

Received on 10 June 2019; received in revised form, 12 July 2019; accepted, 28 July 2019; published 31 August 2019

PAMAM DENDRIMER MEDIATED FORMULATION, DEVELOPMENT AND CHARACTERIZATION OF FLURBIPROFEN

Nagendra Singh*, Devender Singh, Ram Narayan Prajapati and S. K. Prajapati

Department of Pharmaceutics, Institute of Pharmacy, Bundelkhand University, Jhansi - 284128, Uttar Pradesh, India.

ABSTRACT: The aim of the present study was comprehensively establishing the utility of PAMAM dendrimers in solubility enhancement of Flurbiprofen. It can be concluded that PAMAM dendrimers in the solubility of Flurbiprofen but also helped to localize the drug at the site of action and the drug can provide better therapeutic efficacy with lower dose by reducing its adverse effect. From the solubilization study, it was observed that the increase in solubility was due to the dendrimeric complexation or encapsulation. From this paper, it can also be concluded that formulation containing more amount of dendrimer concentration provides a higher flux than formulation containing a lower amount of dendrimer. This may be due to an increased thermodynamic activity of the drug in dendrimeric formulation at a lower concentration of dendrimer.

Keywords: PAMAM dendrimers, Flurbiprofen, Formulation development, and characterization

Correspondence to Author:

Nagendra Singh

Research Scholar (M. Pharm.), Department of Pharmaceutics, Institute of Pharmacy, Bundelkhand University, Jhansi - 284128, Uttar Pradesh, India.

E-mail: nssat123@gmail.com

INTRODUCTION: Dendrimers are highly branched polymer of nano size. These are three dimensional, monodisperse, globular macromolecules having the high number of functional groups on their surface. Dendrimers are synthesized by a series of repetitive steps. The idea of repetitive growth with branching was first reported by Vogtle¹. This was followed by independent development of the divergent, macromolecular synthesis of "true dendrimers" by^{2, 3}. They were the first who coined the term dendrimer for these macromolecules and described the synthesis of poly (amidoamine) (PAMAM) dendrimer in a detailed manner.

Then a convergent synthetic method of dendrimer synthesis was introduced by Frechet⁴. Afterward, there was an explosion of scientific interest in dendrimers as a new class of polymeric nanomaterial because of its various cutting edge structural advantages over simple polymers. Dendrimers are highly branched polymers which have special characteristics like different functional end groups, higher density, and lesser viscosity⁵⁻⁸. Due to these unique features this class of polymeric nanomaterial has various applications in different fields like as drug delivery⁹⁻¹³, dendrimer-based nanomedicine¹⁴, gene delivery¹⁵, light-harvesting¹⁶, dendritic nanomaterials¹⁷, electrode design¹⁸, solubility enhancers¹⁹ and for various biotech applications²⁰.

Structure of Dendrimers: Dendrimers are built form a starting atom, such as nitrogen, after a repeating series of chemical reactions, carbon, and other elements was added into it; produce a spherical branching structure.

	<p>QUICK RESPONSE CODE</p>
	<p>DOI: 10.13040/IJPSR.0975-8232.IJLSR.5(8).125-37</p>
<p>The article can be accessed online on www.ijlsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJLSR.5(8).125-37</p>	

As the process repeats, the result is a spherical macromolecular structure. Dendrimers possess three distinguished architectural components, namely a central core which is either a single atom or an atomic group, generation in which branching emanating from the core composed of repeating units, which is radially in position and many terminal functional groups generally located in the exterior of the macromolecules. Dendrimers are playing an efficient role in enhancing the solubility of poorly aqueous soluble drugs²¹. Dendrimers are synthesized by a divergent approach using ethylenediamine and methyl acrylate. Dendrimers consist of three major parts-

- Initiator core
- Interior layer composed of repeating units, radically attached to the interior core
- Exterior (terminal functionality) attached to the outermost interior generations²².

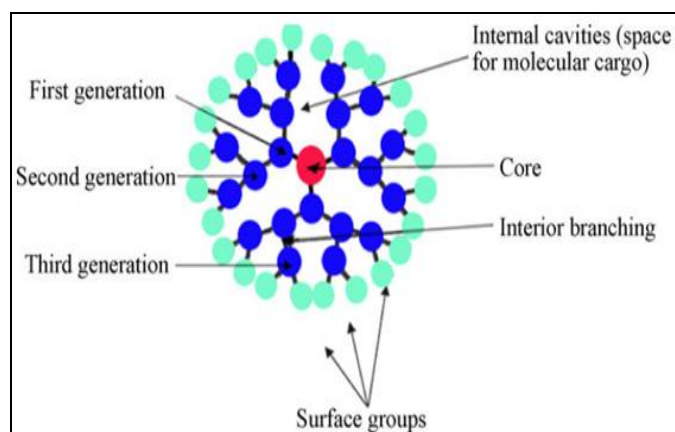


FIG. 1: STRUCTURE OF DENDRIMERS

MATERIALS AND METHODS: Various chemicals, solvents, instruments, and glassware are used during project work are listed below in **Table 1** and **Table 2**.

Pre-formulation Studies:

Identification of Drug: Infrared Spectroscopy (IR), Ultra Violet (UV) and melting point are used for identification and purity of drug sample. Flurbiprofen was identified by various techniques which are the following:

Organoleptic Property of the Drug: Drug (Flurbiprofen) was Physically Characterized based on color, odor, and taste. All these parameter were recorded and compared with standard.

TABLE 1: LIST OF CHEMICALS

S. no.	Chemicals	Source
1.	Flurbiprofen	Yarrow Chem, Mumbai
2.	Ethylenediamine	CDH, New Delhi
3.	Methyl acrylate	CDH, New Delhi
4.	Methanol	Shreya Life Science, Roorkee
5.	n-octanol	CDH, New Delhi
6.	Buffering agent	CDH, New Delhi
7.	Deionized water	CDH, New Delhi
8.	Carbopol 934p	CDH, New Delhi

TABLE 2: LIST OF EQUIPMENT

S. no.	Equipment	Source
1.	UV-spectrophotometer	Model 1700, Shimadzu, Japan
2.	FTIR spectrophotometer	Perkin Elmer, Singapore, Pvt., Ltd.
3.	Melting point apparatus	Jyoti Scientific Industries, Gwalior
4.	Electronic balance	Roy Electronics, India
5.	Franz diffusion apparatus	Jyoti Scientific Industries, Gwalior
6.	Magnetic stirrer	Jyoti Scientific Industries, Gwalior
7.	Mechanical shaker	Jyoti Scientific Industries, Gwalior
8.	Brookfield viscometer	Jyoti Scientific Industries, Gwalior
9.	pH meter	VSI-IB, VSI Electronics, India
10.	Thermometer	Jyoti Scientific Industries, Gwalior
11.	Rotatory Vacuum Evaporator	Microtech Scientific Instruments, New Delhi

Identification of Drug by U.V. Spectroscopy: 10 mg of Flurbiprofen was taken in volumetric flask and volume make up to 100 ml with methanol, 10 ml of above solution is diluted with methanol up to 100 ml, and then it was scanned between 200 nm to 400 nm. The solution showed an absorbance maximum at 245 nm in **Fig. 2**.

Identification of Drug by I.R. Spectroscopy: The FTIR spectral analysis was carried out by the pressed pellet technique. IR spectrum of any substance gives information about the group present in a specific substance. An IR spectrum of the drug was taken using (KBr potassium bromide) pellets. Small quantities of drug sample were mixed with oil, and a drop was placed between KBr pellets and spread uniformly. The pellets were placed in the holder, and an infrared spectrum was taken. The range of scanning was 400-4000 cm^{-1} . Different peaks in the infrared spectrum were interpreted for the presence of the various group in

the structure of the drug. The observed IR spectra of the drug are shown in **Fig. 3**, and **Fig. 4**.

Melting Point Determination: The temperature at which the solid and liquid phases are in equilibrium is called the melting point of substance. The melting point of a drug can be measured using three techniques:

- Hot stage microscopy
- Capillary melting method
- Differential scanning calorimeters thermal analysis

A melting point determination is a good first indication of purity since the presence of a relatively small amount of impurities can be detected by lowering as well as widening in the melting point range. The melting point of Flurbiprofen was determined by a capillary method using melting point apparatus. 10 mg of the drug sample was weighed accurately and placed into a capillary tube. The tube was placed in the melting point apparatus and was heated to a temperature below 5-10 °C of the temperature at which powder started to melt, and temperature at which the sample started to melt was observed.

Solubility Determination: The solubility study of drug was performed in different solvents (*e.g.*, methanol, ethanol, acetone, ethyl acetate, 0.1N HCl). A known quantity of the drug was transferred in a series of different solvents having volume 5 ml in test tubes. Excess amount of drug was added to different solvents till the solution became saturated and these test tubes were shaken by mechanical shaker for 1 h under constant vibration at a constant temperature. After this period, the solution was centrifuged. The supernatant was then analyzed by U.V. spectrophotometer (Shimadzu-1700, Japan) at λ_{\max} 245nm with appropriate dilution. Three determinations were carried out before each sample to calculate the solubility of Flurbiprofen in different solvents **Table 3**.

Determination of Partition Coefficient of Drug: Partition coefficient of a drug is a measure of its hydrophilic-lipophilic balance (HLB). It can be defined as the ratio of the unionized drug distributed between the organic and aqueous phase

in equilibrium. Partition coefficient (solid water quotient of drug distribution) has several applications which are relevant to pre-formulation.

- Solubility both in aqueous and in mixed solvents.
- Drug absorption *In-vivo*: applied to a homologous drug series for structure-activity relationships.
- Partition chromatography: choice of column (HPLC) and choice of mobile phase (eluent).

The partition coefficient of the drug sample was determined by the shake flask method. An equal volume of water (or phosphate buffer pH 6.8) and *n*-octanol were taken in glass stoppered flask and added accurately weight amount (10 mg) of Flurbiprofen. The mixture was shaken for 24 h at room temperature with the help of wrist action shaker.

The two phases are separated by separating funnel, and the aqueous phase was analyzed spectrophotometrically at 273 nm for drug content after appropriate dilution. The drug concentration in *n*-octanol phase was determined by subtracting the amount in aqueous phase from the total quantity of the drug. The partition coefficient P is expressed as by the equation:

$$\text{Log } P = \frac{\text{Concentration in } n\text{-octanol}}{\text{Concentration in water}}$$

n-octanol is used because the properties of *n*-octanol are thought to resemble those of lipid bilayer membranes. It has therefore been suggested that distribution of chemicals into *n*-octanol simulates, to a certain extent, their ability to passively diffuse across biological membranes **Table 4**.

The procedure of Standard Curve Preparation: Standard Stock Solution of Flurbiprofen: Accurately weighed 10 mg of Flurbiprofen and was dissolved in 100 ml of methanol, from this stock solution, 10 ml was withdrawn and transferred into 100 ml volumetric flask. Volume was made with methanol to get a standard stock solution containing 100 µg/ml.

Standard Graph of Flurbiprofen: Form this standard stock solution, a series of dilution (10, 20,

30, 40, 50 µg/ml) were prepared using methanol. The absorbance of these solutions was measured spectrophotometrically against a blank solution of methanol at 245 nm for Flurbiprofen **Fig 5**.

Formulation Development & Characterization:

Synthesis of PAMAM Dendrimer:

Method of preparation of PAMAM Dendrimers:

Synthesis of PAMAM dendrimer at lab scale was carried out by the divergent method. Synthesis of EDA core PAMAM dendrimers consists of two steps: (a) Michael addition of primary amine (EDA in the very first step) to methyl acrylate (b) Amidation of formed multitester (tetra ester at the very beginning) of EDA.

Synthesis of 0.5 Generation PAMAM

Dendrimer: Ethylene diamine and methyl acrylate were mixed separately in methanol. Both of the above solutions were mixed and kept aside for several hours. The reaction was monitored through copper sulfate test for progress, and the solvent was removed under vacuum at 50 °C. The semisolid mass formed was 0.5 generation PAMAM dendrimer.

Synthesis of 1 Generation PAMAM Dendrimer:

0.5G PAMAM and EDA was mixed separately with a small quantity of methanol respectively. Both of the above solutions were mixed and kept aside for several hours. The reaction was monitored through copper sulfate test, and the solvent was removed under vacuum at 50 °C. The semisolid mass formed was 1.0 generation PAMAM dendrimer.

Synthesis of Further Generation PAMAM

Dendrimer: 1.0G PAMAM and Methyl acrylate mixed separately with a small quantity of methanol. Both of the above solutions were mixed and kept aside for several hours. The reaction was monitored through copper sulfate test. After completion of the reaction, the solvent was removed under vacuum at 50 °C. The semisolid mass formed was 1.5 generation PAMAM dendrimer.

The further generations were prepared by reacting a small quantity of the previous generation alternatively with ethylenediamine and methyl acrylate respectively. EDA was dissolved in methanol, and the solution was cooled in an ice bath down to -30 °C. In another reaction vessel,

half-generation dendrimer (multi ester) was dissolved in methanol, and the flask was also cooled down at -30 °C.

The cold multi ester solution was gradually added to the EDA solution at a rate keeping the temperature below -25 °C. After the addition was completed, the mixture was allowed to warm to room temperature, and the reaction continued for several days. The excess of EDA and solvents were removed under vacuum at the temperature below 50 °C. As a result, the pale-amber-colored syrup was obtained. The reaction was carried out using methanol as a medium. Redistilled EDA was used whenever required.

To avoid direct light and moisture, the RBF was corked tightly, and it was well covered with carbon black paper and silver foil. The whole reaction was carried in the dark at 25 °C. The reactions were followed by removal of excess reagents by rotary vacuum evaporator under reduced pressure at 55-60 °C, in every step. Addition reaction was allowed to complete in two days (48 h), whereas the amidation reaction took place four days for completion. Reiteration of this reaction sequence results in the synthesis of half and full generation intermediates, respectively.

Characterization of Pamam Dendrimer:

The solubility of PAMAM Dendrimers: 0.5% w/v dendrimers were kept in amber-colored vials in an aqueous and non-aqueous solvent such as water, methanol, ethanol, acetone, diethyl ether, chloroform, cyclohexane, dimethyl sulfoxide, *etc.* Previously accurately weighed the quantity of dendrimers were taken with all solvents separately and kept in an incubator for 6hr followed by 3hr equilibrium period to check the solubility of dendrimers **Table 5**.

Identification of PAMAM Dendrimers by UV-

Spectroscopy: Absorption maxima (λ_{max}) were recorded for 4.0G dendrimers and surface modified dendrimers. The dendrimer solution was analyzed over the UV range between 200 to 600 nm in a UV visible spectrophotometer (UV-vis 1601 Shimadzu, Japan) to analyze the effect of solubilization **Fig 6**.

FT-IR Spectroscopy: FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. FTIR (a

mathematical process) is required to convert the raw data into the actual spectrum. FT-IR (Perkin Elmer 2000, Department of Chemistry, IIT Kanpur) analysis was performed using the KBr pellet method **Fig 7**.

NMR Spectroscopy: Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy or magnetic resonance spectroscopy (MRS), is a spectroscopic technique to observe local magnetic fields around atomic nuclei. NMR (JEOL 400 MHz spectrometer, 1999, IIT Kanpur), the PAMAM dendrimers was solubilized in deuterated methanol and analyzed at 300 MHz **Fig 8**.

Differential Scanning Calorimetry (DSC): DSC is a thermo-analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. The result of a DSC experiment is a curve of heat flux versus temperature or versus time. In the DSC process, the changes in endothermic peak were performed and analyzed through apparatus (Perkin Elmer, CDRI Lucknow) **Fig 9**.

Formulation Development:

Drug Loading: The loading of the drug was carried out by dissolving 10 mg of Flurbiprofen in 10 ml methanol, which gave a concentration of 1000 µg/ml, 1 ml of this solution was mixed with 100 mg of 4.0 G PAMAM dendrimers. The final volume was made up to 10 ml. The resultant final concentration of drug in the formulation was 100µg/ml, and of dendrimers, it was 10000 µg/ml **Fig 10** and **11**.

Determination of Effect of PAMAM dendrimer Concentration: For determination of the effect of PAMAM dendrimer concentration on the solubility of Flurbiprofen by adding an excess amount of Flurbiprofen into screw-capped amber-colored vials containing various concentrations (0.5% to 0.4% w/v) of G4-PAMAM dendrimer in double-distilled water. The separately excess drug was added in a vial containing only double distilled water and used as a control. The vials were shaken at room temperature for 24 h in a shaking bath and allowed to stand for 12 h to attain equilibrium.

After equilibration, the solutions were filtered through 0.45 µm membrane filter. Aliquots (0.5 ml) of the filtrate were withdrawn from each vial and were diluted with the appropriate quantity of double-distilled water. These samples were analyzed for drug content by UV-spectrophotometer at 245 nm using distilled water as a blank. The calculated results are recorded and expressed as drug concentration vs. dendrimer concentration **Table 8**.

Preparation of Gel Base: Carbopol 934p (1, 2, 3, 4, 5% w/w) and purified water were taken in a beaker and allowed to soak for 24 h. To this required amount of Flurbiprofen loaded 4.0G PAMAM dendrimer was dispersed in water and then carbopol 934p was then neutralized with sufficient quantity of triethanolamine. Glycerine as a moistening agent, methylparaben and propylparaben as preservatives were added slowly with continuous gently stirring until the homogenous gel was formed ²³.

Preparation of Dendrigel: Based on effects of PAMAM dendrimers concentration on the solubilization of Flurbiprofen, F5 was selected for the formulation of gel. 0.4% of dendrimer (0.4 g of 4.0G PAMAM dendrimers were diluted to 100 ml with distilled water to make 0.4% of dendrimer). Carbopol 934p was used in varying proportion (1-5 gm) with other ingredients for the dendrigel formulation as per **Table 9**.

Evaluation of Dendrigel: The prepared Flurbiprofen dendrigel formulations were inspected visually for their color, homogeneity, consistency, pH, spreadability, viscosity.

Percentage Yield: The empty container was weighed in which the gel formulation was stored then again, the container was weighed with gel formulation. Then subtracted the empty container weighed with the container with gel formulation then it gives the practical yield. Then the percentage yield was calculated by the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Determination of Drug Content: Weighed 10gm of each gel formulation were transferred in 250 ml of the volumetric flask containing 20 ml of methanol and stirred for 30 min. The volume was

made up to 100 ml and filtered. 1 ml of the above solution was further diluted to 10 ml with methanol, and again 1 ml of the above solution was further diluted to 10 ml with methanol. The absorbance of the solution was measured spectrophotometrically at 245 nm. Drug content was calculated by the following formula²⁴.

$$\text{Drug content} = \text{Absorbance} / \text{slope} \times \text{Dilution factor} \times 1/1000$$

Determination of pH: Weighed 50 gm of each gel formulation were transferred in 10 ml of the beaker and measured it by using the digital pH meter. The pH of the topical gel formulation should be between 3-9 to treat skin infections.

Spreadability: Spreadability was determined by a modified wooden block and glass slide apparatus. The apparatus consisted of a wooden block with a fixed glass slide and a pulley. A pan was attached to another glass slide (movable) with the help of a string. For the determination of spreadability measured amount of gel was placed in the fixed glass slide, the movable glass slide with a pan attached to it, was placed over the fixed glass slide, such that the gel was sandwiched between the two slides for 5 min. Now about 50 g of weight was added to the pan. Time taken for the slides to separate was noted. The spreadability of the gel formulation was determined, by measuring the diameter of 1 gm gel between horizontal plates (20 × 20 cm²) after 1 min. The standardized weight tied on the upper plate was 125 gm²⁵. Spreadability was determined using the following formula:

$$S = M.L/T$$

Where S is the spreadability in g.cm/s, M is the mass in grams, and T is the time in sec.

Viscosity Estimation: The viscosity of gel was determined by using a Brookfield viscometer DVII model with a T-Bar spindle in combination with a helipath stand.

a) Selection of Spindle: Spindle T 95 was used for the measurement of viscosity of all the gels.

b) Sample Container Size: The viscosity was measured using 50gm of gel-filled in a 100ml beaker.

c) Spindle Immersion: The T-bar spindle (T95) was lowered perpendicular in the center, taking

care that spindle does not touch the bottom of the jar.

d) Measurement of Viscosity: The T-bar spindle (T95) was used for determining the viscosity of the gels. The factors like temperature, pressure and sample size, etc. which affect the viscosity was maintained during the process. The helipath T- bar spindle was moved up and down giving viscosities at a number of points along the path. The torque reading was always greater than 10%. The average of three readings taken in one minute was noted as the viscosity of gels²⁶.

Ex-vivo Skin Permeation Studies: *Ex-vivo* skin permeation studies were performed on a Franz diffusion cell with an effective diffusion area of 2.54 cm² and 45 ml of receiver chamber capacity, using cellophane membrane. The membrane was washed with phosphate buffer before further use. Initially, the donor compartment was empty, and the receiver chamber was filled with phosphate buffer. The receiver fluid was stirred with a magnetic rotor at a speed of 600 rpm, and the assembled apparatus was placed in a hot air oven where the temperature was maintained at 37 °C. The whole phosphate buffer saline (PBS) pH 6.8 was replaced with a fresh one after every 30 min to stabilize the skin. It was found that the receiver fluid showed a negligible peak area after 2.5 hr and beyond indicating complete stabilization of the membrane. After complete stabilization of the membrane, 2 ml dendritic formulation was placed into the donor compartment and sealed with paraffin film to provide occlusive conditions. Samples were withdrawn at regular intervals (0, 1, 2, 3, 4, 5, 6) h filtered using 0.45 µm membrane filter and analyzed for drug content by UV at 245 nm **Fig 12**.

Stability Study: The dendrigel formulation was kept in tightly closed glass vials. The sample was kept in the dark (amber colors vials) and light (in colorless vials) at 0 °C, Room temperature (25 - 30 °C) and 45 °C for a period of seven 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.

RESULTS:

Pre-formulation Studies: Flurbiprofen was procured from Yarrow Chem, Mumbai. It was identified and characterized as per the identification test is given in the Indian Pharmacopoeia (2010) and United State Pharmacopoeia.

Identification of the Drug:**Organoleptic Property:**

- Colour: White or slightly yellow
- Odor: Odourless
- Taste: Bitter
- Appearance: White or slightly yellow crystalline

Identification of Drug by U.V Spectroscopy:

Flurbiprofen was scanned between 230 nm to 360 nm. The solution showed an absorbance maximum at 245 nm. (Spectra of Flurbiprofen in methanol shown below in Fig. 2).

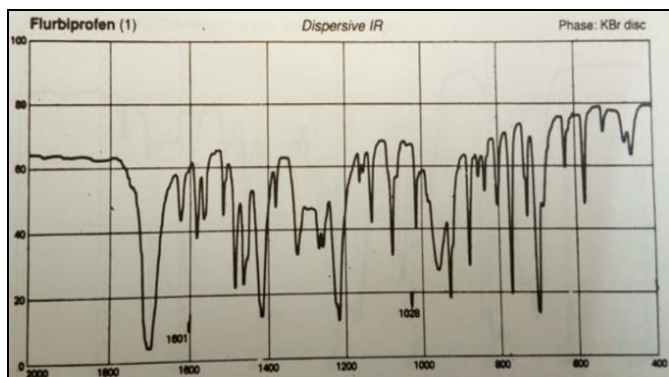


FIG. 3: IR SPECTRA ANALYSIS OF STANDARD FLURBIPROFEN (I.P. 1996)

The peaks of sample drug are very close to the peaks of the standard drug, so it indicates the sample of Flurbiprofen is authentic.

Determination of Melting Point: Melting point range of the drug having from 110 °C to 112 °C and Melting point of the drug was found to be 111 °C. So the drug was found to be suitable for the formulation.

Solubility Study of the Drug: It was found that Flurbiprofen was soluble in most of the organic solvent and insoluble in water, as shown in Table 3.

TABLE 4: PARTITION COEFFICIENT OF FLURBIPROFEN

Water: n- octanol (ml)	Conc. of the drug in water (µg/ml)	Conc. of drug in n-octanol (µg/ml)	Log P
1:1	4.52	18.8	4.16

FTIR Spectroscopy: The IR spectrum of the obtained sample was done acc. to the procedure mention in material & method portion and complied with the IR spectrum of the reference standard of Flurbiprofen. IR spectra of sample drug show similar characteristic peaks. Fig. 3 shows IR spectra analysis of standard drug Flurbiprofen and Fig. 4 shows the IR spectra of sample drug, and the interpretation is shown in the table.

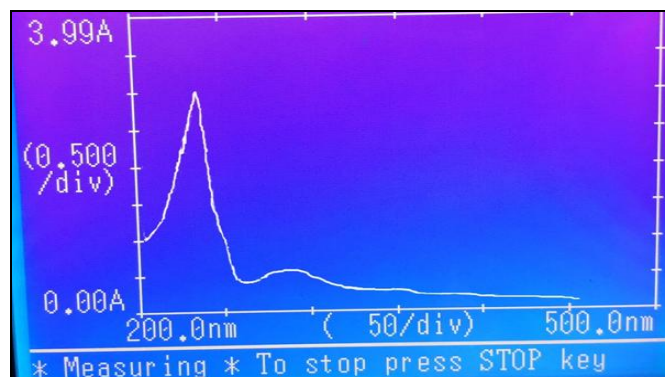


FIG. 2: UV SCAN OF FLURBIPROFEN IN METHANOL (245 nm)

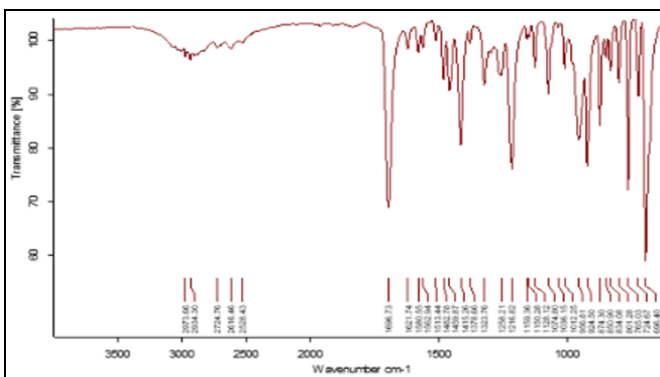


FIG. 4: IR SPECTRA ANALYSIS OF FLURBIPROFEN (SAMPLE)

TABLE 3: SOLUBILITY STUDY OF DRUG

S. no.	Solvent	Interference
1	Water	Insoluble
2	Ethanol	Freely Soluble
3	Methanol	Soluble
4	Chloroform	Soluble
5	Acetone	Soluble

Partition Coefficient of Flurbiprofen: Partition coefficient of the drug was determined by the procedure mention material & method portion and shown in Table 4. The value of log P was found out to be 4.16. The standard value of log P for the drug is 4.20.

Preparation of Calibration Curve of Flurbiprofen: Calibration curve of Flurbiprofen was prepared as per the procedure mentioned above.

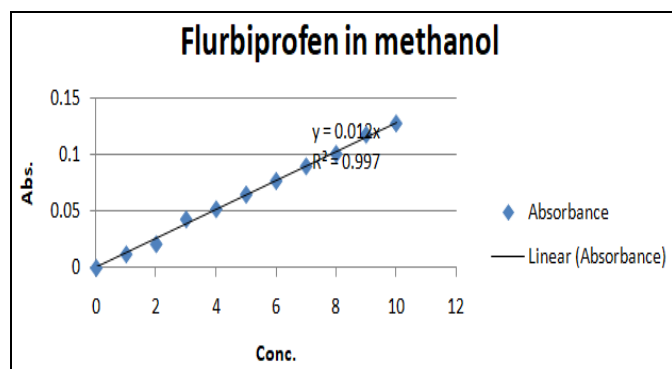


FIG. 5: CALIBRATION CURVE OF FLURBIPROFEN IN METHANOL AT λ_{\max} 245 nm

Formulation Development & Characterization:

Synthesis: The PAMAM dendrimers were synthesized using ethylenediamine as initiator core and methyl acrylate as repeating unit. Synthetic progress involves Michael addition and exhaustive amidation to complete the cycle. An increasing amount of reactant in every progressive step was

added to avoid an incomplete reaction and hence to improve the yield. Completion of the reaction was confirmed by the copper sulfate solution reaction.

The whole generation gave a purple color, whereas half-generation gave a deep blue color, due to copper chelation at the terminal group of dendrimers. All the steps were found to be complete by the color reactions. Progress of Synthesis and differentiation of 3.5G and 4.0G was confirmed by UV, IR, and NMR spectroscopy. The formed dendritic systems were subjected to FTIR spectroscopy. The IR peaks confirmed the progress of PAMAM dendrimer.

Characterization of Pamam Dendrimer:

The solubility of PAMAM dendrimers: For determination of the effect of PAMAM dendrimer concentration on the solubility of Flurbiprofen, adding an excess amount of Flurbiprofen into screw-capped amber-colored vials containing various concentrations (0.05% to 0.4% w/v) of G4-PAMAM dendrimer in distilled water.

TABLE 5: SOLUBILITY BEHAVIOUR OF PAMAM DENDRIMERS

S. no.	Solubility	Solvent
1	Soluble	Dimethylacetamide, ethanol, ethylene glycol, methanol, and water
2	Insoluble	Chloroform, cyclohexane, methyl cyclohexane, pentane and toluene

TABLE 6: PHYSICAL CHARACTERISTIC OF PAMAM DENDRIMERS GENERATIONS

S. no.	Generation of Dendrimers	Colour as Concentrated	Physical state	Identification by CuSO ₄ test
1	-0.5 G	Light yellow	Oily	Deep blue
2	0.0G	Light greenish-yellow	Oily	violet
3	0.5 G	Greenish-yellow	Viscous liquid	Deep blue
4	1.0G	Light Reddish yellow	Thick oily	violet
5	1.5 G	Reddish yellow	Very viscous liquid	Deep blue
6	2.0G	Light Reddish yellow	Very viscous oily	violet
7	2.5 G	Reddish yellow	Very viscous liquid	Deep blue
8	3.0G	Light reddish yellow	Very viscous oily	violet
9	3.5 G	Honey like yellow	Very viscous liquid	Deep blue
10	4.0G	Honey like yellow	Viscous glassy mass	violet

TABLE 7: PHYSICO-CHEMICAL CHARACTERISTIC OF PAMAM DENDRIMERS

S. no.	Generation of Dendrimers	λ_{\max} of PAMAM dendrimers (nm)
1	-0.5 G	282.0
2	0.0G	277.5
3	0.5 G	285.5
4	1.0G	277.0
5	1.5 G	283.5
6	2.0G	277.5
7	2.5 G	283.5
8	3.0G	276.5
9	3.5 G	283.5
10	4.0G	282.0

Concentrations were analyzed for drug content by UV-spectrophotometer at 245 nm using distilled water as a blank. It results that the solubilization system/solubility is directly proportional to concentration, which means if the concentration is increased; the solubilization system/system is also increased. 0.5 % w/v dendrimers were kept in amber colored vials in an aqueous and non-aqueous solvent such as water, methanol, ethanol, acetone, diethyl ether, chloroform, cyclohexane, dimethyl sulfoxide, etc. Previously accurately weighed the quantity of dendrimers were taken with all solvents separately and kept in an incubator for 6 h followed by 3 h equilibrium period to check the solubility of dendrimers.

Identification of PAMAM Dendrimers by UV-Spectroscopy: Absorption maxima (λ_{\max}) were recorded for 4.0G dendrimers and surface modified dendrimers. The dendrimer solution was analyzed over the UV range between 230 to 360 nm in a UV visible spectrophotometer (UV-vis 1601 Shimadzu, Japan) to analyze the effect of solubilization.

FT-IR Spectroscopy: Infrared spectrum of Flurbiprofen confirms the presence of different

groups. The peaks obtained in the IR spectrum matched with the IR spectrum given in the official pharmacopeia. The FTIR spectrum of Flurbiprofen confirmed characteristic absorption band of C-C group at 1215.9. In IR spectrum peaks of N-H stretch for primary amine were obtained at 3218.61 cm^{-1} which was due to NH_2 periphery of 4.0G PAMAM dendrimers, C-H stretching at 2834.22 cm^{-1} , C=O stretch of the carbonyl group at 1650.81.

Half generation carboxyl terminated shows intense peaks in the -C=O region while full generations show intense peaks in the -N-H stretch for primary amine. Appearance - disappearance and reappearance of characteristic peaks provide the proof of synthesis.

NMR Spectroscopy: In NMR spectra terminal amino group proton peaks ($-\text{CH}_2\text{NH}_2$) were obtained at 3.84 ppm, and 2.68, carbonyl methylene proton ($-\text{CH}_2\text{C}=\text{O}$) were obtained at 2.93, 3.03 ppm. Characteristic shifts in NMR spectra of 3.5G PAMAM dendrimers was due to terminal groups of $-\text{COOCH}_3$ at 3.73 ppm, and 4.0G PAMAM dendrimers were for terminal groups of $-\text{NHCH}_2\text{CH}_2\text{NH}_2$ at 3.84 ppm.

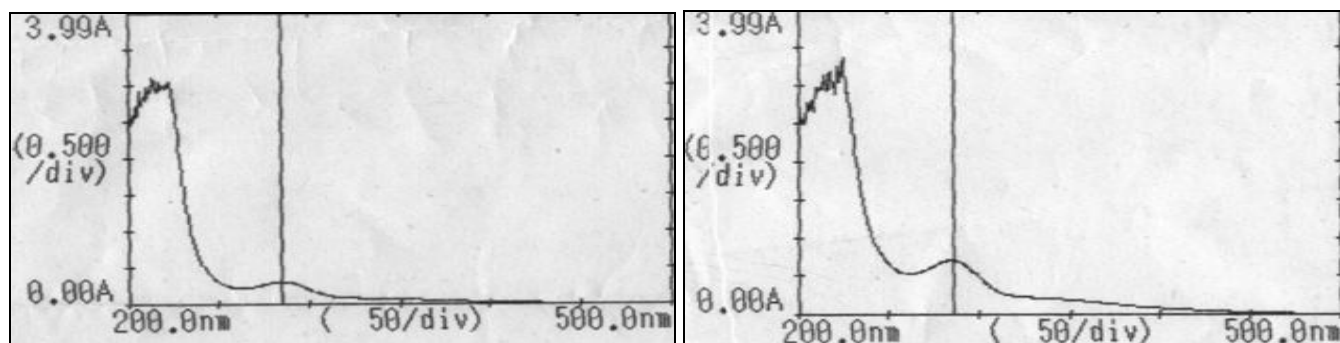


FIG. 6: UV SPECTRA OF 3.5G AND 4.0G PAMAM DENDRIMERS

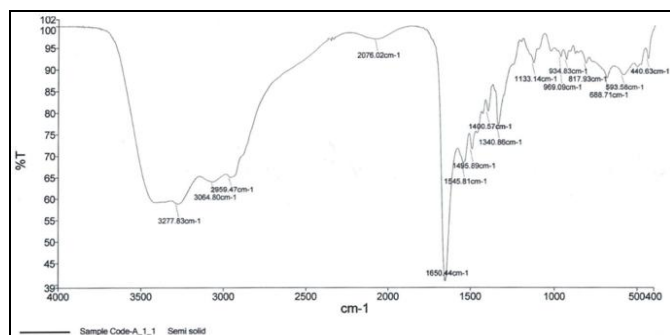


FIG. 7: FTIR SPECTRA OF 4.0G PAMAM DENDRIMERS

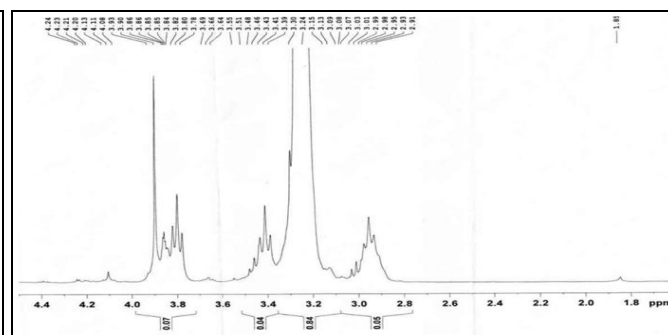


FIG. 8: NMR SPECTRA OF 4.0G PAMAM DENDRIMERS

Differential Scanning Calorimetry (DSC): Differential Scanning Calorimetry (DSC) results in the changes in endothermic peak from 120.03 to

120.56 $^{\circ}\text{C}$ were observed, which shows the change in the structure of PAMAM dendrimers.

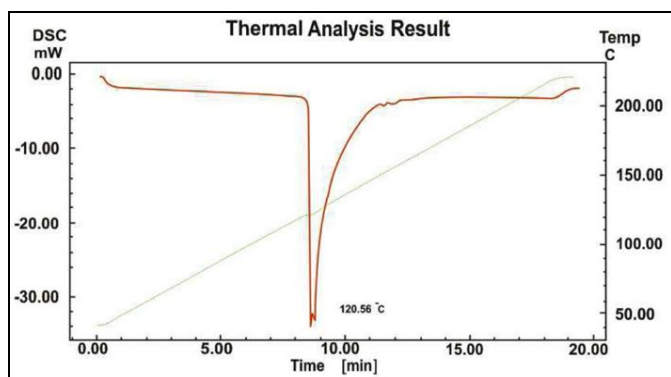


FIG. 9: DSC SPECTRA OF 4.0G PAMAM DENDRIMERS

Formulation Development:

Drug Loading:

1) The drug loading confirmed by IR spectra. The change in characteristic peaks of 4.0G PAMAM dendrimers indicates the complex formation.

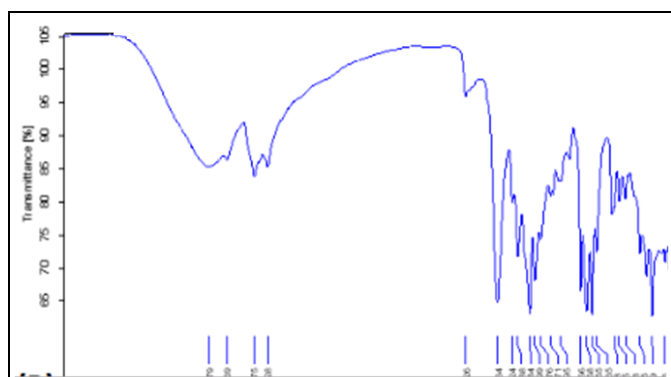


FIG. 10: IR SPECTRA OF DRUG LOADED 4.0G DENDRIMER

2) The drug loading was further confirmed by NMR spectra. The characteristic shifts in NMR spectra of 4.0 G PAMAM dendrimers were changed in drug-complexed NMR spectra, which indicates the complexation.

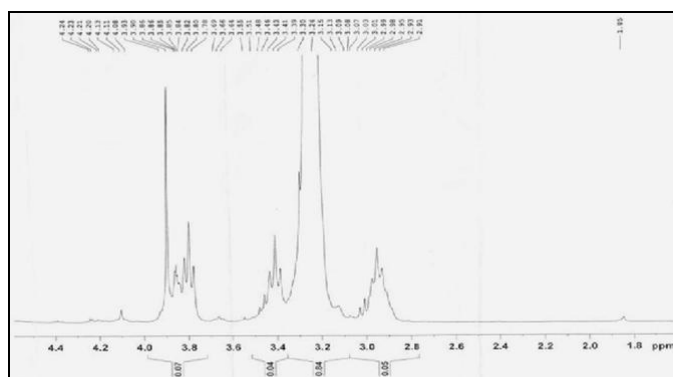


FIG. 11: NMR SPECTRA OF DRUG LOADED 4.0G DENDRIMER

Determination of Effect of PAMAM dendrimer Concentration:

TABLE 8: EFFECT OF PAMAM DENDRIMER CONCENTRATION ON THE SOLUBILIZATION OF FLURBIPROFEN

Solubilizing system	Flurbiprofen solubility at various concentration of PAMAM dendrimers (µg/ml)
Distilled water	14.04
RAA ₁	23.72
RAA ₂	31.48
RAA ₃	65.58
RAA ₄	91.05

Where, RAA₁, RAA₂, RAA₃, RAA₄ represent 0.05%, 0.1%, 0.2%, 0.3% and 0.4% respectively aqueous solutions of corresponding generations of PAMAM dendrimers

Preparation of Gel Base:

Preparation of Dendrigel: Based on effects of PAMAM dendrimers concentration on the solubilization of Flurbiprofen, DAA₄ was selected for the formulation of gel. Various formulation (F1, F2, F3, F4, F5) were developed by using a suitable polymer (carbopol 934p) and penetration enhancer.

TABLE 9: FORMULATION DESIGN FOR DENDRIGEL

Ingredients	F1	F2	F3	F4	F5
Flurbiprofen loaded 4.0G PAMAM dendrimer (mg)	2	2	2	2	2
Methanol (ml)	4	4	4	4	4
Carbopol (gm)	1	2	3	4	5
Water (ml)	50	48	47	46	45.5
Propylene glycol	48	46.5	46	45.5	45

Developed formulations of Flurbiprofen dendrigel were evaluated for the physiochemical parameters such as drug content, viscosity, spreadability.

Evaluation of Dendrigel:

Percentage Yield: After studying and performing the gel formulation, percentage yield was found to

be 94.549%, 97.034%, 86.99, 97.002% and 98.00% for formulation F1, F2, F3, F4, and F5 respectively.

Drug Content: Drug content was found to be 79.49%, 82.16%, 88.04%, 91.10% and 95.03% for formulation F1, F2, F3, F4 and F5 respectively.

pH Determination: pH determination was found to be 4.5, 4.4, 4.2, 4.3 and 4.5 for formulation F1, F2, F3, F4 and F5 respectively.

Spreadability: Spreadability was found to be 11.55, 11.8, 10.64, 10.59, and 10.20 for formulation F1, F2, F3, F4, and F5 respectively. Spreadability test which was carried out for all the formulations, spreadability of the gel formulation was decreases with the increases in the concentration of the polymer. The spreadability is very much important as to show the behaviour of gel comes out from the tube.

Viscosity Estimation: Viscosity plays a major role in the stability of gel. For all 5 formulations viscosity is determined by Brookfield viscometer. F5 has more viscosity, and less extrudability compare to all formulations F4 is having ideal viscosity and extrudability.

Ex-vivo Skin Permeation Studies: *Ex-vivo* study was performed using the best gel formulation F5 because it has more solubility behavior rather than other four formulations. From the study, it has been found that solubility is directly proportional to concentration. Therefore, F5 shows greater solubility than other formulation.

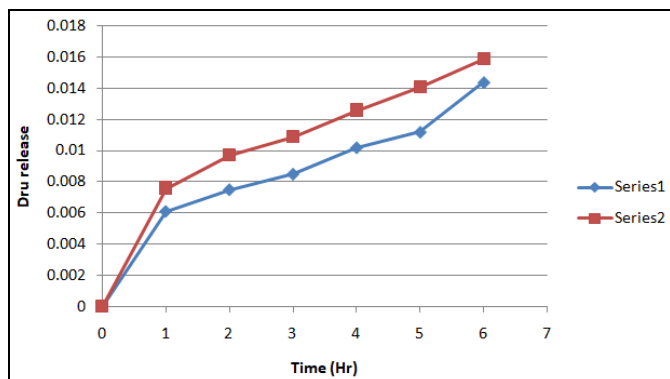


FIG. 12: EX-VIVO SKIN PERMEATION OF DENDRIMERIC GEL FORMULATION AND FLURBIPROFEN. Series 1-F5, Series 2-flurbiprofen

DISCUSSION: Present study aims to develop the PAMAM dendrimer mediated transdermal formulation of Flurbiprofen and explore the potential of PAMAM dendrimer as novel drug delivery to enhance the skin permeation and to avoid the serious toxic effects caused by oral and other topical formulation available. Dendrimer drug delivery is in its infancy; it offers several attractive features. This novel class of polymers and their

derivatives exhibit unique physicochemical and biological properties, which have great potential for use in a variety of applications. It has greater flexibility in design. High control over branching length, shape and size allow modification according to the delivery system, so these serve as an ideal carrier for drug and other application.

The objective of the present study was comprehensively establishing the utility of PAMAM dendrimers in solubility enhancement of Flurbiprofen. It can be concluded that PAMAM dendrimers in the solubility of Flurbiprofen but also helped to localize the drug at the site of action and the drug can provide better therapeutic efficacy with lower dose by reducing its adverse effect. From the solubilization study, it was observed that the increase in solubility was due to the dendrimeric complexation or encapsulation.

Dendrimers are used in drug delivery application because it contains hydrophilic branching and hydrophilic core, so it is suitable for all type of drug and shows better absorbance in both (hydrophilic and lipophilic) medium. It produces sustain release action for the drug that has low plasma half-life. It increases the bioavailability of the drug by increasing its stability. The solubility efficacy of dendrimers comparable to sodium lauryl sulfate (SLS), cyclodextrin, micelles is more because dendrimers could solubilize as low as 5×10^{-7} mol/lit while SLS and micelle can solubilize concentration as 8.1×10^{-3} mol/lit.

PAMAM dendrimers have emerged as a promising drug delivery device in the modern medicine system owing to their unique characteristics such as predictable and nanoscopic size range, low polydispersity, and globular structure. Their branched architecture, multivalent surfaces, and high drug loading capacity make these dendrimers as suitable carrier candidates for drugs, genes, targeting moieties, and imaging agents.

Despite the high efficiency of PAMAM dendrimers in the pharmaceutical applications, there is conflicting evidence regarding their biological safety, since cationic PAMAM dendrimers have been shown to possess concentration and generation-dependent cytotoxicity and hemolytic activity, properties that are associated with their

terminal amine groups. Hydrophobicity and poor solubility of drugs is a major drawback encountered during product development and presents a major hindrance in the achievement of satisfactory bioavailability. Flurbiprofen is practically insoluble in water whereas dendrimers play a major role in the solubilization of poorly water-soluble drug. Flurbiprofen is kind of non-steroidal anti-inflammatory drug. Flurbiprofen is used to relieve pain from various conditions such as headaches, muscle aches, tendonitis, dental pain, menstrual cramps, swelling, joint stiffness caused by arthritis, bursitis, and gout attacks.

However, the improvement of the 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 the drug penetration into the skin. It increases the solubility of low water-soluble drug.

Dendrimers are used in drug delivery application because it contains hydrophilic branching and hydrophilic core, so it is suitable for all type of drug and shows better absorbance in both (hydrophilic and lipophilic) medium. It produces sustain release action for the drug that has low plasma half-life. It increases the bioavailability of the drug by increasing its solubility. The solubility efficacy of dendrimers comparable to sodium lauryl sulfate (SLS), cyclodextrin, a micelle is more because dendrimer could solubilize as low as 5×10^{-7} mol/lit while SLS and micelle can solubilize concentration as 8.1×10^{-3} mol/lit.

The physical appearance of the drug sample was identified to that mentioned in official monographs. The absorption maxima (λ_{\max}) of the drug Flurbiprofen was matched with standards. FTIR spectrum of Flurbiprofen confirmed the presence of different groups and matched with the values as reported in the official pharmacopeia. The melting point determined of Flurbiprofen found to be 111 °C. Flurbiprofen was found to be soluble in methanol, ethanol, 0.1N HCl, ethyl acetate, acetone and practically insoluble in water. The lipophilicity of Flurbiprofen was determined as log P value, which is found to be 4.16 in n-octanol. Flurbiprofen shows it is lipophilic.

The U.V. spectroscopy of Flurbiprofen in methanol was observed. The λ_{\max} was found to be 245 nm. The UV spectra showed no significant interaction of the drug with any of the polymers to be used in the formulation.

In the solubilization studies, the effect of dendrimer concentration, pH of the solution of Flurbiprofen was investigated. Flurbiprofen with a pKa of 4.15 exhibits pH-dependent solubility. The pH dependence of the Flurbiprofen with dendrimers was investigated based on pH/solubility profiles. In the presence and absence of dendrimers as a function of pH. As can be seen, both in the presence and absence of dendrimer, Flurbiprofen exhibited pH-dependent solubility. Its solubility increases with increasing pH.

The addition of dendrimers results in a solubility profile as a function of pH similar in shape to that obtained in the absence of the complexing agent. However, it shows a significant rise in the solubility of Flurbiprofen at all pH values tested. The formation of complexes between drug molecules and dendrimers were characterized by the FTIR spectra, showing the appearance of the bond formed between the functional groups of the drug and dendrimers. *In-vitro* release of pure Flurbiprofen and drug-dendrimer formulations were performed in phosphate buffer saline pH 6.8 by the help of Franz diffusion cell.

The content of dendrimer concentration also played an important role in the formulation, and it affected the skin permeation rate directly. The transdermal flux of Flurbiprofen gel was too less this may be due to the large content of Flurbiprofen may have substantially reduced partition coefficient between the skin and vehicle for the drug, which can counteract the benefit of the increased concentration gradients effects and thereby actually decrease the transdermal flux.

CONCLUSION: From the above study, it can also be concluded that formulation containing more amount of dendrimer concentration provides a higher flux than formulation containing a lower amount of dendrimer. This may be due to an increased thermodynamic activity of the drug in dendrimeric formulation at a lower concentration of dendrimer.

ACKNOWLEDGEMENT: NIL

CONFLICTS OF INTEREST: The author declared no conflict of interest.

REFERENCES:

- Buhleier E, Wehner W and Vogtle F: "Cascade" and "Nonskid-Chain-like" syntheses of molecular cavity topologies. *Synthesis* 1978; 2: 155-8.
- Tomalia DA, Dewald JR, Hall MR, Martin SJ and Smith PB: Reprints of the 1st SPSJ International Polymer Conference, Society of Polymer Science Japan, Kyoto 1984: 65.
- Tomalia DA, Baker H, Dewald J, Hall M, Kallos G and Martin S: A new class of polymers: Starburst-dendritic macromolecules. *P Polym J (Tokyo)* 1985; 17: 117-32.
- Hawker CJ and Fréchet JMJ: Preparation of polymers with controlled molecular architecture: A new convergent approach to dendritic macromolecules. *J Am Chem Soc* 1990; 112: 7638-47.
- Fréchet JMJ, Hawker CJ, Gitsov I and Leon JW: Dendrimers and hyperbranched polymers: two families of three-dimensional Macromolecules with similar but clearly distinct properties. *J Macromol Sci Pure Appl Chem* 1996; A33: 1399-25.
- Jikei M and Kakimoto M: Hyperbranched polymers: a promising new class of materials. *Prog Polym Sci* 2001; 26: 1233-85.
- Aulenta F, Hayes W and Rannard S: Dendrimers: A new class of nanoscopic containers and delivery devices. *Eur Polym J* 2003; 39: 1741-71.
- Voit BI and Lederer A: Hyperbranched and highly branched polymer architecture - Synthetic strategies and major characterization aspects. *Chem Rev* 2009; 109(11): 5924-73.
- Florence AT: Dendrimers: a versatile targeting platform. *Adv. Drug Delivery Rev* 2005; 57: 2101-86.
- Gillies ER and Fréchet JMJ: Dendrimers and dendritic polymers in drug delivery. *Drug Discovery Today* 2005; 10: 35-43.
- Emanuele AD and Attwood D: Dendrimer-drug interactions. *Adv Drug Delivery Rev* 2005; 57: 2147-62.
- Wolinsky JB and Grinstaff MW: Therapeutic and diagnostic applications of dendrimers for cancer treatment. *Adv Drug Delivery Rev* 2008; 60: 1037-55.
- Medina SH and El-Sayed MEH: Dendrimers as carriers for delivery of chemotherapeutic agents. *Chem Rev* 2009; 109: 3141-57.
- Majoros IJ and Baker JR: Dendrimer based nanomedicine. Singapore: Pan Stanford Publishing 2008; 1st edition: 440.
- Mintzer MA and Simanek EE: Non-viral vectors for gene delivery. *Chem Rev* 2009; 109: 259-02.
- Adronov A and Fréchet JMJ: Light-harvesting dendrimers. *Chem Commun* 2000; 18: 1701-10.
- Grimsdale AC and Mullen K: The chemistry of organic nanomaterials. *Angew Chem Int Ed* 2005; 44(35): 5592-29.
- Guldi DM and Prato M: Electrostatic interactions by design. Versatile methodology towards multifunctional assemblies/nanostructured electrodes. *Chem Commun* 2004; 22: 2517-25.
- Gupta U and Agashe BH: Dendrimers: Novel polymeric nanoarchitectures for solubility enhancement. *Bio-macromolecules* 2006; 7: 649-6-57.
- Grinstaff MW: Dendritic macromers for hydrogel formation: Tailored materials for ophthalmic, orthopedic, and biotech applications. *J Polym Sci Part A: Polym Chem* 2008; 46(2): 383-00.
- Pillai O and Panchagnula R: Polymers in drug delivery. *Curr Opin Chem Biol* 2001; 5: 447-51.
- New Kone GR: Dendrimers and dendrons: concepts, synthesis, applications. *Int J Pharm* 2001; 4: 25-29.
- Vaibhav D, Dinesh M and Jain N: Melatonin loaded ethanolic liposomes: physicochemical characterization and enhanced transdermal delivery. *Eur J Pharm Biopharm* 2007; 67(5): 398-05.
- Bhalaria M, Naik S and Misra A: A novel delivery system for antifungal drugs in the treatment of topical fungal disease. *Indian J Exp Biol* 2009; 47(5): 368-75.
- Sera UV and Ramana MV: *In-vitro* skin absorption and drug release-a comparison of four commercial hydrophilic gel preparations for topical use. *The Indian Pharmacist* 2006; 73: 356-60.
- Jain S, Padsalg BD, Patel AK and Moale V: Formulation development and evaluation of fluconazole gel in various polymer bases. *Asian J Pharm* 2007; 1(8): 63-68.

How to cite this article:

Singh N, Singh D, Prajapati RN and Prajapati SK: PAMAM dendrimer mediated formulation, development and characterization of Flurbiprofen. *Int J Life Sci & Rev* 2019; 5(8): 125-37. doi: 10.13040/IJPSR.0975-8232.IJLSR.5(8).125-37.

All © 2015 are reserved by International Journal of Life Sciences and Review. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Play store)