Regular Article

Design, Development and Evaluation of Dendritic Architechture for the Delivery of Ofloxacin against Resistance Producing Strains

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Dendrimer represents hyperbranched, monodisperse, three-dimensional macromolecules with host-guest entrapment properties having defined molecular weight. Dendrimer allows defined control of size, shape and position of functional groups. For the above said reason, dendrimers have become more popular application in many fields.The present study was aimed at developing and exploring the use of novel PEGylated 0.5G EDA-PPI (poly propyeleneimine) dendritic architecture for the delivery of an antibacterial drug, Ofloxacin. The dendritic architecture was synthesized by double Michael addiction reaction using ethylenediamine as a core moiety and the synthesized system was PEGylated using PEG-4000 and the Ofloxacin was loaded by equilibrium dialysis method into the system. The prepared Ofloxacin loaded PEGylated 0.5G EDA-PPI dendritic architecture was evaluated for FTIR studies, solubility studies, drug entrapment efficiency, *in-vitro* drug release studies and anti-bacterial assay. The results showed that there was enhanced rate of drug release, drug solubility with significant antimicrobial activity compared to plain Ofloxacin. The tablets of Ofloxacin loaded PEGylated 0.5G EDA-PPI dendrimer were prepared by direct compression method and evaluated for various parameters such as hardness, thickness, weight variation, drug content and *in-vitro* drug release studies. The *in-vitro* drug release of Ofloxacin loaded 0.5G EDA-PPI dendrimer compared with that of marketed Ofloxacin tablet. The results revealed that there is enhanced rate of dissolution of Ofloxacin loaded PEGylated 0.5G EDA-PPI dendrimer than that of marketed Ofloxacin tablet formulation.

Key Words: Dendrimers, Ofloxacin, PEGylation, Dendron.

INTRODUCTION

Development of bacterial resistance to currently available antibacterial drugs by either new mutations or the exchange of genetic information, that is, putting old resistance genes into new hosts is very common. In many healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming the norm, complicating treatment and increased both human morbidity and financial costs.

About forty percent of newly developed drugs are rejected by the pharmaceutical industry and will never benefit a patient because of poor bioavailability due to low water solubility and/or cell membrane permeability. New delivery technologies could help to overcome this challenge. Nanostructures with uniform and well-defined particle size and shape are of eminent interest in biomedical applications because of their ability to cross cell

membranes and to reduce the risk of premature clearance from the body. The high level of control over the dendritic architecture (size, branching density, surface functionality) makes dendrimers ideal carriers in these applications. These have unique characteristics including monodispersity and modifiable surface functionality, along with highly defined size and structure constituted of three distinct domains: (i) a central core which is either a single atom or an atomic group having at least two identical chemical functions, (ii) branches emanating from the core, constituted of repeat units having at least one branch junction, whose repetition is organized in a geometrical progression that results in a series of radially concentric layers called generations, and (iii) many terminal functional groups, generally located in the exterior of the macromolecule, which play a key role in the properties. This makes these polymers attractive candidates as carriers in drug delivery applications. Many commercial small drug molecules with anticancer, anti-inflammatory, and antimicrobial activity have been successfully associated with Dendrimers (Sushma *et al.*, 2010). Drug delivery can be achieved by coupling a drug to polymer through one of two approaches. Hydrophobic drugs can be complexed within the hydrophobic dendrimer interior to make them water-soluble or drugs can be covalently coupled onto the surface of the dendrimers. The loading ability of drug molecules and other bioactive agents can be altered by varying dendrimer generations, the water solubility, biodistribution, circulation time in blood and therapeutic efficiency of drugs in dendrimer-based formulations can be tuned by varying dendrimer surface components, the release of drugs from dendrimer scaffolds can be controlled by using different degradable linkers between dendrimers and drugs, and the specific accumulation of the dendrimer-based therapeutics can be achieved by further modifying the dendrimers with targeting moieties. These properties together prove dendrimer perfect candidate in the design of new drug delivery systems (Mishra, 2011).

Dendrimers are repeatedly branched macromolecules or nano-sized, radially symmetric molecules with well-defined, homogeneous and mono-disperse structure consisting of tree-like arms or branches. The name comes from the~Greek~word Dendron which translates to tree. Dendrimers are globular or spheroid nanostructures that are engineered to encapsulate the molecules into their interior void spaces or to attach onto the surface (Nanjwade *et al.*, 2009)

Although dendrimers have vast application in biomedical field, their use is restricted due to RES uptake, drug leakage, immunogenicity, stability, hemolytic toxicity, hydrophobicity etc. PEGylation of dendrimers can generally overcome these limitations. Polyethylene glycol (PEG) conjugation or linking with the dendritic system is called PEGylation. This can, in addition, increase the solubility of hydrophobic drugs. The term PEG is used for polyethylene glycol, which is often referred for polymer chains with a molecular weight below 20,000. Poly ethylene glycol (PEG) is typically a clear, colorless, odourless substance that is soluble in water, stable to heat, inert to many chemical agents, that does not hydrolyze or deteriorate, and is generally non-toxic, PEG is considered to be biocompatible, which is to say that PEG is capable of coexistence with living tissue or organisms without causing harm. It has been shown that covalent attachment of poly (ethylene glycol) to proteins decreases their immunogenicity and increases their circulation time. Moreover, a number of studies have demonstrated that poly (ethylene glycol) chains grafted to surface of polymer micelles and liposomes suppress their interaction with plasma proteins and cells and prolong their blood elimination half-life. On the basis of these findings, it seems that dendrimers covered with poly (ethylene glycol) grafts are attractive compounds as drug carriers in *in-vivo*. Such molecules are expected to encapsulate drugs in their dendrimer moiety and reveal biocompatibility due to their hydrophilic shell consisting of poly (ethylene glycol) grafts (Virendra *et al.*, 2007; Roberts *et al.*, 2002).

The present study was aimed at developing and exploring the use of novel PEGylateddendritic architecture for the delivery of anti-bacterial drug Ofloxacin.

MATERIALS AND METHODS:

Materials used: PEG 4000, Dimethyl sulphoxide, 4-Dimethyl amino pyridine, Dioxane, Triehylamine and Acrylonitrile was purchased from Himedia laboratories Pvt Ltd, Mumbai, India. Ethylene diamine and N, N- Dicyclohexylcarbodimide was purchased from Lobachemie, India. Cellulose dialysis bag (MWCO 12-14kda) was purchased from Himedia laboratories Pvt Ltd, Mumbai, India. Ofloxacin (OFL) was purchased from Yarrow chemical Mumbai, India. All other materials were used are of analytical grade.

Methods:
Construction of standard calibration curve for Ofloxacin in different solutions
Preparation of stock solution of Ofloxacin (OFL) in Methanol:-
　　50 mg of pure Ofloxacin was weighed and transferred to 100 ml volumetric flask. Ofloxacin was dissolved in 35 ml methanol by gentle shaking and volume was made up to the mark with methanol to obtain final concentration of 500 µg/ml and labeled as 'Std Stock solution I'. From the 'Std Stock solution I' 2 ml of aliquot was pipetted out in a 25 ml volumetric flask and the volume was made up to the mark with methanol to obtain final concentration of 40 µg/ml and labeled as 'Std Stock solution II' (Gandhi *et al.*, 2013).

Determination of λ max of Ofloxacin in Methanol:-
　　From the above Std stock solution II aliquot of 2.5ml was pipetted out in a 10ml volumetric flask and volume was made up to the mark with methanol to obtain 10 µg/ml of drug solution. UV scanning was done for this 10 µg/ml of drug solution between 200-400nm using methanol as a blank in UV/ VIS spectrophotometer. The wavelength maximum was found to be 295nm.

Construction of calibration curve of Ofloxacin in Methanol:-
　　From the above Std stock solution II aliquots of 0.5, 1, 1.5, 2.0, 2.5 and 3ml was pipetted out and transfer to 10 ml volumetric flask and volume was made up to the mark with methanol to get working standards of 2-12µg/ml. The absorbance of these solutions was measured at 295nm against methanol as a blank. Calibration curve was constructed by plotting concentration versus absorbance.

Construction of standard calibration curve of Ofloxacin in 0.1N HCl buffer:-
　　The standard stock solution of Oflaxacin was prepared by transferring accurately weighed 10 mg of drug to 10 ml volumetric flask and dissolving it with 0.1N HCl to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100µg/ml and was kept as the stock solution. The prepared stock solution was diluted with 0.1N HCl solution to get working standard solutions of concentrations 02-12 µg/ml (Arun *et al.*, 2011).

Determination of λ max of Ofloxacin in 0.1N HCl:-
　　The standard solution of Ofloxacin (10 µg/ml) was scanned in the wavelength region of 200-400 nm and the λmax was found to be 297 nm.

**Preparation of standard calibration curve of Ofloxacin in 0.1N HCl:-**
　　The working standard solutions of Ofloxacin were scanned in the UV region and the absorbances were observed against 0.1N HCl solution as blank at 297nm. Finally the calibration curve was plotted between concentration (x-axis) and absorbance (y-axis).

SYNTHESIS OF OFLOXACIN LOADED DENDRITIC ARCHITECHTURE:-
Synthesis of 0.5G EDA-PPI Dendrimers:-
　　The half generation EDA-dendrimer-(CN) 4n (where n is generation of reaction or reaction cycle) was synthesized by double Michael addiction reaction between acrylonitrile (2.5 molar times per terminal NH_2 group of core amine moiety) and aqueous solution of

ethylenediamine or previous full generation dendrimers. After the initial exothermic phase, the reaction mixture was heated at 80ºC for 1 h to complete the addiction reaction. The excess of acrylonitrile was then removed by vaccum distillation (Bhadra*et al.,* 2005)

Ethylenediamine (1mole)   Acrylonitrile (5 mole)   **0.5 G Nitrile terminal**
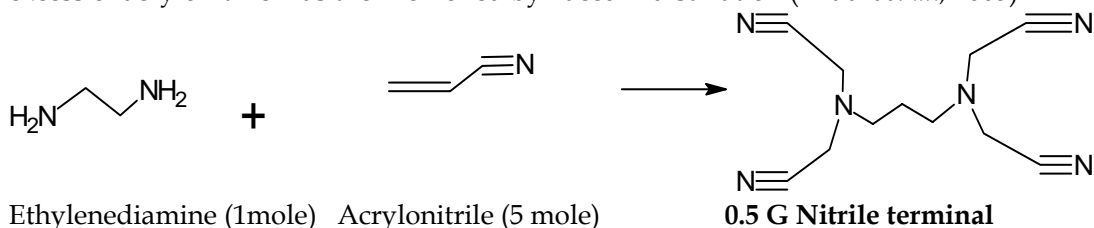
Synthesis of PEGylated 0.5G EDA-PPI Dendrimers:-
To a solution of 0.5G EDA-PPI dendrimer (0.01 mmol) in dimethyl sulfoxide (DMSO) (10 ml), PEG 4000 (0.32 mmol) in DMSO (10 ml) and N, N dicyclohexylcarbodiimide (DCC) (0.32 mmol) in DMSO (10 ml) were added and the solution was stirred for 5 days at room temperature. The product was precipitated by addition of water, filtered and dialyzed (MWCO 12-14 Kda, Himedia, India) against double distilled water for 24 h to remove free PEG 4000, DCC and partially PEGylateddendrimers (Reddy *et al.,* 2012)

Drug Loading in Formulation:-
The loading of drug was carried out by equilibrium dialysis method. The known molar concentrations of PEGylated 0.5G dendrimers was dissolved separately in methanol and mixed with methanolic solution of Ofloxacin (2 mol). The mixed solutions were incubated with slow magnetic stirring (50 rpm) using teflon beads for 24 h. These solutions were twice dialyzed in cellulose dialysis bag against double distilled water under sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically to determine indirectly the amount of drug loaded within the system (Gajbhiye *et al.,* 2008).

CHARACTERIZATION OF PREPARED DENDRITIC ARCHITECHTURE:-
Fourier Transform Infrared Spectroscopy (FTIR):-
The prepared half generation EDA-PPI dendrimer was subjected for FTIR for detection of functional groups which proves the development of dendritic architechture. The drug-loaded dendrimer was also subjected for FTIR to check drug-dendrimer compatibility.

In-vitro Drug Release:-
The drug release from the known amount of OFL loaded PEGylated 0.5G EDA-PPI Dendrimers were determined by using 0.1N HCl as a dissolution medium. The dialysis bag were filled with a known amount of OFL loaded PEGylated 0.5G PPI Dendrimers and were placed in 50 ml of 0.1N HCl at 37±20C with slow magnetic stirring under sink conditions. Aliquots of 1 ml were withdrawn from the external solution and replenished with an equal volume of fresh media. The drug concentration was detected by UV spectrophotometer at 297nm λmax (Gajbhiye*et al.,* 2008).

Antibacterial assay (Agar well diffusion method):-
Two human pathogenic bacteria *Staphylococcus aureus* and *E.coli* were tested against the Ofloxacin loaded dendrimer. The antimicrobial activity of Ofloxacin loaded dendrimer was determined by agar well diffusion method against different bacteria as described. In this method, pure isolate of each bacterium was sub-cultured in nutrient broth at 37ºC for 24h. One hundred microliters of each test bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton Agar plate so as to achieve a confluent growth. The plates were

allowed to dry and a sterile cork borer of diameter 6.0mm was used to bore wells in the agar plates. Subsequently, a 50μL volume of the dendrimer, pure Ofloxacin and drug loaded dendrimer was introduced in triplicate wells into Muller-Hinton Agar plate. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37°C for 24h. The zone of inhibition was recorded to the nearest size in mm. (Reddy *et al.*, 2012).

Determination of solubility of Ofloxacin loaded dendrimercomplex:-

The solubility study of Ofloxacin loaded dendrimer complex was determined by adding an excess amount of dendrimer-drug complex (Equivalent to10 mg of drug) to 10 ml of different solvents in conical flasks. The flasks were kept at 37±0.50 °C on mechanical shaker for 24 hrs to reach equilibrium. The samples were removed from shaker and filtered through whatmann filter paper. The concentration of OFL was determined from samples after suitable dilution by using UV- visible spectrophotometer at 295nm (Shyamala, 2003).

Preparation of tablets containing Ofloxacin loaded dendrimer by direct compression method:-

The tablets of Ofloxacin loaded EDA-PPI dendrimer was prepared by direct compression technique. About 5 gm of Ofloxacin was loaded in 0.5G EDA-PPI dendrimer by method mentioned above. The dried powder was directly used for the preparation of tablets by mixing it with Micro crystalline cellulose, Magnesium stearate and Talc in a geometric ratio and passed through sieve no. 60. The powder blend was subjected to preformulation studies prior to compression.

Table 1. Formulation chart for tablet

Ingredients (mg)	F0	F1
Ofloxacin	200	0
Ofloxacin loaded 0.5 EDA-PPI dendrimer equivalent to 200mg of Ofloxacin	0	300
MCC	150	50
Mg. stearate	25	25
Talc	25	25

Tablet compression

The tablets were compressed by using single-station tableting machine. The compressible weight of each tablet was 500 mg. The tablet was compressed using 6 mm flat-faced punches. The hardness was adjusted to 2.5 to 3.0 kg/cm².

Preformulation Studies of Powder Blend-

Different preformulation studies such as angle of repose, bulk density, tapped density, carr's index, hausner's ratio of powder blend were carried out (Upendra *et al.*, 2011; Lakshmi *et al.*, 2011).

Evaluation of Tablets

Tablets were evaluated for different post compressional parameters such as hardness, thickness, friability, weight variation, *in-vitro* disintegration and *in-vitro* drug release studies (Rakesh *et al.*, 2011; Nagendrakumar *et al.*, 2010; Kathiresan *et al.*, 2010).

Stability study:

The prepared tablet formulation and drug loaded 0.5G EDA-PPI dendrimer were subjected to stability studies as per I.C.H. Guidelines (ICH guidelines Q1A, 2011).

Following conditions were used for stability studies

✶ 30 °C/65 % RH analyzed at a time interval of 30 days till a period of 60 days
✶ 40 °C/75 % RH analyzed at a time interval of 30 days till a period of 60 days

RESULT AND DISCUSSION

Determination of λmax

The drug was identified by the light absorption in the U.V range of 400-200 nm. The absorbance of drug solutions (10mcg/ml) was measured at λmax 295nm with methanol as a solvent and 297nm with 0.1N HCl as solvent. This was in accordance with reported values. The results were shown in Figure 1 and 2.
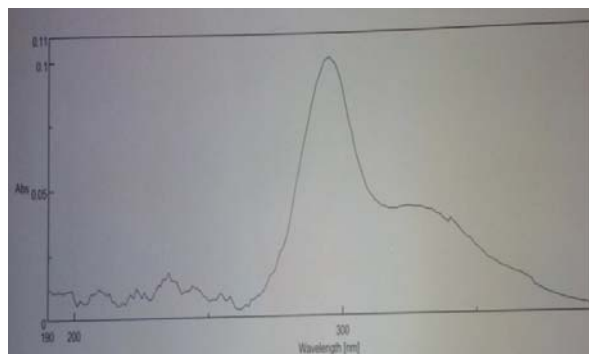

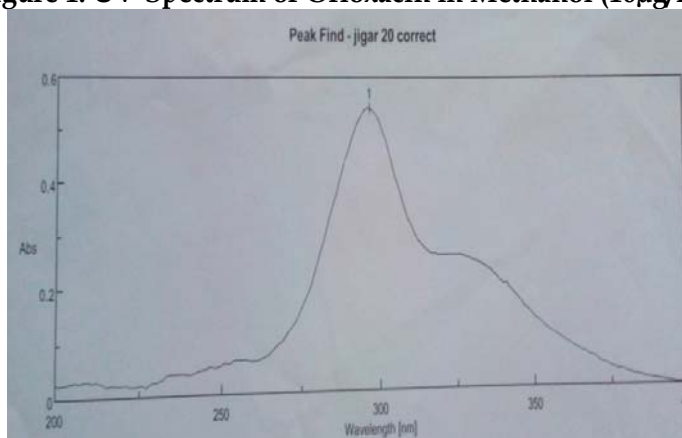**Figure 1: UV-Spectrum of Ofloxacin in Methanol (10µg/ml)**

Figure 2: UV-Spectrum of Ofloxacin in 0.1N HCl (10µg/ml)

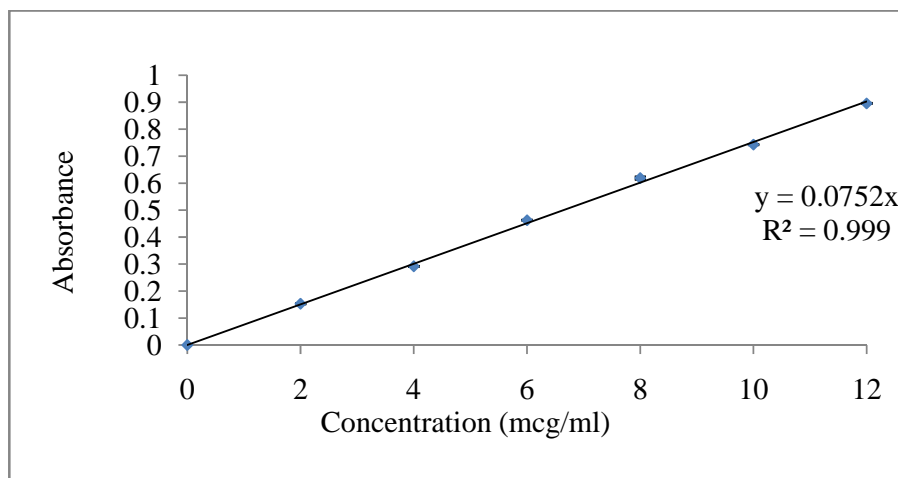**Standard plot of Ofloxacin in Methanol at λmax 295nm**

$$y = 0.0752x$$
$$R^2 = 0.999$$

Figure 3: Standard plot of Ofloxacin in Methanol at 295 nm

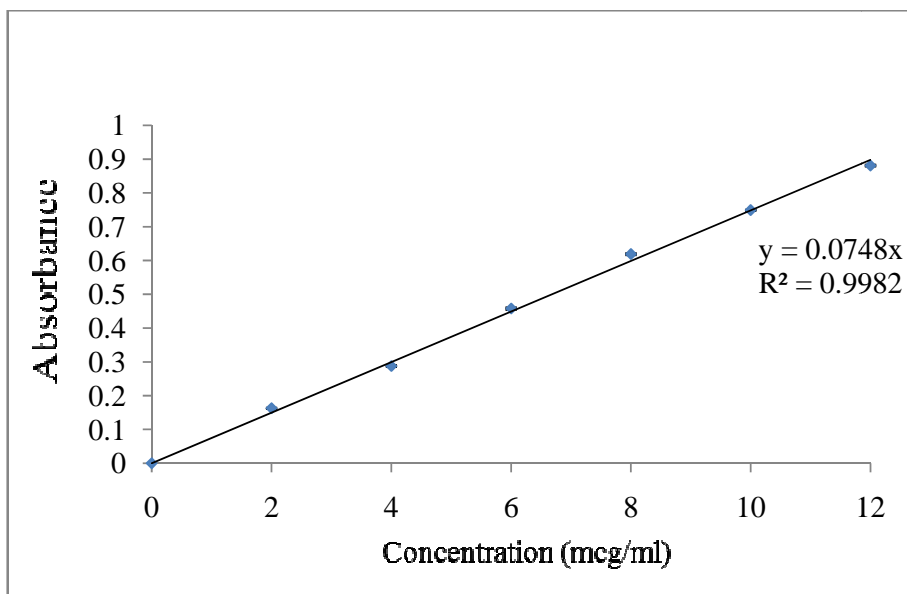**Standard plot of Ofloxacin in 0.1N HCL at λmax 297nm**

Figure 4: Standard plot of OFL in 0.1N HCl at 297nm

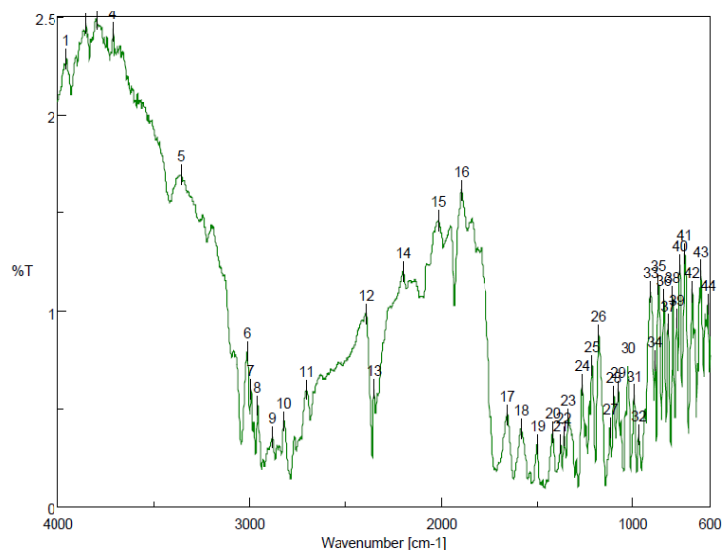**Characterization of Prepared Dendritic Architechture**
FTIR Spectra:-

Figure No. 5: FTIR spectra of Ofloxacin
Table 2: FTIR frequencies in pure drug (Ofloxacin)

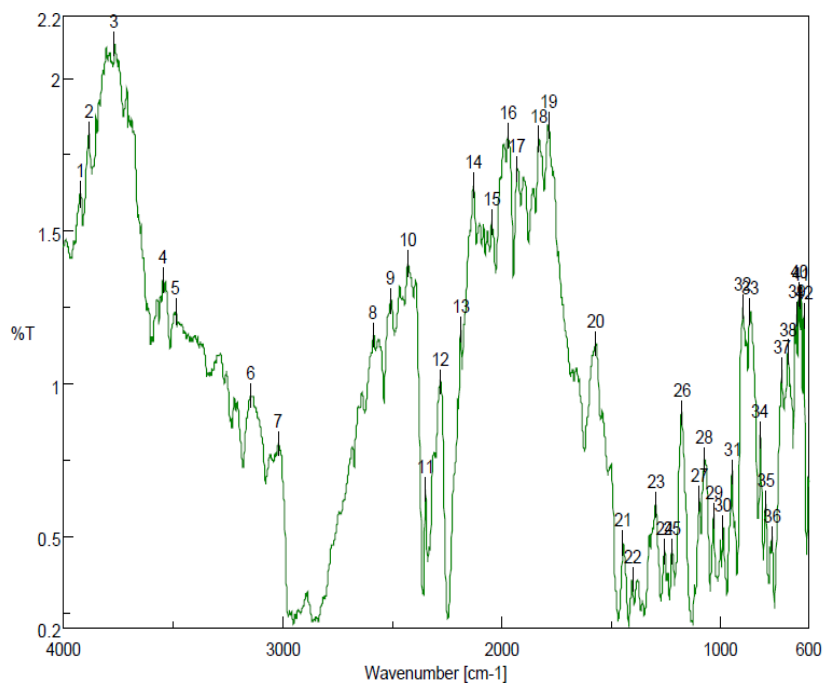| Sr. no	Functional Group	Reported Frequency cm^{-1}	Observed Frequency cm^{-1}
1	C=O	1600-1690	1654
2	C-H (aromatic)	2785	2705
3	C-H (aliphatic)	2960	2957
4	C-F	1000-1100	1027
5	N-H	3400-3500	3361
6	Benzene ring	3070	3012

Figure 6: FTIR spectra of 0.5G EDA-PPI Dendrimer

Table 3: FTIR frequencies in 0.5G EDA-PPI Dendrimer

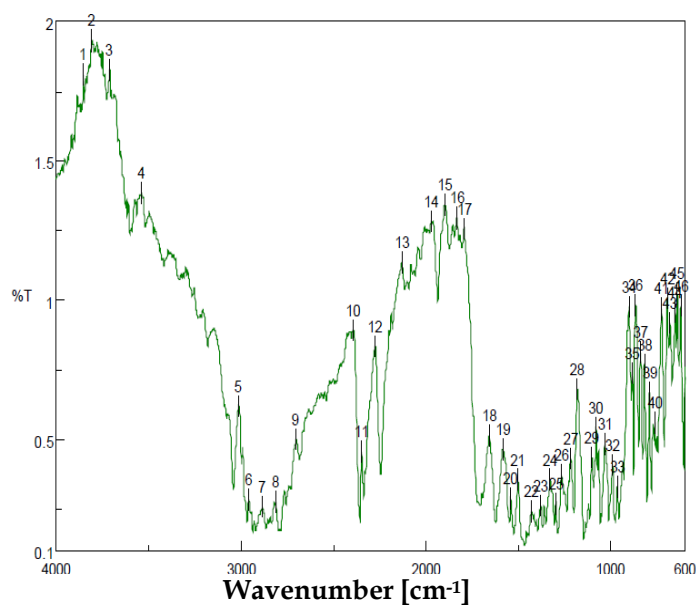| Sr. no	Functional Group	Reported Frequency cm^{-1}	Observed Frequency cm^{-1}
1	CN	2250	2281
2	C-H	1260	1255.43
3	N-H	3400-3500	3488.6

Figure 7: FTIR spectra of Ofloxacin and Dendrimer

Table 4: FTIR frequencies in Ofloxacin and Dendrimer

Sr. no	Functional Group	Reported Frequencycm^{-1}	Observed Frequencycm^{-1}
1	C=O	1600-1690	1658.48
2	C-H (aromatic)	2785	2702.75
3	C-H (aliphatic)	2960	2959.23
4	C-F	1000-1100	1031.73
5	N-H	3400-3500	3540
6	Benzene ring	3070	3014.19

The IR spectra of the drug loaded dendrimer formulation was compared with the standard spectrum of pure drug Ofloxacin and the characteristic peaks associated with specific functional groups and bonds of the molecule and their presence/absence were noted.

The prominent peaks associated with C=0, C-H, C-F, N-H and benzene ring (alone and with dendrimer) were analysed.

The range of peak values were found to be the same indicating that there were no interaction of Ofloxacin with dendrimer confirming the stability of drug in the formulations.

FTIR peak mainly of nitrile at 2281 cm^{-1}, confirmed synthesis of half generation poly propylene imine dendrimer(Figure 6).

Drug loading in the Formulation

Table 5: Drug Loading in the Formulation

Formulation code	% Drug Entrapment
PEGylated 0.5G EDA-PPI Dendrimer	71.59±1.45

The non-covalent interaction between Ofloxacin and PEGylated EDA-PPI 0.5G dendrimes, such as hydrophobic interaction and hydrogen bonding contributed to the physical binding of drug molecules inside the dendritic micelles and surface of PEG layers. PEGylation increases the Ofloxacin loading capacity of the 0.5G PPI dendrimers due to the more interaction of drug and PEG at the peripheral portions of Dendrimers. Number of Ofloxacin moles entrapped in one mole of PEGylated dendritic architecture was found to be 71.59±1.45 moles.

In-vitro **drug release study of Ofloxacin loaded PEGylated 0.5G EDA-PPI dendrimer:-**
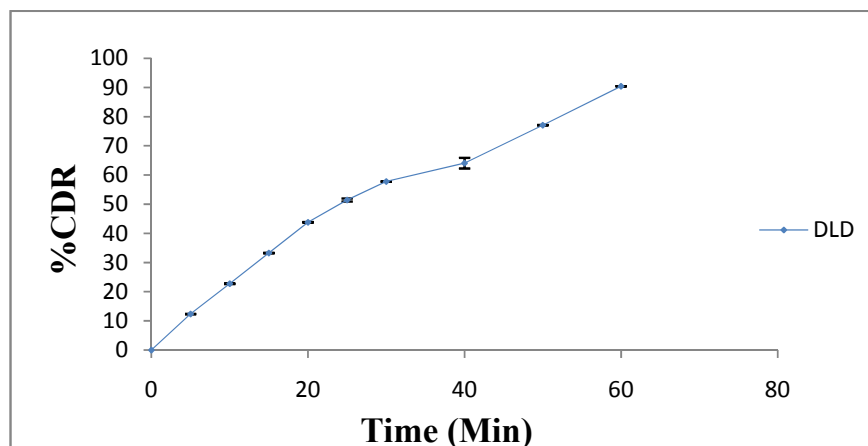
Figure 8.*In vitro* **release of Ofloxacin loaded PEGylated 0.5G EDA-PPI dendrimer**

The *in-vitro* release of Ofloxacin loaded PEGylated PPI 0.5G dendrimer was enhanced from the formulation. This is possibly due to contribution of tertiary nitrogens within the dendritic cavities and primary nitrogens in the periphery. The nitrogens of tertiary amines are strongly basic (Gajbhiye *et al.*, 2008). These quaternized nitrogens bind with the counter ions such as carboxylate ions in the case of Ofloxacin. Some retardation in the drug release was seen due to PEGylation. When the drug will be released from the dendrimer, drug release got struck in the PEG chains and got slow release in the environment. But due to low pH, the dendrimer became more and more protonated and the branches expanded and both attached as well as encapsulated drug showed a burst of release from the dendrimer within a very short period of time. The protonation of primary as well as tertiary amine groups in PPI dendrimer affects size and density of the dendrimer molecule, which contributed to sustained drug delivery at higher pH and faster drug release at lower pH. The % cumulative drug release from Ofloxacin loaded 0.5G EDA-PPI dendrimer was found to be 90.43±0.015 after 60 mins. The results are shown in Figure 8.

Antibacterial Assay:-

Figure 9. No zone of inhibition produced by Control solvent DMSO against *E. coli*

Figure 10. No zone of inhibition produced by Control solvent DMSO against *S. aureus*

Figure 11. Zone of inhibition produced by Ofloxacin against *E. coli*

Figure 12. Zone of inhibition produced by Ofloxacin against *S. aureus*

Figure13. Zone of inhibition produced by DLD against *E. coli*

Figure 14. Zone of inhibition produced by DLD against *S. aureus*

The antibacterial activity of drug loaded dendrimer was performed by agar well diffusion method. The results were compared with pure Ofloxacin and thus, it reveals that the PEGylated drug loaded dendrimer shows potent activity than that of plain drug on both the selected organisms (Table 6 and Figure 9 to 14). Further, it was observed that the Ofloxacinshows lesser inhibitory zone. Hence it clarifies that the selected organisms are prone to produce resistant to Ofloxacin. Thus, the synthesized PEGylatedpolypropylenei-

minedendrimer is an effective carrier to target any antibacterial agent for resistant producing organism.

Table 6 Antibacterial activity of Ofloxacin loaded Dendrimers against resistant producing microorganism

S. No	Treatment	*Staphylococcus aureus*	*E-coli*
1.	Control (DMSO)	NZI	NZI
2.	Standard (Ofloxacin 100µg/ml)	16.25±0.15	18.3±0.25
3.	DLD (100µg/ml)	26.7±0.23	28.48±0.1

NZI-No zone of Inhibition; DLD- Drug loaded dendrimer; DMSO- Dimethyl sulphoxide.
Average of three trials (n=3); Zone of inhibition in mm

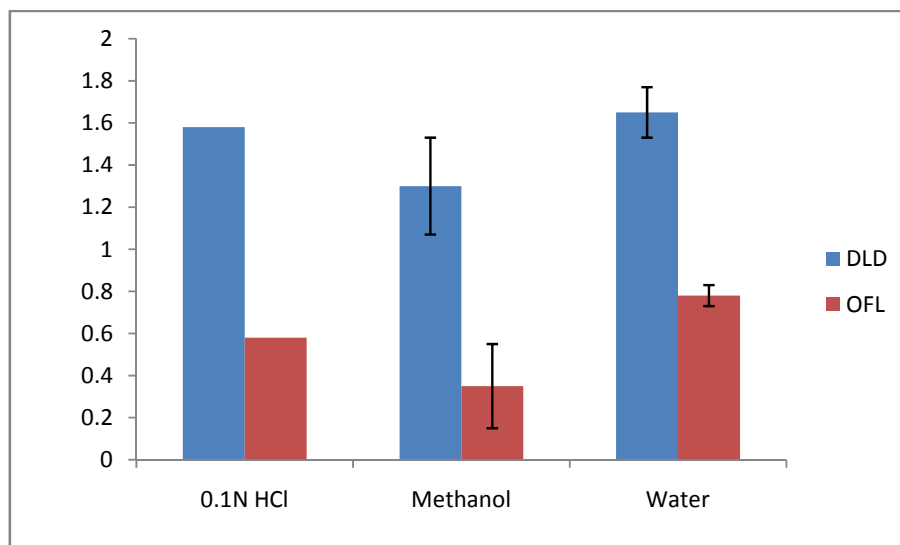**Determination of solubility of Ofloxacin loaded Dendrimer complex**

Figure 15. Comparison of solubility of plain OFL and OFL loaded dendrimer complex

The solubility study of OFL loaded dendrimer complex was determined by adding an excess amount of dendrimer-drug complex (Equivalent to10 mg of drug) to 10 ml of different solvents such as 0.1N HCl, Water and Methanol and compared with plain Ofloxacin. The results revealed that the solubility of OFL loaded dendrimer complex shows better solubility than that of plain Ofloxacin. The results are shown in Figure 15.

PREFORMULATION STUDIES OF POWDER BLEND

Table 7.Micromeritic properties of pre-compressional powder blend.

Batch no.	Angle of repose (Θ)± SD	Bulk Density (gm/cc)± SD	Tapped Density (gm/cc)± SD	Hausner's Ratio± SD	Carr's Index (%)± SD
F0	28.54±0.54	0.2772±0.3045	0.4021±0.0035	1.241±0.0025	18.63±0.853
F1	27.63±0.53	0.293±0.2957	0.3468±0.0063	1.226±0.0079	16.77±0.411

All values are expressed as mean ± SD, n=3

EVALUATION OF OFLOXACIN LOADED DENDRIMER TABLETS
Table 8(A). Evaluation of Ofloxacin Loaded Dendrimer Tablets

Batch no.	Weight variation* (mg) ±SD	Hardness+ (kg/cm²)±SD	Thickness+ (mm)±SD
F0	398.67±2.05	4.0±0.05	4.64±0.0249
F1	396.33±1.7	3.0±0.08	4.70±0.0163

All values are expressed as mean ± SD, n = 10+, 20*

Table 8(B). Evaluation of Ofloxacin Loaded Dendrimer Tablets

Batch no.	%Friability†±SD	Disintegration Time(sec)± SD	Drug Content (%)±SD
F0	0.698±0.0029	40.67±1.25	97.41±0.32
F1	0.583±0.0037	39.00±0.24	98.07±0.10

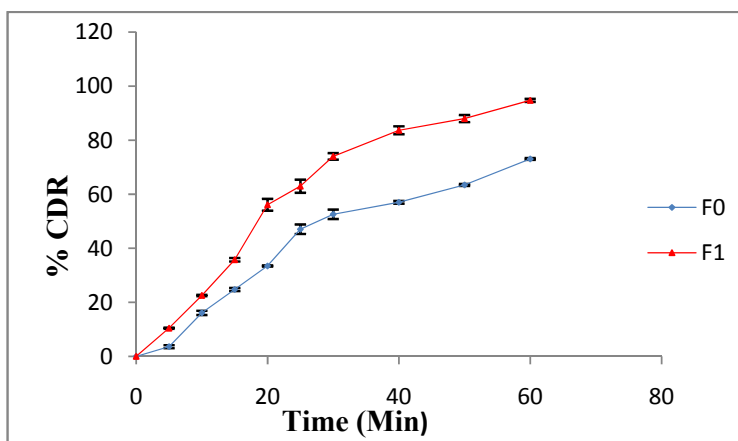All values are expressed as mean ± SD, n = 20†

***In-vitro* drug release study of Ofloxacin loaded dendrimer tablets**

Figure16.*In-vitro* drug release study of Ofloxacin loaded dendrimer tablets and plain Ofloxacin tablets

Comparison of Dissolution profile of Ofloxacin loaded dendrimer tablet with Marketed Ofloxacin Tablet

Both the formulations were subjected for *in vitro* dissolution studies using tablet dissolution tester USP type II in 900 ml of 0.1N HCl buffer pH 1.2 dissolution medium at 50 rpm at 37°C ± 0.5°C. The percentage of Ofloxacin released as a function of time for F0 is 73.09±0.35 for 60 mins. The percentage of Ofloxacin released as a function of time for formulation F1 was found to be 94.77±0.57 for 60 mins. The rapid drug dissolution was observed in F1 because the protonation of primary as well as tertiary amine groups in PPI dendrimer affects size and density of the dendrimer molecule, which contributed to faster release of drug at lower pH. The *in-vitro* drug release studies of formulation F0 and F1 is depicted in Figure 16.

The *in-vitro* dissolution studies of formulation F1 was compared with plain Ofloxacin conventional tablet available in market. The study shows better dissolution profile of Ofloxacin loaded 0.5G EDA-PPI dendrimer tablet i.e. formulation F1 than that of marketed

available Ofloxacin tablet. The comparison of Dissolution profile of formulation F1 and markted formulation is depicted in Figure 17.
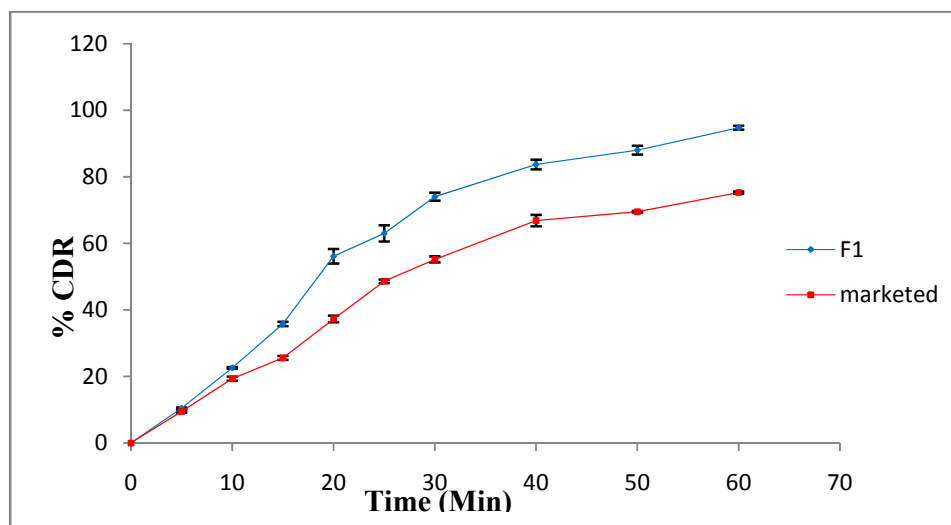

Figure 17.Comparision of Dissolution profile of Ofloxacin loaded dendrimer tablet with Marketed Ofloxacin Tablet

STABILITY STUDIES
Table No. 9Physico-chemical characterization of formulation F1 during stability studies

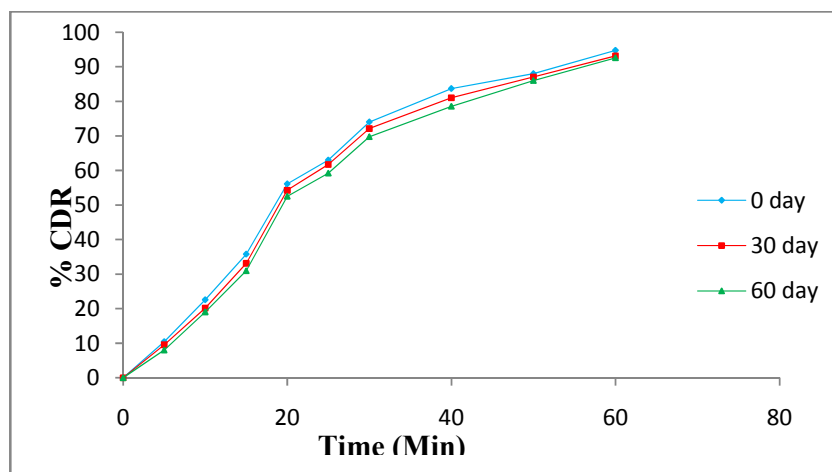| Time (Days)		Hardness (kg/cm²)	Friability (%)	Disintegration time (sec)	Drug Content(%)
		F1	F1	F1	F1
0	-	3.0	0.583	39.00	98.07
30	At 30 ± 2 °C/65 ± 5 % RH	2.90	0.570	36.0	96.0
	At 40 ± 2 °C/75 ± 5 % RH	2.80	0.561	34.0	94.32
60	At 30 ± 2 °C/65 ± 5 % RH	2.70	0.55	33.0	94.05
	At 40 ± 2 °C/75 ± 5 % RH	2.50	0.5	30.0	93.55

Figure 18.*In-vitro* dissolution study of F1 (A) after stability studies
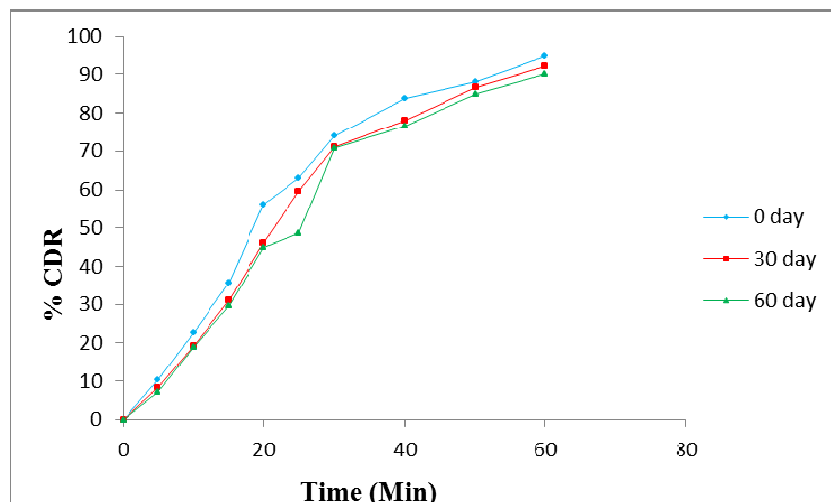
Figure 19. *In-vitro* **dissolution study of F1 (B) after stability studies**

The stability studies were carried out for the formulation F1 at 30 ± 2°C/65 ± 5% RH and 40 ± 2°C/75 ± 5% RH for two month. The results indicated that the tablets did not show any physical changes (hardness and friability) during the study period and the drug content was found above 93.55% at the end of two month. There were no significant differences found in the percentage cumulative drug release after stability study. It is shown in Fig. 18 and 19. This indicates that tablets are fairly stable at storage condition.

CONCLUSION

From the present study it can be concluded that the half generation polypropyleneimine dendrimer can be efficiently synthesized by double Michael addiction reaction and further it can be PEGylated successfully using PEG-4000. The synthesized system was found to be suitable for the delivery of Ofloxacin against resistant producing strains and further this system is found to be a helpful tool in enhancement of solubility, *in-vitro* drug release i.e. bioavailability and efficacy of Ofloxacin and hence can be used as drug delivery tool for any of the anti-bacterial agent.

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