposure. Most pharmaceutical drug substances are lipophilic compounds, which are practically insoluble in water. For skincare and the topical treatment of dermatological disease, a wide choice of vehicles ranging from solids to semisolids. Itraconazole is effective against several fungal strains such as *Candida albicans* and *Candida topicalis*, which are responsible for topical candidiasis in more than 25% of patients suffering from this condition. Candida-related fungal infection is a common skin disease affecting two-thirds of all persons at least once during their lifetime Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal, and skin as topical routes. Topical formulations apply a wide spectrum of preparations,both cosmetic and dermatological, to their healthy or diseased skin [19-20].

MATERIALS AND METHOD: Itraconazole standard (API) powder was kindly purchased by PVR Life Science from India Mart Limited. Some chemicals and reagents used were obtained from the Laboratory, Institute of Pharmacy Bundelkhand University Jhansi and were of analytical grade.

Preformulation and Characterization Study: Pre-formulation is an integral part of the entire development process. These studies concentrate on the physicochemical properties of the medicament that could affect its performance and the development of an efficient dose form. A detailed understanding of these properties may eventually explain formulation design, or support the need for molecular remaking. Pre-formulation studies are mandatory prior to formulation development. It helps in determining the physiochemical properties of the drug to cause the process of formulation development, important parameters to be evaluated during pre-formulation studies include physical appearance, melting point, IR spectra, absorption, partition coefficient, solubility, and development and validation of analytical methods for quantification of the drug in different experimental protocols. These are stated as the phase of research and development in which pre-formulation studies characterize the physical and chemical properties of a drug molecule in order to develop a safe, effective, and stable dosage form. Critical information provided during the formulation can enhance the successful and rapid introduction of new therapeutic entities for humans [21]. Hence, pre-formulation studies are essential to characterize drugs for the proper design of drug delivery systems.

Physicochemical Characterization of ICZ: The ICZ was checked for its physical parameters such as organoleptic properties, and solubility. Melting point, and then compared with the values/description reported in the literature. A typical preformulation program should begin with a description of the organoleptic qualities of the drug substance.

Organoleptic Properties of Drug ICZ: Organoleptic properties of the drug (API) were found to be as per the I.P. monograph. Different organoleptic properties like appearance, colour, odour, and taste were determined. Data show in 4.1 [24].

Physical Appearance: The drug sample (ICZ) was analyzed by different means in order to prove the authenticity of the drug. The color, odor, and texture were observed. The physical appearance and melting point of the drug sample under investigation were found to be concordant with the reported values. The drug was found to be white in color and odorless. The result is shown in **Table 1**.

Determination Melting point of ICZ: The melting point of a substance is the temperature at which it changes state from solid to liquid. At the melting point, the solid and liquid phase exists in equilibrium. Melting point determination of the obtained drug sample was done because it is a good indication of the purity of the sample since the presence of a relatively small amount of impurity can be detected by lowering as well as widening the melting point range. The melting point of Itraconazole was determined by the capillary melting method.The result is shown in **Table 2.**

Capillary Fusion Method: This method involves placing the sample in a capillary tube and running an experiment that will heat the sample until it reaches the melting point. The melting point of itraconazole was determined by a capillary method using a digital melting point apparatus. (EIE Instruments pvt. Ltd., Ahmedabad, India). The observation value of the melting point is recorded in **Table 2.**

Determination of λ max of Drug [25]: Methanol was selected as the ideal solvent for the spectrophotometric analysis of Itraconazole the UV spectrum is generally recorded as a plot of absorbance versus wavelength. A double-beam UV-visible spectrophotometer (Shimadzu Corporation, UV-1700, Kyoto, Japan) was used to determine the λmax of the drug.

A stock solution of the drug (1mg/ml) in methanol was prepared. The sample was then diluted with methanol to obtain (100 µg/ml). The result solution was scanned in the range of 200-400 nm to determine the λmax of the drug. The result is mentioned in **Fig. 1.**

FTIR Spectroscopy of ICZ: An FTIR spectrum of ICZ was obtained by using an FTIR Spectrophotometer (Innovation Center in Bundelkhand University, Jhansi). FTIR spectrum of any drug helps in determining the functional groups present in that particular compound. The infrared spectral assignment of ICZ was carried out using an FTIR spectrophotometer (Shimadzu Corporation, UV–1700, Kyoto, Japan) result is shown in **Fig. 2** & **Table 3.**

Determination of Partition Coefficient [26]: The partition coefficient is defined as the ratio of the unionized drug distributed between the organic and aqueous phases at equilibrium. For a drug delivery system. Lipophilic Hydrophilic balance is a contributing factor to the rate and extent of drug absorption. Partition coefficients provide a means of characterizing the Lipophilic/Hydrophilic nature of the drug. The partition coefficient directly influences the permeability of the drug through the bio-membrane and could be approximated by measuring the drug's partition coefficient in n-octanol/water.

The partition coefficient of the drug was determined by allowing 10.0 mg of the drug to equilibrate in a mixture of n-octanol/water containing 10.0 ml of each by shaking on a shake flask method for 30 min. and storing it overnight for 24 hours at 25°C± 2°C in a separating funnel. The two layers were separated and the concentration of the drug in the two layers was determined by a UV spectrophotometer (Shimadzu Corporation, UV–1700, Kyoto, Japan) the partition coefficient was determined by using the formula given formula. The result is mentioned in **Table 4.**

Partition coefficient = Concentration of drug in organic phase / Concentration of drug in aqueous phase

Determination X-ray Diffraction Analysis ICZ Powder: The angle 2θ in the setup of the XRD fluctuates in a range of 10–90 degrees at a measurable step of 0.01 degrees. The analysis of the XRD is based on the peak observed.

If a sharp peak is observed, it infers a crystalline nature; otherwise, an amorphous nature of the surface is inferred.

X-ray diffraction (Phillips Pert PRO analytical, Japan) of ICZ was performed at room temperature. Experimental parameters are as follows: generator voltage 40kV, tube current 40mA, anode material Cu, and kα1& α2 are filters.

Each experiment was performed on a 2-theta range of 5-500. All samples were placed on the sample holder and samples were flatted to restricted particle orientation during the analysis (RigakuD/max-2000pc diffractometer) the result is shown in figure4.3.

Solubility Study of the ICZ [27]: The solubility study of the drug was performed in different solvents (e.g., distilled water, alcohol, methanol, 0.1N HCL, *etc*.). A known quantity of the drug was transferred in a series of different solvents having a volume of 5ml in four different test tubes. An excess amount of drug was added to different solvents till the solution became saturated and these test tubes were shaken by a mechanical shaker (Jyoti Scientific Industry) for 1-hour constant vibration at a constant temperature. After this period the solution was centrifuged. The supernatant was then analyzed by UV spectrophotometer (Shimadzu-1700, Japan) at 263 nm with appropriate dilution [28]. Three determinations were carried out before each sample to calculate the solubility of Itraconazole in different solvents. The result is given in **Table 5.**

Determination Calibration Curves of ICZ:

Preparation of Calibration Curve of Itraconazole in Methanol:

Preparation of Stock Solution and Calibration Curve: Accurately weighed of 10 mg itraconazole was dissolved in 100 ml of methanol, and from this stock solution, 10 ml was withdrawn and transferred into a 100 ml volumetric flask. Volume was made with methanol to get a standard stock solution containing 100 µg/ml. serial dilution was done (1, 2, 3, 4, 5, 6, 7, 8, 9, 10µg/ml) at 70:30 (PBS pH 5.5 and Methanol) and taking the absorbance at 263 nm by using an Ultraviolet-Visible Spectrophotometer (Model 1700 Shimadzu, Japan) result mentioned in **Fig. 4** & **5** and **Table 6** & **7.**

Method of Preparation of PAMAM Dendrimer: Synthesis of PAMAM dendrimer at a lab scale was carried out by the divergent method. Synthesis of EDA core PAMAM dendrimers consists of two steps

- Michal addition of primary amine EDA in the very first step to methyl acrylate.
- Amidation of formed multitester (tetra ester at the very beginning of EDA).

Identification of Dendrimer [29, 30, 31]:

Identify Dendrimer by UV Spectroscopy: Absorption maxima (max) were recorded for 4.0G dendrimers and surface modified dendrimers. The dendrimer solution was analyzed over the UV range between 200 to 400 nm in a UV-visible spectrophotometer (UV-1700 Shimadzu, Japan) to analyze the effect of solubilization.

Copper Sulphate Test: A copper sulphate test was done to confirm the formation of half and full generations of dendrimers. Ten percent of 3ml copper sulphate solution was added to dendrimers and colour change was observed. The results of the identification are exhibited in **Fig. 5.**

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis of Dendrimer: Infrared spectroscopy is a commonly used spectroscopic technique because of its ability to display the result and also easiness in sample preparation. This technique provides both quantitative and qualitative information about a given sample. The infrared (IR) portion of the electromagnetic spectrum is where the radiation wavelength is between 4000-400 cm^{-1}. The Fourier transform infrared (FTIR) spectroscopic experiments were performed with a Bruker Optics Alpha-T. Result discussed in **Fig. 6.** & **Table 7.**

Particle size and PDI of 4.0.G PAMAM Dendrimerand 4.0 G PAMAM Dendrimer & Drug Complex: Nano formulation particle size

determination is very important parameter for development of novel Nano formulation or Nano-carrier now days which can easily determine by zeta sizer. If formulation has some large particle (micro range) which can easily be determined by master sizer. Hence 4.0 G PAMAM dendrimer particle size was determined by zeta-sizer (Malvern-panalytical) where Particlesize of PAMAM dendrimer was found to be78.00nm which shown in **Table 7.** The PDI was also determine by zeta-sizer that found to be 0.242, which indicates homogeneously dispersion of the PAMAM. When PDI ≤ 0.3 indicates uniform size distribution was shown in **Table 8** and **Fig. 7** and 4.0 G PAMAM dendrimer & drug complex shown in **Table 11** and **Fig. 9.**

Zeta potential of 4.0 G PAMAM Dendrimer and 4.0 G PAMAM Dendrimer & Drug Complex: Nano formulation Zeta potential determination is a very important parameter for the development of suitable and targeted Nano-carrier nowadays which can easily determine by zeta sizer (Malvern panalytical).

The surface charge of PAMAM dendrimer was found to be 13.9 mV due presence of terminal amine groups which confirms the successfulul synthesis of PAMAM dendrimer. If the increased concentration of PAMAM dendrimer increases the zeta potential of the formulation due presence of more amine groups at the end of the terminal the data of surface charge (Zeta-potential) was shown in **Table 9** and **Fig. 8.** And 4.0 G PAMAM dendrimer & drug complex shown in **Table 12** and **Fig. 10.**

Drug-polymer Interaction 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 polymers. A physical mixture of drug, polymer and other excipients was prepared and mixed with suitable quantity. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer.

FTIR studies revealed that there is no chemical interaction between the drug and gel polymers. This study was governed between drug, dendrimer and gel base (carbapol-934) **Fig. 13** and & **Table 17.**

Characterization of Itraconazole-Loaded Dendrigel:

Preparation of ICZ-4.0G PAMAM Dendrimer Complex: 1% of dendrimer (1g of generation 4 dendrimers were diluted to 10 ml with distilled water in order to make 1% of dendrimer) in varying proportions (1-6 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 shown in the **Table 13** & **11.**

Physiochemical Characterization of Dendrigel: The gel was characterized for physical appearance, viscosity by Brookfield viscometer, and pH by digital pH meter.

Colour Test: Physical parameters such as color and appearance were checked visually was shown in **Table 14.**

Determination of pH: The formulation was transferred in 10 ml of the beaker and measured by using the digital pH meter the pH of the topical gel formulation should be between 3-9to treat the skin infection pH as shown in **Table 14** [32, 33].

Homogeneity: All developed gels were tested for homogeneity by visual inspection after the gels had been set in the container. They were checked for their appearance and the presence of no aggregate data was shown in **Table 14.**

Spreadability: The spreadability was measured by placing 0.5 g of gel within a pre-marked circle of diameter 1 cm on a glass plate over which a second glass plate was placed and 50 g weight was allowed to rest on the upper glass plate for a period of 5 min. The spreading of the gel caused an increase in the diameter of the circle, which was measured in cm and noted down. These results were taken as comparative values for spread ability. Data was shown in **Table 14** [34].

Drug Content Determination [35]: The preparation of dendrigel loaded with the drug was carried out by the simple physisorption method. Itraconazole and dendrigel polymer were mixed in the ratio of 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 in the all-ratios drug content capacity of dendrigel was calculated. After perceiving the concentration of the unbound drug in the supernatant with the assistance of UV- Vis

spectrophotometry (Shimadzu Corporation, UV–1700, Kyoto, Japan), outcomes predict that the loading capacity system for the ICZ is as high as 1.82 mg/ml alongside the early ICZ concentration. Data was shown in the **Table 19.**

Entrapment Efficiency: The % EE of different prepared batches of gel was estimated by quantitating free mass drug in the diffused phase of gel solution after centrifugation. In brief, 1g of gel was diffused with ethanol and vortexes for 5 minutes to ensure proper extraction of drugs in ethanol. Then, obtained mixture proceeded for centrifugation at 15000 rpm for 60 minutes at 4 °C temperature. Supernatant collected from the centrifuged mixture and allowed to analyze for quantitative analysis spectrophotometric [36]. The EE percentage was calculated from the equation as follows; whichwas shown in **Table 18.**

EE % = W (Added drug) -W (free drug) / W (Added drug) x 100

Where, W = (initial drug) is the mass of drug added initially, W= (free drug) is the mass of free drug detected in supernatant after centrifugation.

***In-vitro* Drug Release and Kinetics Study [37-38]:** The drug release and kinetics profiling of optimized formulation F1 dendrigel were evaluated by *in-vitro* drug release profiling methods using the dialysis bag technique. 1g of gel sample was accurately weighed and placed on a cellulose dialysis membrane. The membrane was tied with thread and placed in a flask containing 50 ml acetate buffer solution (pH 5.5). The container was placed on a magnetic stirrer at 37 °C with constant stirring at 50 rpm. Thereafter, 1 ml of the sample was withdrawn at regular intervals of 0, 1, 2, 3, 4, 6, 8, 12, and 24, and the withdrawn amount was replenished with dissolution media at the same time withdrawn. Released mass of dendrigel entrapped ICZ quantitated spectrophotometrically at 263 nm in respect of blank. Each measurement is taken in triplicate. *In-vitro* drug release profiles of prepared ICZ-loaded dendrigel formulation were evaluated statistically by various kinetic models named zero-order, first-order, and Higuchi and Korsemeyer-Peppas models. Kinetics models were determined statistically to enlighten the mechanism of drug release profiling. The high regression coefficient value is considered to be more effective for initialization and acceptance of kinetics orders results was shown in the **Table 20** & **Fig. 16**. All mathematical release zero, first higuchi, korsmeyer-Peppas order data shown in **Fig. 16** & **Table 21**, **Fig. 17** and **Table 22**, **Table 23** and **Fig. 18, Table 24** and **Fig.19.**

Rheology Study [39]: The viscosity was directly dependent on the polymeric content of the formulations. Addition of a higher concentration of carbopol-934 which have pseudo-plasticity behavior in nature. So, carbapol-934 was used as a predetermined ratio for the preparation of gel base of all formulation (F1-F6). All optimized formulations which were gives good results. The formulation which is in the gel form has an optimum viscosity that will allow for easily apply on the topical surface of the skin which would undergo a rapid gel-to-sol transition. These results further confirmed that the ICZ-loaded dendrites gel that Produces a strong anti-fungal effect due to the combined effect of drug-loaded dendri-gel along with its superior release profile as compared to other formulations. Data is shown in **Table 18** and **Fig. 14**.

Antifungal Activity of Dendri-gel [40]: The antifungal activity of the dendri-gel 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 15 min. Once the nutrient agar has been autoclaved, allow it cool but not solidify. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified. Then four wells were made in each Petri plate, and in the first well dendrigel, the second good gel base, the third well free drug solution, and the fourth PAMAM were filled in these wells, and the remaining well was blank. Place the plates into an incubator for 7 days. And then after 7 days observe effects. The result is shown in the **Table 25** 4.26 and **Fig. 20, 21, 22.**

Stability Study: Stability study of optimization dendrigel formulation as per ICH guideline. It can be observed that the dendrigel formulation was kept in tightly closed glass vials colourless vials at 0°C, Room temperature 25°- 30°C and 45°C for a period of eight weeks. After this storage period,

physicochemical and pH is shown in the **Fig. 25, 26, 27** & **28** & **Table 28, 29, 30** [41].

***Ex-vivo* Permeation Study:** All data shown in **Fig. 24** & **Table 27.**

RESULT AND DISCUSSION:

Pre-formulation Study of Drug: Pre-formulation is an integral part of the entire development process. These studies concentrate on the physicochemical properties of the medicament that could affect its performance and the development of an efficient dose form. A detailed understanding of these properties may eventually explain formulation design, or support the need for molecular remaking. In simple cases, these pre-formulation examinations may simply confirm that there are no significant walls to the emulsion's development. These studies are necessary protocols for developing safe, effective, and stable drug forms. The attained sample was linked by colourful logical methodologies like IR spectroscopy, UV spectroscopy, and Melting point.

Identification of Drug: It was identified and characterized as shown in the pre-formulation studies. The drug was identified in the laboratory by observing its physical appearance, melting point determination, UV spectroscopy, and FTIR spectroscopy.

Physical Appearance: The drug sample (ICZ) was analysed by different means to prove the drug's authenticity. The colour, odour, and texture were observed.

TABLE 1: OBSERVATION OF ORGANOLEPTIC PROPERTIES OF ITRACONAZOLE

S. no.	Test	Specification	Observation
1.	Physical Appearance	Crystalline	Crystalline Powder
2.	Color	White	Off White Powder
3.	Odor	Odorless	Odorless

Determination of Melting Point: The melting point of Itraconazole was studied in the mid of 100-200°C which was found to be within the limits of the declared scale 165 ±1°C indicating the authenticity of the drug. (EIE Instruments Pvt. Ltd., Ahmedabad, India).

TABLE 2: VALUE OF MELTING POINT

S. no.	Drug	Specification	Observation
1.	Itraconazole	166^0C	165±1

Determination of λ max of Drug: Methanol was selected as the ideal solvent for the spectrophotometric analysis of Itraconazole the UV spectrum is generally recorded as a plot of absorbance versus wavelength. A double-beam UV-visible spectrophotometer (Shimadzu Corporation, UV-1700, Kyoto, Japan) was used to determine the λmax of the drug. A stock solution of the drug (1mg/ml) in methanol was prepared. The sample was then diluted with methanol to obtain (100µg/ml). The resulting solution was scanned in the range of 200-400 nm to determine the λmax of the drug.

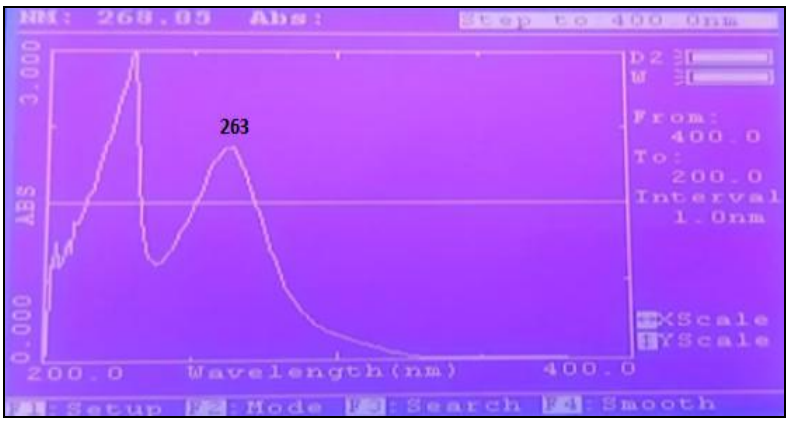


FIG. 3: UV SPECTURAM OF ITRACOAZOLE IN METHANOL AT 229 NM

Observation Fourier Transform Infrared Spectroscopy (FTIR): The IR spectrum of Itraconazole has high peaks at 1697 cm^{-1} indicating that there is a typical C=O stretching. Many prominent peaks can be characterized for pure Itraconazole, such as the peaks at 1506 aromatic (C=C) and 1376 cm^{-1} Aliphatic bending, the peak at 1216 cm^{-1} for alkyl ketone group, and the peak at 1042 cm^{-1} alkyl amine. IR spectra match the reported standard literature confirming the selected drug's identity and purity.

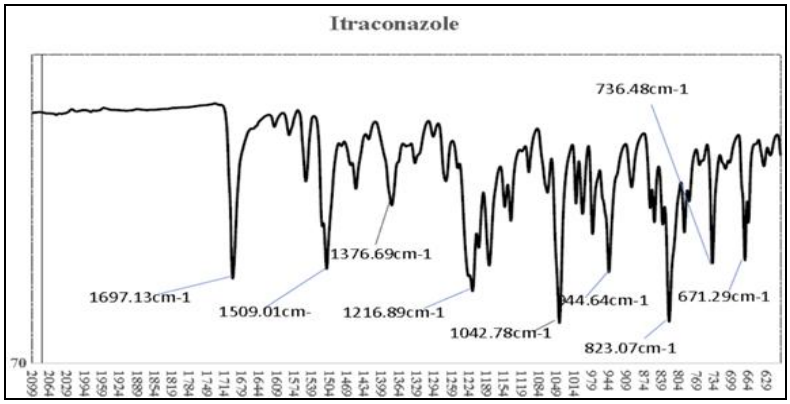


FIG. 4: FTIR SPECTRUM OF ITRACONAZOLE

TABLE 3: OBSERVATION PEAK OF FTIR SPECTRA OF DRUG

S. no.	Characteristic functional group	Standard Range (cm^{-1})	Observed Peaks(cm^{-1})
1.	C=O Stretching	1850-1680	1697.13
2.	Aromatic ¬C=C	1680-1450	1509.01
3.	CH & CH_2Aliphatic bending	1440-1350	1376.69
4.	Alkyl ketone group	1325-1215	1216.89
5.	N-CH_3stretching (Alkyl amine)	1250-1000	1042.78
6.	C-O, C-C	1000-800	944.64, 823.07
7.	CH, out of plane deformation	760-735	736.48
8.	C-O-H twist broad	680-650	671.29

Observation of ICZ by XRD: The angle 2θ in the setup of the XRD fluctuate in a range of 10–90 degrees at a measurable step of 0.01 degrees. The analysis of the XRD is on the basis of the peak observed. If a sharp peak is observed, it infers a crystalline nature; otherwise, an amorphous nature of the surface is inferred. X-ray powder diffraction (XRD) is an analytical tool primarily used for phase identification of crystalline material. The x-rays (Cu K-alpha) were produced using a sealed tube and the samples were scanned over a 20 range of 2°-50° with a scanning rate of 5/minute. The x-rays were detected using a fast-counting detector based on Silicon strip technology (Bruker Lynx Eye detector) Itraconazole was found crystalline in nature as its XRD peaks were obtained at 20 values of 8, 10, 17, 14, and XRD pattern of ICZ revealed information of crystalline nature.

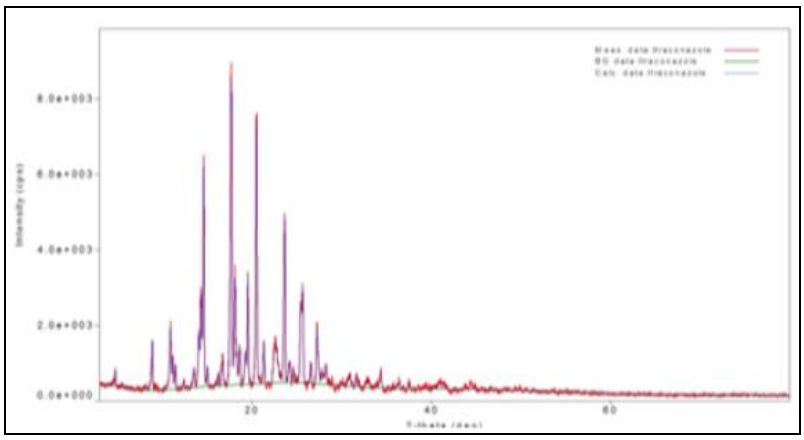


FIG. 5: XRD SPECTRUM OF DRUG (ITRACONAZOLE)

Partition Coefficient Determination: The partition coefficient of itraconazole in an n-octanol: water mixture Log P greater than one indicates that the drug is lipophilic in nature.

Determined by UV spectrophotometer (Shimadzu Corporation, UV–1700, Kyoto, Japan) the partition coefficient was determined by using the formula as given below:

Partition coefficient = Concentration of drug in organic phase / Concentration of drug in aqueous phase

Partition coefficient = 2.540/0.430
=5.906976

TABLE 4: AVERAGE VALUE OF PARTITION COEFFICIENT OF ICZ

S. no.	Drug	Observed Partition coefficient (Log P)	Nature of the drug
1.	Itraconazole	5.906	Lipophilic

Solubility Determination: The solubility determination of ICZ was performed in different aqueous and organic solvents, ICZ was soluble in dichloromethane, methanol, and Acetate buffer pH 5.5, poorly soluble in water, 0.1N HCl, Phasphate buffer.

TABLE 5: VALUE OF SOLUBILITY OF ITRACONAZOLE IN DIFFERENT SOLVENTS

S. no.	Medium	Inferences
1.	Distilled water	Insoluble
2.	0.1N HCl	Insoluble
3.	Phosphate Buffer pH 5.5	Insoluble
4.	Ethanol	Very slightly soluble
5.	Methanol	soluble
6.	Dichloromethane	Soluble

Preparation of Calibration Curve:

Calibration Curve of ICZ in Methanol: Accurately weighed 10 mg itraconazole was dissolved in 100 ml of methanol, and from this stock solution, 10 ml was withdrawn and transferred into a 100 ml volumetric flask. Volume was made with methanol to get a standard stock solution containing 100 µg/ml serial dilution was done (1, 2, 3, 4, 5, 6, 7, 8, 9, 10µg/ml) at methanol and taking the absorbance at 263 nm by using Ultraviolet-Visible Spectrophotometer (Model 1700 Shimadzu, Japan).

TABLE 6: ABSORBANCE DATA OF ICZ IN METHANOL

S. no.	Conc.	Abs.
1.	0	0
2.	1	0.11±0.0125
3.	2	0.21±0.145
4.	4	0.395±0.26
5.	6	0.58±0.45
6.	8	0.82±0.68
7.	10	0.995±0.93

All data SD mean value (mean value =2)

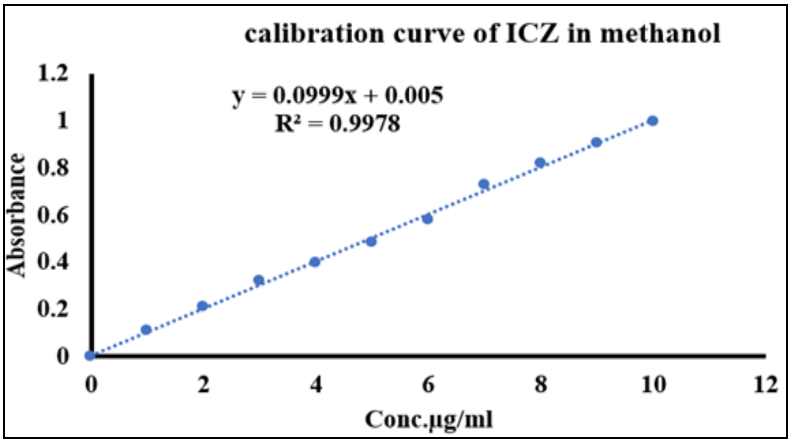


FIG. 6: STANDARD CURVE OF ICZ IN METHANOL

Calibration Curve of ICZ in Acetate buffer pH 5.5: Accurately weighed 10 mg Itraconazole was dissolved in 100 ml of acetate buffer pH 5.5, and from this stock solution, 10 ml was withdrawn and transferred into a 100 ml volumetric flask. Volume was made with methanol to get a standard stock solution containing 100 µg/ml. serial dilution was done (1, 2, 3, 4, 5, 6, 7, 8, 9, 10µg/ml) at methanol and taking the absorbance at 263 nm by using an Ultraviolet-Visible Spectrophotometer (Model 1700 Shimadzu, Japan).

TABLE 7: ABSORBANCEDATA OF ICZ IN ACETATE BUFFER PH 5.5

S. no.	Conc.	Abs.
1	0	0.0
2	1	0.1±0.012
3	2	0.19±0.025
4	3	0.28±0.085
5	4	0.39±0.12
6	5	0.48±0.28
7	6	0.58±0.45
8	7	0.69±0.68
9	8	0.78±0.78
10	9	0.87±0.89
11	10	0.98±1.0

All data can show SD value (SD mean=3)

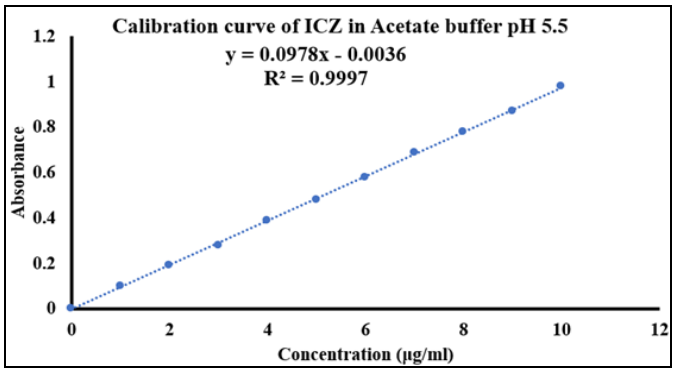


FIG. 7: STANDARD CURVE OF ICZ IN ACETATE BUFFER PH 5.5

Synthesis of PAMAM Dendrimer: Prepared a PAMAM dendrimer by the divergent method. I was taken 25ml ethylene diamine and 12ml methyl acrylate mixed with 40 ml methanol and held on the refrigerator for 30 all chemicals were chilled and then stirred for 30 minutes. After 30 minutes, I used a rotatory evaporator to completely removable methanol from the sample for 24 hrs and I got a concentrated solution.

After 24 hrs, I added again 24ml ethylene diamine in the previous solution for the formation of amide functional groups on the surface and kept 48h to finally get zero generation PAMAM dendrimer (0.G PAMAM Dendrimer).

This whole process was repeated eight times with various chemical concentrations to obtain 4-Generation PAMAM dendrimer (4.G PAMAM Dendrimer).

Evaluation of Pamam Dendrimer:

Copper Sulphate Test: PAMAM dendrimers and acetylated derivatives were treated with an aqueous solution of copper sulphate 7 (1%w/v).

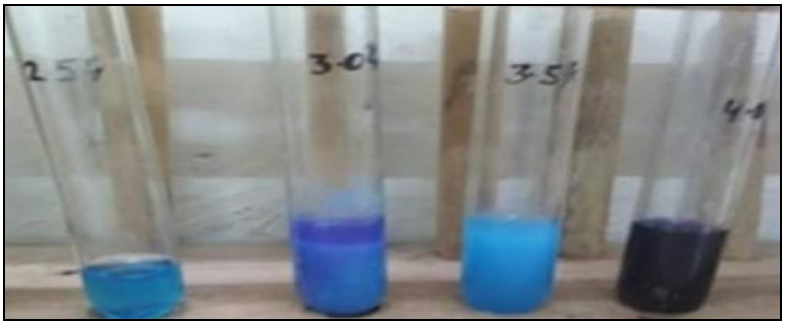


FIG. 8: COLOUR TEST OF PAMAM DENDRIMER

UV Spectroscopy: 0.01%w/v concentration of PAMAM dendrimers was scanned in the 200nm to 400 nm range in against methanol. Observed λ max was 279 find during λ max determination.

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis of Dendrimer: FTIR spectra of Dendrimer showed the presence of different types of N-H stretching of primary amine functionalities was confirmed at 3352.62 cm^{-1}, whereas N-H stretching of anti-symmetric primary amine vibrations was observed at 2923.89 cm^{-1}. C-H stretching for aliphatic was observed at 2814.84 cm^{-1} and N-H bending of N substitute amine find 1590.70, 1493.67, and C-C bending at 1170.82 cm^{-1} were observed. The spectra of 4.0 G PAMAM dendrimer are shown in **Fig. 6.**

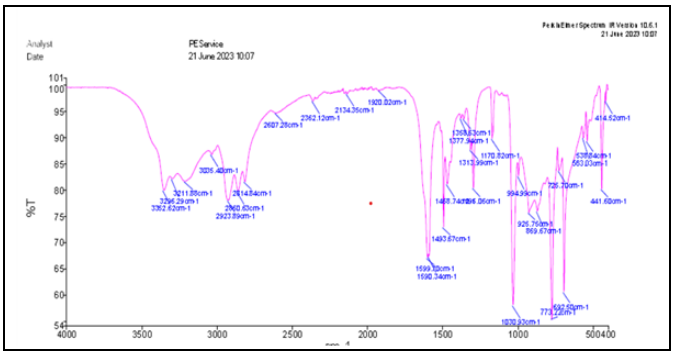


FIG. 9: FTIR SPECTRA OF 4.0 G PAMAM DENDRIMER

TABLE 8: OBSERVED PEAKS OF 4.0 G PAMAM DENDRIMER FUNCTIONAL GROUPS FREQUENCY

S. no.	Characteristic functional group	Reference Range (cm^{-1})	Observed Peaks(cm^{-1})
1.	N-H Stretching of primary amine	3315.74	3352.62 cm-1
2.	N-H stretch anti- symmetric primary amine	2991.69	2923.89 cm-1
3.	C-H stretch for aliphatic	2827.74	2814.84 cm-1
4.	N–H bending of N substituted amine	1514.17,1448.59	1590.70,1493.67,1468.74 cm-1
5.	C-C bending	1149.61	1170.82 cm-1

Particle size and PDI (Polymer Dispersion Index) of 4.0 G-PAMAM dendrimer: Particlesize of PAMAM dendrimer was found to be78.00nm nm shown in table. The PDI was found to be 0.242, which indicates homogeneously dispersion of the PAMAM. When PDI ≤ 0.3 indicates uniform size distribution.

TABLE 9: OBSERVED PARTICLE SIZE AND PDI OF 4.0 G-PAMAM DENDRIMER

S. no.	Particle size (nm)	PDI
1	78.00	0.242

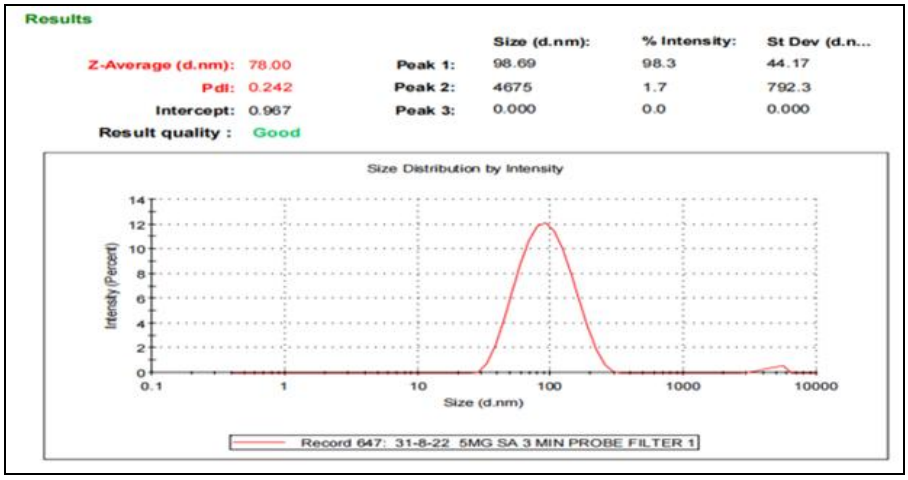


FIG. 10: PARTICLE SIZE & PDI SPECTRA OF 4.0 G-PAMAM DENDRIMER

Zeta potential of 4.0 G-PAMAM Dendrimer: The surface charge of 4.0 GPAMAM dendrimer was found to be 13.9 mV due presence of terminal aminegroups which confirm the successfully synthesis of PAMAM dendrimer. If increased concentration of 4.0 G-PAMAM dendrimer to increased zeta potential of the formulation's due presence of more amine group in the end of terminal.

TABLE 10: OBSERVED ZETA POTENTIAL OF 4.0 G-PAMAM DENDRIMER

S. no.	Zeta potential
1	13.9 mV

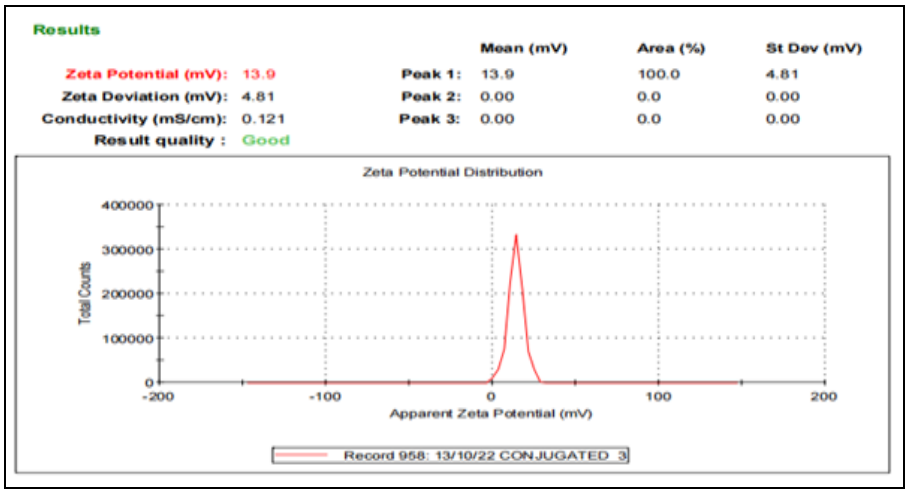


FIG. 11: ZETA POTENTIAL SPECTRA PEAK OF 4.0 G-PAMAM DENDRIMER

Optimization of ICZ-4.0 G-PAMAM Dendrimer Complex Batches Based on their Study of PAMAM Dendrimer Size, PDI and Zeta Potential: We was prepared six ICZ-PAMAM dendrimer batches. In this batches, we was varying amount the ICZ so increased size, PDI and Zeta potential all batches. F1 was optimized batch because size and PDI of this batch is less than other than batches complex names as F1, F2, F3, F4, F5 and F6 were formulated.

TABLE 11: OPTIMIZED DATA OF ICZ-4.0 G-PAMAM DENDRIMERS

Code of formulation	ICZ (mg)	PAMAM Dendrimer (ml)	Size (nm) (Mean±SD)	PDI (Mean±SD)	Zeta-Potential(mV) (Mean±SD)
F1	10	1ml	141.3±2.12	0.200±0.034	21.7±1.34
F2	10	2ml	159.8 ±3.32	0.187±0.054	25.3±1.76
F3	10	3ml	167.6±2.45	0.169±0.024	29.4±2.98
F4	10	4ml	175.1±4.14	0.166±0.074	34.5±2.73
F5	10	5ml	179.9±5.34	0.145±0.064	39.7±2.82
F6	10	6ml	187.6±6.32	0.85±0.054	43.8±2.65

Optimized Formulations of ICZ- 4.0 G-PAMAM Complex (F1): The particle size of the ICZ-4.0 G-PAMAM complex was found to 141.3 nm. The size of PAMAM is increased due entrapment of ICZ inside the 4.0 G-PAMAM branches. The PDI was found to be 0.242 and 0.20 respectively, which indicates homogeneously dispersion of the 4.0 G-PAMAM dendrimer. When PDI ≤ 0.3 indicates uniform size distribution.

TABLE 12: OBSERVED PARTICLE SIZE AND PDI OF ICZ-4.0 G-PAMAM DENDRIMER COMPLEX

S. no.	Particle size	PDI
1	141.3	0.242

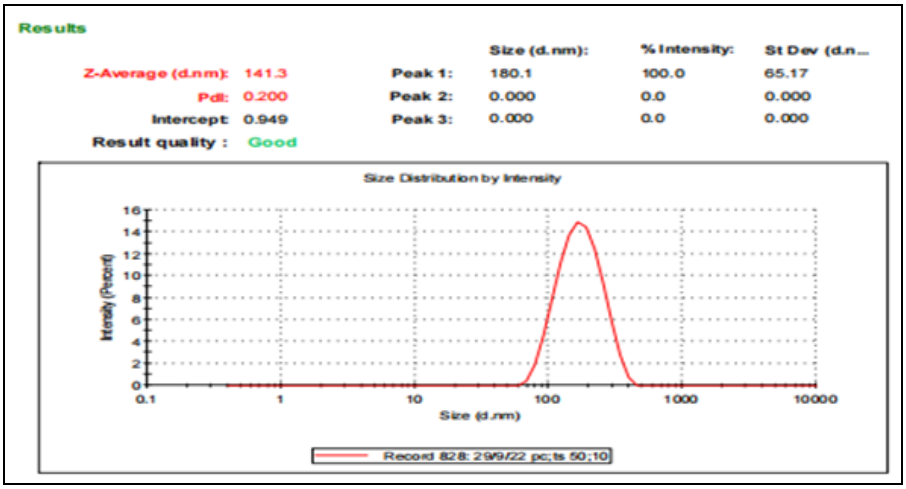


FIG. 12: PARTICLE SIZE AND PDI SPECTRA OF ICZ-4.0 G-PAMAM DENDRIMER COMPLEX

Zeta Potential of Optimized ICZ-4.0 G-PAMAM Complex (F1): The surface charge was found of the ICZ-4.0 G-PAMAM dendrimer complex was found to be 21.7 mV. We observed in optimization table of ICZ-4.0 G-PAMAM dendrimer if increased concentration of PAMAM dendrimer to increased zeta potential of the formulation's due presence of more amine groups on the terminal ends of 4.0 G-PAMAM dendrimer.

TABLE 13: OBSERVED ZETA POTENTIAL OF ICZ-4.0 G-PAMAM DENDRIMER COMPLEX (F1)

S. no.	Batch code	Zeta potential (mV)
1	ICZ-4.0 G-PAMAM dendrimer complex	21.7

TABLE 14: OPTIMIZATION OF ICZ-LOADED 4.0 G-PAMAM DENDRIMER COMPLEX (F1) GEL BATCHES

S. no.	Formulation code	Carbopol-934 (g)	TEA (ml)	Ethanol	Methyl Paraben: Propyl-paraben (4:1)	ICZ-4.0 G-PAMAM Complex (F1)	Distilled water (ml)

							up to 100
1.	ICZ-DG 1	1.0 g	0.5 ml	0.5 ml	4:1	1 ml	up to 100
2.	ICZ-DG 2	1.5 g	0.8 ml	0.5 ml	4:1	1 ml	up to 100
3.	ICZ-DG 3	2.0 g	1.2 ml	0.5 ml	4:1	1 ml	up to 100
4.	ICZ-DG 4	2.5 g	1.5 ml	0.5 ml	4:1	1 ml	up to 100
5.	ICZ-DG 5	3.0 g	1.8 ml	0.5 ml	4:1	1 ml	up to 100
6.	ICZ-DG 6	3.5 g	2.5 ml	0.5 ml	4:1	1 ml	up to 100

All data are represent as mean ± SD (n=3)

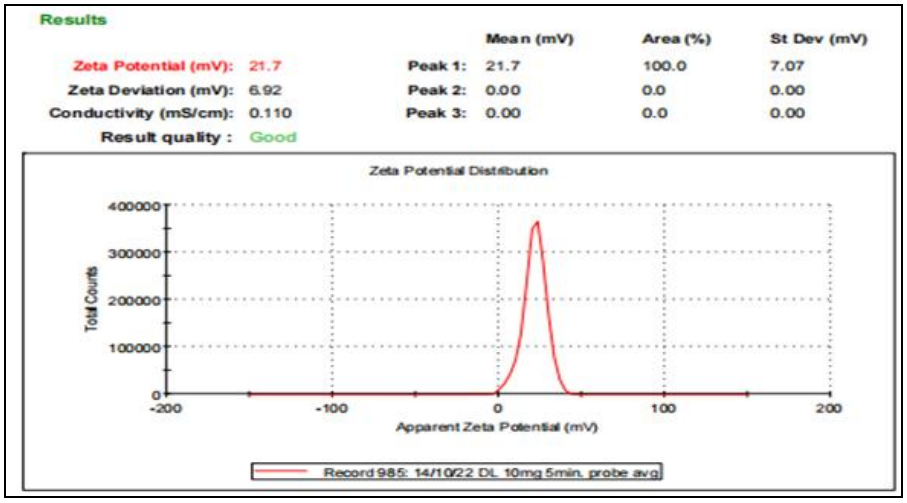


FIG. 13: ZETA POTENTIAL SPECTRA PEAK OF ICZ-4.0 G-PAMAM DENDRIMER COMPLEX (F1)

FIG. 14: FORMULATIONS OF DENDRIGEL

Physicochemical Evaluation of Dendrigel: All gel preparations including control gel were evaluated for their physical characteristics like organoleptic properties, pH, homogeneity, spreadability, viscosity. Colour of all the gels was yellowish colour and pH were in range of all gel preparations including control gel were evaluated for their physical characteristics like organoleptic properties, pH, homogeneity, spreadability.

TABLE 15: PHYSICOCHEMICAL EVALUATION OF DENDRIGEL

Formulation	Colour	PH	Homogeneity	Spreadability
ICZ-DG 1	light Yellowish	5.5±0.2	Good	5.56±0.1
ICZ-DG 2	Yellowish	5.01±0.2	Good	5.23± 0.1
ICZ-DG 3	Yellowish	4.80±0.3	Good	5.15±0.3
ICZ-DG 4	Yellowish	4.50±0.1	Good	4.85±0.2
ICZ-DG 5	Yellowish Brown	4.0±0.1	Good	4.64±0.2
ICZ-DG 6	Yellowish Brown	3.80±0.3	Good	4.45±0.2

Characterization of Dendrigel:

FTIR study for Dendrigel (ICZ-DG 1):

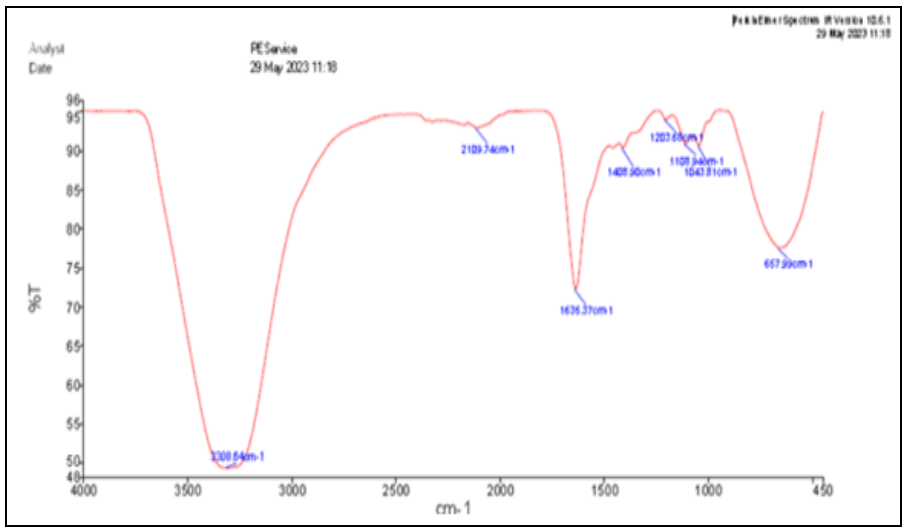


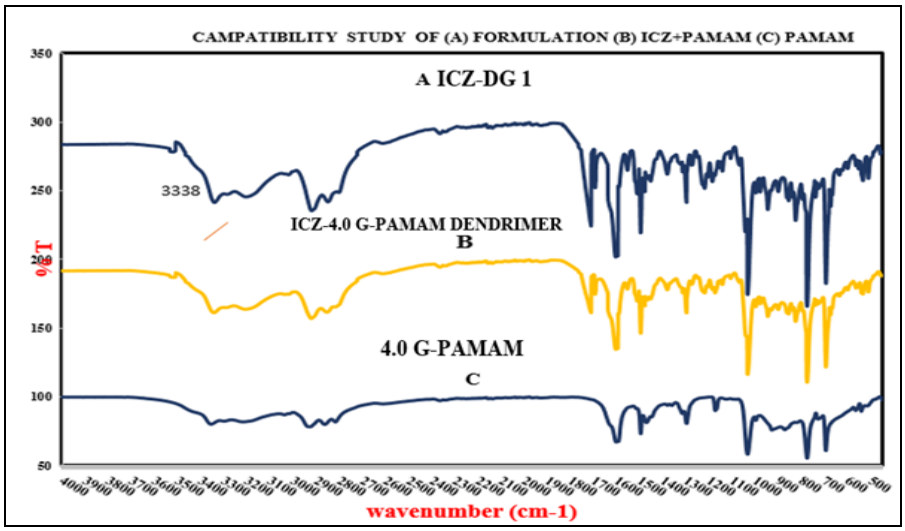
FIG. 15: FTIR SPECTRA OF ICZ-DG1 FORMULATION

TABLE 16: OBSERVED PEAKS OF 4.0 G PAMAM DENDRIMER FUNCTIONAL GROUPS FREQUENCY

S. no.	Characteristic functional group	Observed Peaks(cm^{-1})
1.	N-H Stretching of primary amine	3308cm^{-1}
2.	C=C Stretching bond of alkynes molecule	2109.7cm^{-1}
3.	C-O aromatic	1636.37cm^{-1}
4.	CH out of plane aromatic bond	667.99cm^{-1}

Drug-polymer Interaction 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 polymers. A physical mixture of drug, polymer and other excipients was prepared and mixed with suitable quantity. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer. FTIR studies revealed that there is no chemical interaction between the drug and gel polymers. This study was governed between drug, dendrimer and gel base (carbapol-934) **Fig. 14.**

Compatibility Study of Formulation with ICZ+4.0 G PAMAM Dendrimer-complex and 4.0 G-PAMAM Dendrimer:



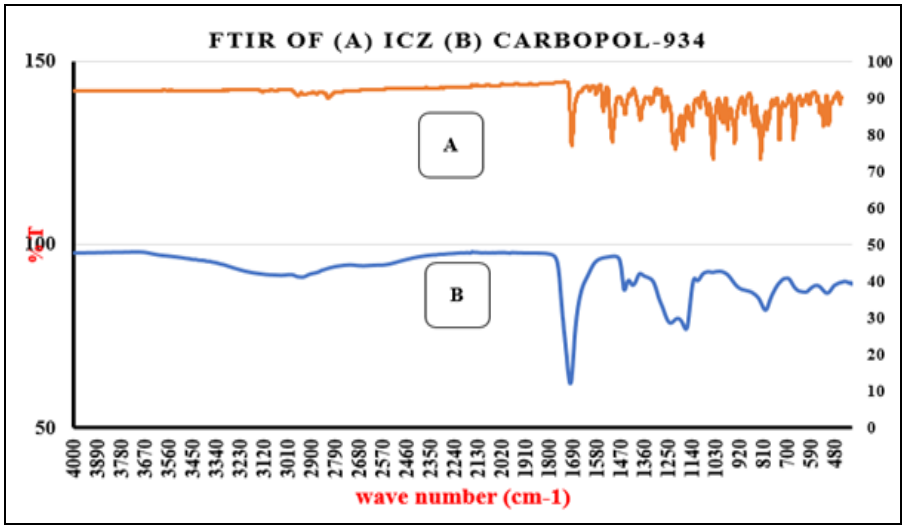


FIG. 16: COMPATIBILITY STUDY OF FTIR SPECTRA

TABLE 17: FUNCTIONAL GROUP OF FTIR SPECTRUM

S. no.	Functional group dendrimer	Observed peaks
1.	N-H Stretching of primary amine	3352.62
2.	N-H stretch anti- symmetric primary amine	2923.89
3.	C-H stretch for aliphatic	2814.84
4.	N–H bending of N substituted amine	1590.70,1493.67,1468.74
5.	C-C bending	1170.82
6.	**Functional group ICZ**	**Observed peaks**
7.	C=O Stretching	1697.13
8.	Aromatic ¬C=C	1509.01
9.	CH & CH_2Aliphatic bending	1376.69
10.	Alkyl ketone group	1216.89
11.	N-CH_3stretching (Alkyl amine)	1042.78
12.	C-O, C-C	944.64, 823.07
13.	CH, out of plane deformation	736.48
14.	C-O-H twist broad	671.29
15.	**Functional group Carbopol-934**	**Observed peaks**
16.	C=O stretching	**1705 cm-1**
17.	CH & CH_2 stretching	1239,1168 cm-1

Determination of Drug Entrapment Efficiency (% EE) of Formulation (ICZ-DG 1): The indirect method was used to determine the percentage drug entrapment efficiency of ICZ-4.0 G-PAMAM Gel formulation (ICZ-DG 1). The percentages EE of ICZ-DG 1 formulation were found to be96.5±0.06% ICZ-PAMAM Gel (ICZ-DG 1) is showing higher drug efficiency than ICZ-PAMAM.

TABLE 18: SHOWDRUG ENTRAPMENT EFFICIENCY (% EE) OF FORMULATION (ICZ-DG 1)

S. no.	Batch code	% EE (mean SD,n=3)
1	ICZ-DG 1	96.5±0.06%
2	ICZ- 4.0 G-PAMAcomplex9(F1)	92.07±02%

Rheological Study: The viscosity of gel formulation (ICZ-DG 1) was determined at 37ºC using a brook field viscometer (Brookfield DV-E viscometer, Brookfield engineering laboratories, USA).

TABLE 18: OBSERVATION TABLE OF VISCOSITY OF (ICZ-DG 1) FORMULATION IN VARIOUS TEMPERATURE

S. no.	Temp [°C]	Viscosity [mPa·s]	S. no.	Temp [°C]	Viscosity [mPa·s]
1	20.45	410.36	27.	35.55	1420.36
2	21.25	423.36	28.	36.06	1495.3
3	22.25	460.23	29.	36.56	1525.36

4	23.36	465.41	30.	37.06	1580.36
5	24.56	470.32	31.	37.56	1623.2
6	23.03	475.23	32.	38.06	1680.26
7	23.54	478.29	33.	35.55	1720.36
8	24.04	478.3	34.	36.06	1800.3
9	24.54	479.45	35.	36.56	1420.36
10	25.05	480.65	36.	37.06	1401.32
11	25.55	510.23	37.	38.56	1377.7
12	26.05	556.32	38.	39.36	1330.1
13	26.55	610.23	39.	40.23	1288.6
14	27.05	670.36	40.	41.25	1235.1
15	27.55	720.36	41.	42.15	1180.25
16	28.05	792.15	42.	43.36	1123.2
17	28.55	825.45	43.	44.56	1096.36
18	29.06	890.45	44.	45.36	1045.4
19	29.55	950.25	45.	46.95	980.26
20	30.06	1010.3	46.	47.89	925.36
21	30.56	1056.36	47.	48.56	850.3
22	31.05	1125.36	48.	49.57	740.26
23	31.56	1185.36	49.	50.25	670.26
24	32.06	1220.36	50.	51.36	634.89
25	32.56	1270.36	51.	52.16	598.36
26	33.06	1350.36	52.	53.15	1420.36

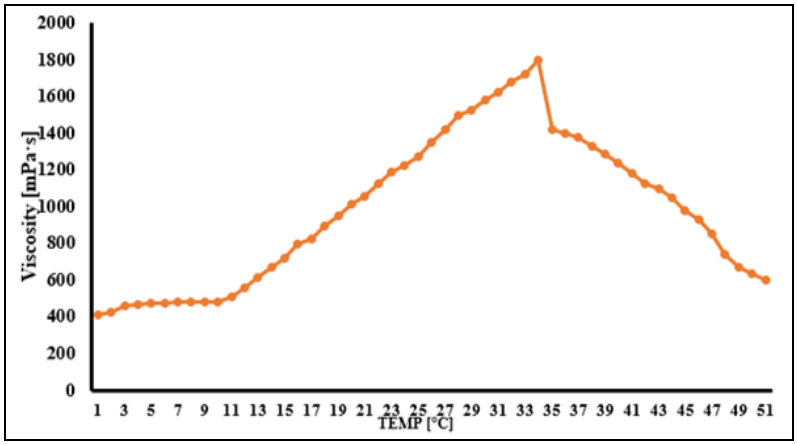


FIG. 17: VISCOSITY OF (ICZ-DG 1) GRAPH IN VARIOUS TEMPERATURE

Drug Content Determination of Formulation (ICZ-DG 1): The drug content estimation was carried out by diluting 1 gm of prepared formulation dissolved in 100 ml distilled water by sonication. Then filter with Whatman filter paper after collecting 5 ml. solution and further diluted with 25 ml distilled water and absorbance were determined by UV- visible spectrophotometer (Shimadzu UV-1700 PC, Shimadzu Corporation, Japan) at 263 nm.

TABLE 19: DRUG CONTENT DETERMINATION OF (ICZ-DG 1) FORMULATION

S. no.	Formulation	Drug content (%)
1	(ICZ-DG 1)	93.03
2	ICZ-4.0G PAMAM dendrimer complex(F1)	96.07

In-*vitro* Release of Formulation (ICZ-DG 1): Franz Diffusion cell was used for the drug release studies. Dendrigel (1gm) was applied onto the surface of placed ona cellulose dialysis membrane. The dialysis membrane was clamped between the donor and the receptor chamber of the 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. Samples were analysed for drug content by UV visible spectrophotometer at 263 nm after appropriate dilutions.

TABLE 20: OBSERVATION OF DRUG RELEASE OF FORMULATION (ICZ-DG 1)

S. no.	Time (Hr.)	Drug (ICZ) % CDR	ICZ- 4.0 G-PAMAM Complex % CDR	Formulation (ICZ-DG 1) % CDR
1.	0	0	0	0
2.	2	11.36	20.67	15.98
3.	4	22.66	35.12	29.53
4.	6	34.56	49.32	43.59
5.	8	39.45	59.68	54.69
6.	10		68.56	63.66
7.	12		79.23	72.59
8.	14		88.25	82.26

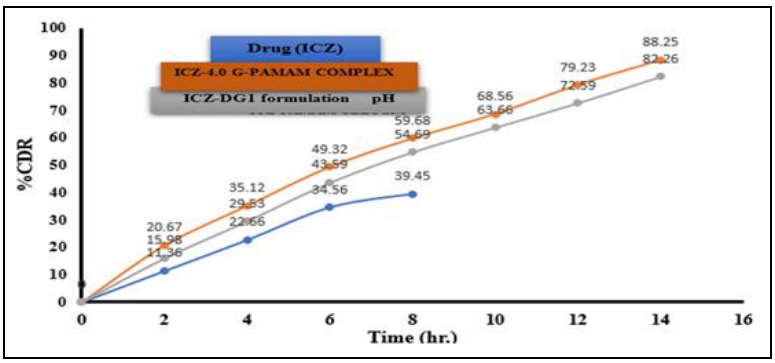


FIG. 18: DRUG RELEASE OF DRUG, ICZ-4.0 G-PAMAM COMPLEX AND FORMULATION (ICZ-DG 1)

Mathematically Drug Release Model: Investigation for the drug release was done by various drug release mathematically model data with zero-ender, first-order kinetics, Higuchi equation and Korsmeyer-Peppas model.

Zero Order Kinetics: When the data is plotted as cumulative "% drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0. Zero-order release would be predicted by the following equation.

$$A_t = A_0 - K_0 t$$

Where, A_t = Drug release at a "time", A_0 = initial drug concentration, K_0 = Zero-Order rate constant (hr-1).

TABLE 21: OBSERVATION OF DRUG RELEASE OF FORMULATION (ICZ-DG 1) AT DIFFERENT HRS

S. no.	Time	Formulation (ICZ-DG 1) pH 5.5 (Drug release) % CDR
1	0	0
2	2	15.98
3	4	29.53
4	6	43.59
5	8	54.69
6	10	63.66
7	12	72.59
8	14	82.26

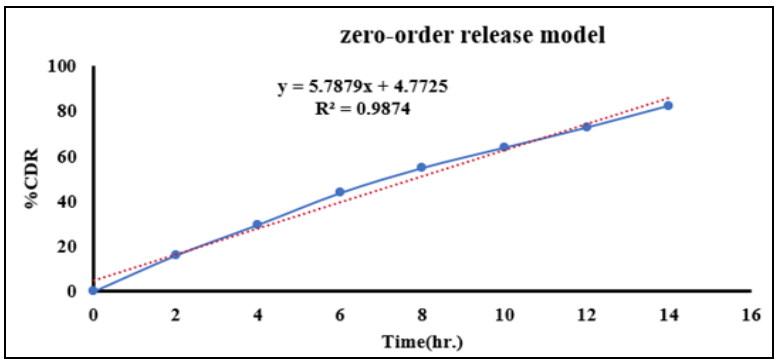


FIG. 19: DRUG RELEASE OF FORMULATION (ICZ-DG 1) ON ZERO ORDER DRUG RELEASE MODEL

First-Order Kinetics: When the data is plotted as log cumulative % drug remaining versus time yields a straight line, indicating that the release follows first-order kinetics. The constant "K" can be obtained by multiplying 2.303 with the slope values. The first-order release would be predicted by the following equation

$$\text{Log } C = \log C_0 - K_t/2.303$$

Where, C = Amount of drug remained at the time "t", C_0 = Initial concentration of drug, K = first-order rate constant (hr-1)

TABLE 22: OBSERVATION OF DRUG RELEASE OF FORMULATION (ICZ-DG 1) OF % CDR LOG VALUE IN DIFFERENT (HR)

S. no.	Time	Formulation (ICZ-DG 1) pH 5.5 (% CDR log value)
	0	0
	2	1.203577
	4	1.470263
	6	1.639387
	8	1.737908
	10	1.803867
	12	1.860877
	14	1.915189

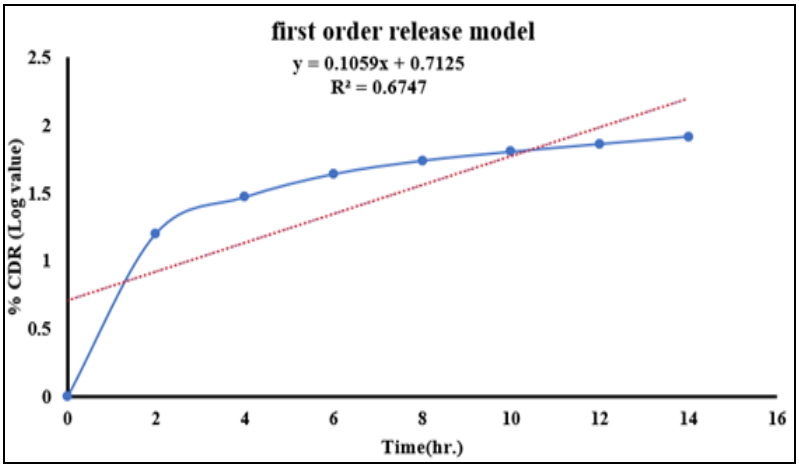


FIG. 20: DRUG RELEASE OF FORMULATION (ICZ-DG 1) PH 5.5 % CDR (LOG VALUE), ON FIRST ORDER DRUG RELEASE MODEL RELEASE ON DIFFERENT (HR)

Higuchi's Model: When the data is plotted as cumulative drug release versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to "K" Drug release from the formulation by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q = [D\,\varepsilon / \varepsilon\,(2A\text{-}CS)\,CSt]_{1/2}$$

Where, Q = Amount of drug released at a "time" D = Diffusion coefficient of the drug in the matrix, A = total amount of drug in the unit volume of a matrix, CS = Solubility of the drug in the matrix, ε = porosity of the matrix, T = Tortuosity

TABLE 23: OBSERVATION OF DRUG RELEASE OF FORMULATION (ICZ-DG 1) PH 5.5 OF % CDR VALUE IN DIFFERENT (HR) WHICH GIVEN IN SQUARE ROOT OF TIME (SQRT)

S. no.	SQRT	% CDR (ICZ-DG 1)
1.	0	0.0
2.	1.414214	15.98
3.	2	29.53
4.	2.44949	43.59
5.	2.828427	54.69
6.	3.162278	63.66
7.	3.464102	72.59
8.	3.741657	82.26

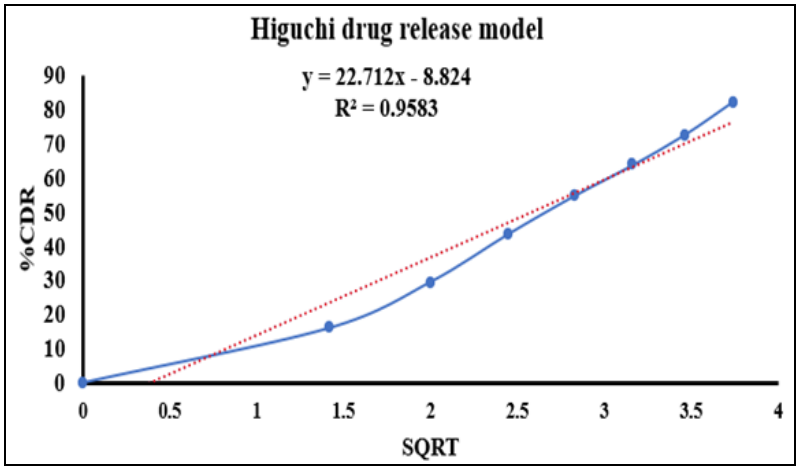


FIG. 21: DRUG RELEASE OF FORMULATION (ICZ-DG 1) PH 5.5 % CDR VALUE, ON HIGUCHI DRUG RELEASE MODEL RELEASE ON DIFFERENT (HR) WHICH GIVEN IN (SQRT)

Korsmeyer Equation/ Peppa's Model: When the data is plotted as a log of drug released versus time, yield a straight line with a slope equal to "n" and the "K" can be obtained from the y-intercept.

To study the mechanism of drug release, the drug release data were also fitted to the well-known exponential equation (Korsmeyer equation/Peppa's law equation), which is often used to describe the drug release behavior from polymeric systems.

$$M_t/M_a = K_{tn}$$

Where, M_t/M_a is the amount of drug released at time t, K = is the release rate constant, and n is the release exponent, n = value is used to characterize the different release mechanisms.

TABLE 24: OBSERVATION OF DRUG RELEASE OF FORMULATION (ICZ-DG 1) PH 5.5OF % CDR (LOG VALUE) IN DIFFERENT TIMES (LOG VALUE)

S. no.	Time (log value)	% CDR (log value) of Formulation (ICZ-DG 1) pH 5.5
1	0	0
2	0.30103	1.203577
3	0.60206	1.470263
4	0.778151	1.639387
5	0.90309	1.737908
6	1.0	1.803867
7	1.079181	1.860877
8	1.146128	1.915189

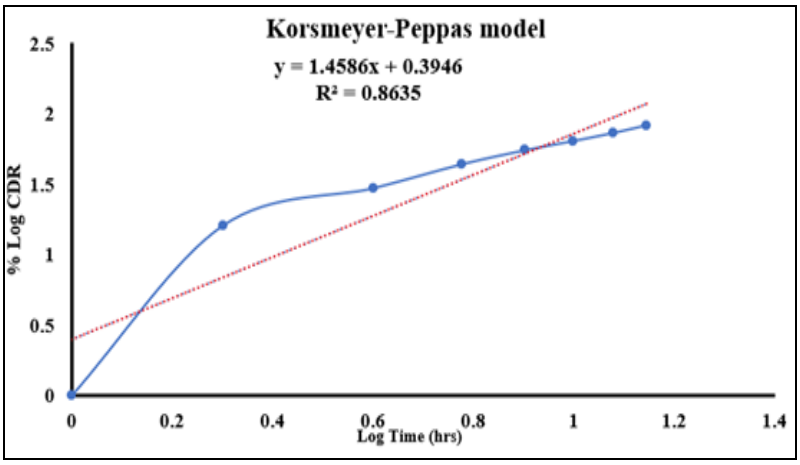


FIG. 22: DRUG RELEASE OF FORMULATION (ICZ-DG 1) PH 5.5 % CDR (LOG VALUE), ON KORS MEYER-PEPPAS DRUG RELEASE MODEL RELEASE ON DIFFERENT TIMES (LOG VALUE)

Antifungal Activity of Dendrigel Formulation (ICZ-DG 1): 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 15 min.

Once the nutrient agar has been autoclaved, allow it cool but not solidify. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.

Then four wells were made in each Petridish plate, and in the first well dendrigel, second well formulation of itraconazole, third well gel base was filled in these wells and the remaining one well was blank. Place the plates into an incubator for 7 days.

TABLE 25: OBSERVATION OF ZOI DIFFERENT FORMULATION

S. no.	Name formulations	ZOI Value IN (mm)
1.	B-Gel	0.98
2.	Drug (ICZ)	2.58
3.	4.0 G-PAMAM	0.35
4.	Formulation (ICZ-DG 1)	5.6

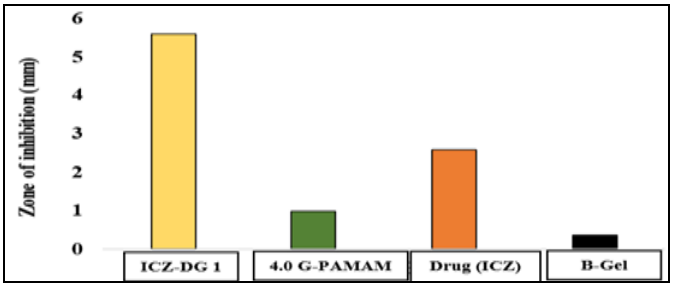


FIG. 23: ZONE OF INHIBITION OF FORMULATIONS WITH BASE-GEL, FREE DRUG, PAMAM

TABLE 26: OBSERVATION OF MINIMUM INHIBITORY CONCENTRATION DIFFERENT FORMULATION

S. no.	Name formulations	Minimum inhibitory conc.
1.	B-Gel	1.01
2.	Drug (ICZ)	2.58
3.	4.0 G-PAMAM	0.17
4.	Formulation (ICZ-DG 1)	5.9

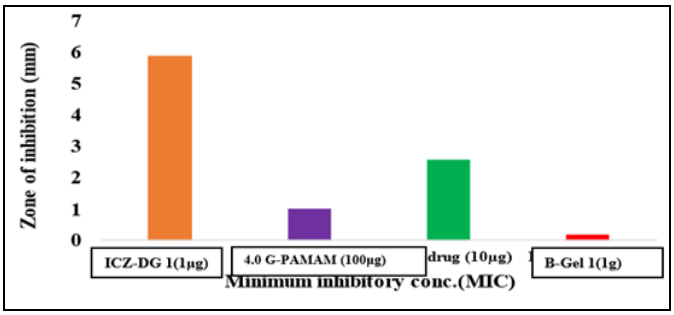


FIG. 24: MINIMUM INHIBITORY CONC. OF FORMULATIONS (ICZ-DG 1) WITH BASE-GEL, DRUG (ICZ), 4.0G-PAMAM

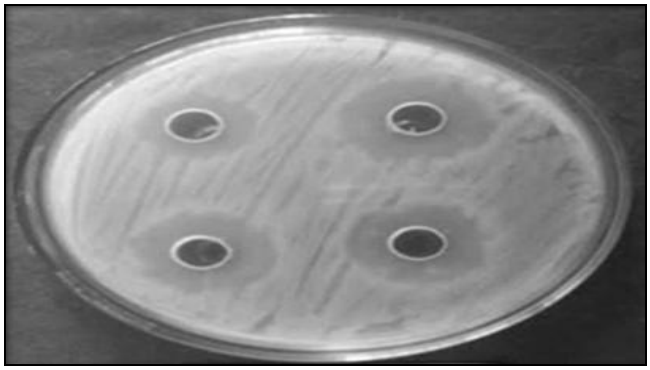


FIG. 25: STUDIES OF ANTIMICROBIAL

Observation *Ex-vivo* Permeability Study (ICZ-DG1): Franz Diffusion cell (Mehdi, 2006) was used to carry out the examination of drug permeation through cellophane membrane. In the donor cell one gram of gel having free or entrapped drug was kept whereas, the receptor cell received twelve millilitres 12ml of the receptor phase. From the receptor cell, about 0.5ml samples were taken at appropriate periods and the drug was studied with the help of a spectrophotometer (Shimadzu UV-1700 PC, Shimadzu corporation, Japan).

TABLE 27: *EX-VIVO* PERMEABILITY STUDY OF OBSERVATION DATA OF FORMULATION (ICZ-DG 1)

S. no.	TIME (hr.)	*Ex-vivo* Permeability of Formulation (ICZ-DG 1) % CDR
1.	0	0
2.	2	16.23
3.	4	28.56
4.	6	42.12
5.	8	55.65
6.	10	66.55
7.	12	76.36
8.	14	84.36

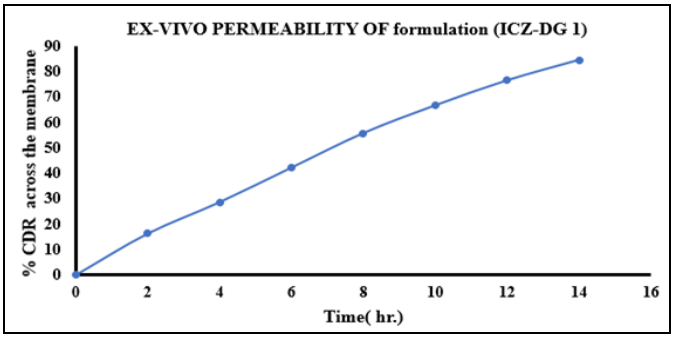
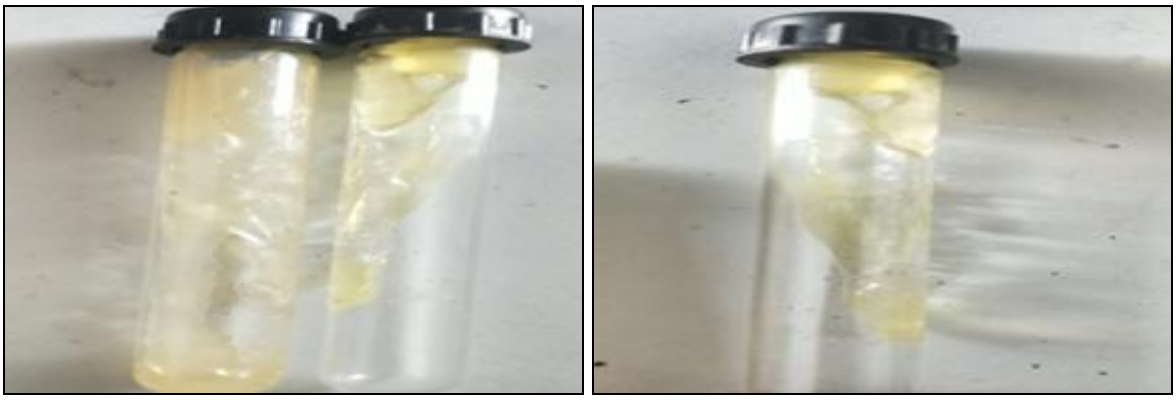


FIG. 26: *EX-VIVO* PERMEABILITY STUDY OF FORMULATION (ICZ-DG 1)

Stability Study of Formulation (ICZ-DG 1): The dendrigel formulation was kept in tightly closed glass vials. The sample were kept in dark in amber colours vials and light in colorless vials at 0°C,

Room temperature 25°-30°C and 45°C for a period of eight weeks. The samples were analyzed initially and periodically after every week for up to seven weeks for change in viscosity, pH, color, 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.



BEFORE STORAGE **AFTER 8 WEEKS STORAGE**

FIG. 27: STABILITY OF FORMULATION (ICZ-DG 1) PRE AND POST STORAGE CONDITION

TABLE 28: OPTIMIZED STABILITY DATA OF TEMPERATURE BASED (5±3^{0}C)

S. no.	(Time) Month	B-4.0 Gpamam	ICZ-4.0 G Pamam Complex (F1)
1	0	78.56	141.3
2	1	81.56	144.65
3	2	85.89	148.32

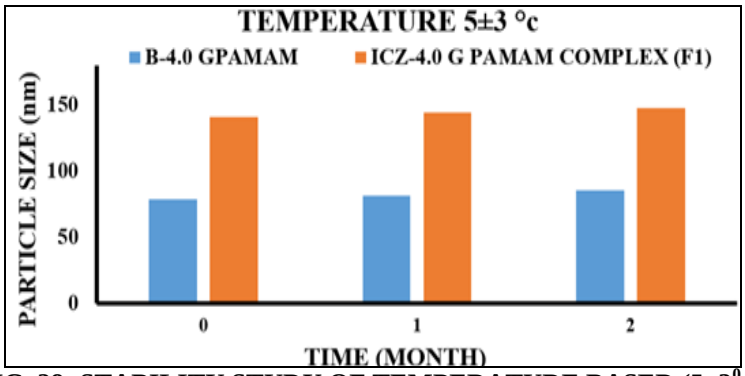


FIG. 28: STABILITY STUDY OF TEMPERATURE BASED (5±3^{0}C)

TABLE 29: OPTIMIZED STABILITY DATA OF TEMPERATURE BASED (40±2^{0}C)

S. no.	**Time** (Month)	B-4.0 G Pamam	ICZ-4.0 G Pamam Complex (F1)
1	0	78.56	141.3
2	1	102.56	169.65
3	2	125.89	188.32

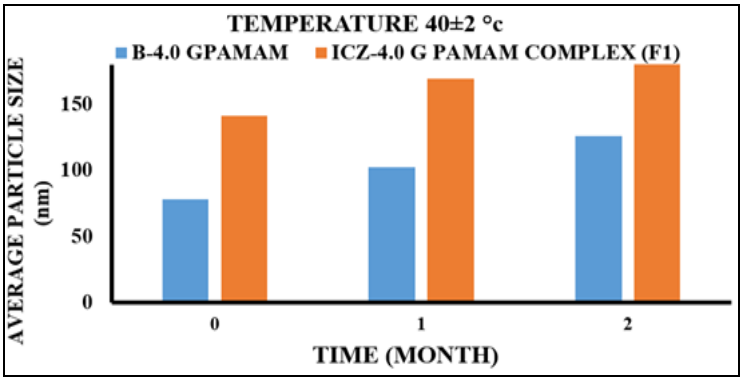


FIG. 29: STABILITY GRAPH STABILITY STUDY OF TEMPERATURE BASED (40±20C)

TABLE 30: OPTIMISED STABILITY DATA OF PH AND SPREAD-ABILITY BASED

S. no.	Time (Days)	pH	Spread-ability
1	0	5.5	5.56
2	1	5.6	5.4
3	2	6.01	5.02

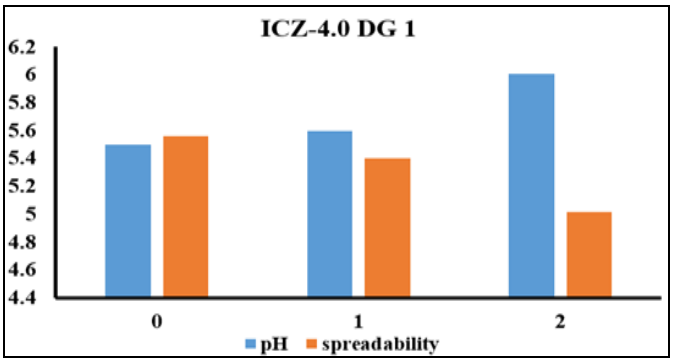


FIG. 30: STABILITY GRAPH OF B-PAMAM AND ICZ-4.0 G PAMAM COMPLEX (F1)

SUMMARY AND CONCLUSION: This study was to develop the PAMAM dendrimer-mediated transdermal formulation of Itraconazole and explore the potential of PAMAM dendrimers as a novel drug delivery to enhance skin permeation and to avoid the serious toxic effects caused by oral. Hydrophobicity and poor solubility of drugs/bioactive is a major drawback encountered during product development and presents a major hindrance in the achievement of satisfactory drug-loaded PAMAM dendrimer-based formulation and their evaluation. Itraconazole is practically insoluble in water, dendrimers play a major role in the solubilization of poorly water-soluble drugs because dendrimers have both in their nature (Hydrophilic and hydrophobic).

However, the improvement of drug permeability through the skin has always been a difficult problem, because of the barrier function of human skin epithelia to exogenous substances. Therefore, the major challenge in topical administration is to increase the drug concentration and the drug penetration into the skin. It increases the solubility of low water-soluble drugs. A dendrimer is used in drug delivery applications because it contains hydrophobic branching and hydrophilic core so it is suitable for all types of drugs and shows better absorbance in both (hydrophilic and lipophilic) mediums. It produces sustained release action for the drug that has a low plasma half-life. It increases the bioavailability of the drug by increasing its solubility. They have broad applicability to interfere with protein-protein interaction.

The surface of dendrimers provides an excellent platform for the attachment of cell-specific ligands, and solubility modifier and reduce the interaction with macro molecule from the body defence system. Pre-formulation studies on the drug were carried out to confirm its identity and purity and to confirm that there are no signification barriers to the proposed formulation of the drug with the enlisted polymer and excipients. The studies besides confirming the compound's identity and purity, showed no significant interaction of the drug with the polymer used.

The physical appearance of the drug sample was identical to that mentioned in official monographs. The absorption maxima of the drug were matched with standards. FTIR spectrum of Itraconazole confirms the presence of different groups and matches the values as reported in research articles the melting point determined of Itraconazole was found to be 165±1. Itraconazole was found to be soluble in ethanol, DMSO, methanol, and dichloromethane and practically insoluble in water.

The lipophilicity of Itraconazole was determined as a log P value which is found to be 5.906 in n-octanol/Distilled water. Itraconazole shows it is lipophilic in nature. The U.V. Spectroscopy of Itraconazole in methanol was observed. The max was found to be 263.0 nm. The UV spectra showed no significant interaction of the drug with any of the polymers to be used in the formulation.

The calibration curves of the drug were prepared using UV at 263.0 mm in calibration curve Itraconazole in PBS pH 4.7 and methanol. The calibration curves were found to be linear in the range of 1-10 pg/ml and a straight line was obtained in all cases. The correlation coefficient values were greater than 0.9978 indicating good linearity of the data. Observation of zeta potential of PAMAM The surface charge of PAMAM dendrimer was found to be 13.9mv due to the presence of the terminal amine group. The enhancement in solubility may be attributed to molecular encapsulation. The formation of the

complexes between drug molecules and dendrimers was characterized by the FTIR spectra of these complexes, showing the appearance of the bond formed between the functional groups of the dendrimers. We had done the formulation using 4.0 G dendrimer diluted 10 ml with distilled water in order to make 1% of dendrimer in varying proportions 1-6 ml was mixed with the 10 mg itraconazole drug to kept for 24 hours to make the dendrimer drug complex. Then it is loaded into the gel for the preparation of dendrigel. Work deals with the formulation and evaluation of Itraconazole topical dendrigel using a gelling agent like carbapol-934 and dendrimers were used as drug carriers in the formulation.

Optimized batch F1- F6 shows yellow colour in appearance. pH of all six batches was found between 5.56, 6. pH of optimized batch (ICZ-DG1) F1 was found 5.5 which lies in the normal PH of the skin. Viscosity is an important parameter for characterizing the gels as it affects spreadability. extrudability and release of the drug, all the formulated batches should increase viscosity as the concentration of gelling agent increased optimized batch ICZ-DG1shows ideal viscosity. All the prepared groups indicate consistency in drug content. Optimized batch ICZ-DG1 shows 93.03 % and ICZ-DG1 formulation entrapment 96.07% drug content which shows uniform drug dispersion in dendrigel. In vitro release studies were carried out by using acetate buffer pH 5.5 release of Itraconazole from all prepared dendrigel formulations was found to be satisfactory. The drug release model follows as well as the zero-order release model. Overall, the study objectives are fulfilled based on the experimental results. It can be inferred that the nanocarrier of dendrimer-itraconazole can be a budding advent to attain applicable transdermal dosage form. Additionally, the nanocarrier of dendrimer itraconazole can be utilized as an individual medicine treatment for the remedy of diverse topical fungal infections. The considerably competent loading extent of ICZ on dendrimers could therefore approach a manner to prepare novel dendrimer-based dendrigel intended for the therapy of diverse topical fungal infections.

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