Research article Rapports De Pharmacie Vol.3 (1), 2017, 341-349
ISSN: 2455-0507

EFFECT OF PEGYLATED POLYPROPYLENE IMINE DENDRIMERS LOADED IMATINIB FOR ANTI-TUMOR ACTIVITY: SYNTHESIS, CHARACTERIZATION AND INVIVO EVALUATION STUDIES

Purushothaman M[1*], Mariyappan G[2]
[1]*Department of Pharmaceutics, Scient Institute of Pharmacy, Telangana, India.*
[2]*Sunrise University, Alwar, Rajasthan, India.*

ABSTRACT
The objective of the present studies is to investigate the efficiency of Imatinib loaded PPI dendrimers for antileukemic activity. The PPI dendrimer is a new concept of drug delivery system for the treatment of cancer. The PPI was prepared by using double Michael addiction reaction procedure to get the various generations of dendrimers. The 5.0 generations of PPI dendrimers was loaded with prednisolone as anticancer drug. The prepared PPI dendrimers were characterized by FTIR and NMR spectroscopy to assess the formation and drug loaded efficiency. The drug loaded PPI dendrimers were evaluated by various characterization procedures like morphology, size and shape, Polydispersity index, Invitro release studies. The optimized formulation was subjected to various stability studies. The stable prednisolone loaded PPI dendrimers were subjected to Hemolytic toxicity studies, ED 50 calculation and antileukemic activity. The experimental results on activity of the Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer showed that an increase in dose levels of equivalent to the free drug led to an increase in the ratio T/C, indicating lower toxicity. The dose of 8.0 mg/kg x 3, i. p., was not toxic (T/ C% = 278.7%). The antileukemic activity of the Prednisolone loaded PEGylated and Imatinib loaded PEGylated dendrimers are shown more significant antitumor activity than the activity of free Imatinib.

Keywords: Dendrimers, Imatinib, Hemolytic toxicity

INTRODUCTION
Cancer is a major cause of morbidity and mortality in developing and developed countries alike. In many low-income and middle-income countries, including India, most of the population does not have access to a well-organized and well regulated cancer care system. The concept of specific targeting has emerged and is crucial to help reduce uptake by normal tissue and increase the payload of the drug inside the tumor. Furthermore, as more than 40% of anticancer drug is poorly soluble in aqueous environment, the ultimate bioavailability and therapeutic efficiency can be significantly hampered [1]. Dendrimer are highly branched synthetic polymers .It consists of center core, integral region and numerous functional end groups. The growth of polymer occurs in outward direction from central core by stepwise polymerization. It is characterized with numerous cavities in the core structure creating channels and cages. Precise control of size can be achieved by the extent of polymerization. The intracellular uptake of dendrimers by receptor mediated endocytosis can be aided using the conjugation of biotin. Dendrimers contribute to drug delivery either by binding the drug on the periphery as prodrug or entrapping the drug in the center core. Recent progress has been made to dendrimer formulary as a biocompatible drug carrier for cancer targeting therapy. Dendrimers used in drug delivery studies typically incorporate one or more of the following polymers [2]: polyamidoamine (PAMAM), melamine, poly (L-glutamic acid) (PG), polyethyleneimine (PEI), poly (propylene imine) [3], and poly(ethylene glycol) (PEG) . Chitin and chitosan have also been incorporated with dendrimers [4]. The objective of the present studies is to investigate the efficiency of Imatinib loaded PPI dendrimers for antileukemic activity.

Address for correspondence:
Prof. Dr. M.Purushothaman, M.Pharm, PhD.,
Principal,
Scient Institute of Pharmacy,
Telangana,
India

MATERIAL AND METHODS
Synthesis of PPI Dendrimers loaded Prednisolone:
EDA-PPI dendrimers were synthesized by the previously reported and established procedure [5]. The half generation EDA-dendrimer-(CN)4*n* (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 vacuum distillation (16 mbar, bath

temperature 40ºC). The full generation EDA-dendrimer-(NH2)4*n* was obtained by hydrogenation in methanol at 40 atm hydrogen pressures and 70ºC for 1 h with Reney Nickel (pretreated with hydroxide and water) as catalyst. The reaction mixture was cooled, filtered and the solvent was evaporated under reduced pressure. The product was then dried under vacuum. EDA-PPI dendrimers up to 5.0G were prepared by repetition of all the above steps consecutively, with increasing quantity of acrylonitrile.

Synthesis of PEGylated 5.0G PPI dendrimers:

To a solution of 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 dicyclohexyl carbodiimide (DCC) (0.32 mmol) in DMSO (10 ml) were added and the solution was stirred for 5 days at room temperature [6]. 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 PEGylated dendrimers followed by lyophilization (Heto drywinner, Germany).

Drug Loading in PEGylated dendrimers:

The known molar concentrations (1:0.5, 1:1, 1:2) of PEGylated-PPI dendrimers were dissolved separately in methanol and mixed with methanolic solution of Prednisolone. 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 (MWCO 1000 Da Sigma, Germany) against double distilled water under sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically at λmax 248 nm to determine indirectly the amount of drug loaded within the system. The dialyzed formulations were lyophilized and used for further characterization.

EVALUATION OF DRUG LOADED PEGYLATED DENDRIMERS

Morphology of the dendrimers:

Morphology of respective drug loaded dendrimers was observed by scanning electron microscope. A small amount of nanoparticles sample has been spread on a metal stub. The stub was then coated with conductive gold by Hitachi 1010 ion sputter and was examined under Hitachi 3000N scanning electron microscope (JSM 5610 LV SEM, JEOL, Japan) chamber. The image was snapped at an acceleration voltage of 20 kV with a chamber pressure of 0.6 mmHg.

Particle Size and polydispersity index determination:

Drug loaded dendrimers size was determined by using a Zetasizer 300 HS (Malvern instruments UK). Samples were diluted with distilled water (2µg/ml) and measured at a temperature of 25 ºC. The diameter was calculated from the autocorrelation function of intensity of light scattered from nanoparticles. The Particles measured are in triplicate. The polydispersity index (PDI) was calculated for dispersion homogeneity and ranges from 0 to 1. The value close to 0 indicated a homogeneous dispersion and greater than 0.3 indicate high heterogeneity [7].

Fourier transforms infrared spectroscopy and Nuclear magnetic resonance spectroscopy:

FTIR spectra of plain dendrimer, PEGylated dendrimer, respective drug and drug loaded dendrimers were determined by using Perkin Elmer RXI model. The pellets were prepared by gently mixing of 1mg sample with 200mg potassium bromide at high compaction pressure. The pellets thus prepared were scanned at resolution of $4cm^{-1}$ from 450 to $4000cm^{-1}$.The Plain and PEGylated dendrimers were analyzed by using Bruker DRX-300, NMR spectroscopy. The dendrimers were solubilized in D_2O using methanol as co solvent and analysed at 300MHz.

In vitro release of drug from PEGylated dendrimers:

Drug releases from known amount of respective drug loaded PEGylated dendrimers were determined using a modified dissolution method. The medium comprised of a 0.05 mol phosphate buffer solution (PBS) (pH 7.4). The dialysis bags were filled with a known mass of plain drug and drug loaded PEGylated dendritic architectures (MWCO 1000 Da) seperatly and the dialysis bags were placed in 50 ml of PBS (pH 7.4) at 37ºC with slow magnetic stirring under sink conditions. Aliquots of 1 ml were withdrawn from the external solution and replaced with the same volume of fresh PBS. The drug concentration was detected in a spectrophotometer at 248 nm.

Stability studies of drug loaded PEGylated dendrimers:

PEGylated dendritic system loaded with Prednisolone was exposed to conditions of temperature and light for 4 weeks. The formulation was taken in different vials and stored in dark (amber color vials) and in light (colorless vials) at , room temperature (40 ± 2°C) in thermostatically controlled oven for a period of 4 weeks. The samples were analyzed every week for any color change, drug content and drug release. The data obtained were used for the analysis of any physical and chemical degradation, the required storage conditions and the precautions required for storage [8]. The samples were initially clear and transparent at 0 °C. The loss of drug from the formulation was ascertained after storage

conditions. The known amount of formulation was kept in benzoylated cellulose tubing (Sigma, USA) and dialyzed across the tubing. The external medium (10 ml methanol) was monitored for the content of the drugs spectrophotometrically. The percentage increase in drug release from the formulation was analyzing the effects of conditions of storage on the formulations.

***EX VIVO* STUDIES**

Hemolytic Toxicity of PEGylated dendrimer:

The RBC suspension was obtained following the reported procedure for hemolytic studies. Briefly, the RBC suspension (5% hematocrit) of the human blood collected in HiAnticlot blood collection vials (Himedia Labs, India). 0.5 ml of suitably diluted Prednisolone encapsulated, PEGylated and non-PEGylated formulations were added to 4.5 ml of normal saline and incubated for 1h with RBC suspension. Similarly, 0.5 ml of drug solution and 0.5 ml of dendrimer solution were mixed with 4.5 ml of normal saline and incubated for 1h with RBC suspension. The drug and dendrimers in separate tubes were taken in such amount that the resultant final concentrations of drug and dendrimer were equivalent in all the cases. The PEGylated system of dendrimer–drug complex was taken in amount such that the resultant final concentrations of drug and dendrimer were equivalent to that in non-PEGylated systems [9]. This allowed comparison of the hemolysis data of the, dendrimer, drug loaded dendrimers and PEGylated dendritic architectures to assess the effect of PEGylation on hemolysis. After centrifugation, supernatants were taken and diluted with an equal volume of normal saline and absorbance was measured at 540 nm. To obtain 0 and 100% hemolysis, RBC suspension was added to 5 ml of 0.9% NaCl solution (normal saline) and 5 ml distilled water, respectively.

Brine Shrimp Lethality Assay:

Brine shrimp lethality assay was used for cytotoxic of formulations, according to method of Brine shrimp [10] (Artemia *salina*) nauplii was hatched in sterile brine solution (prepared by using sea salt 38g/L and adjusted the Ph to 8.5 using 1N NaOH) under constant aeration for 38 h. After hatching, 10 nauplii were placed in each vial and added 25,50,100µg/ml of prednisolone loaded PEGylated PPI dendrimers respectively in a final volume of 5ml in each vial, maintained at 37°C for 24 h under the light of incandescent lamp and surviving larvae were counted. Each experiment was conducted along with control (Vehicle treated), as like test substances. Percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. The ED_{50} values were obtained using Fenny probed analysis software. The result for test compound was compared with the positive control Podophyllotoxine (2.5, 5, 10µg/ml)

RESULTS AND DISCUSSION

FTIR and NMR spectroscopy:

PPI 5.0G dendrimers were synthesized by the procedure reported by using ethylenediamine as initiator core. Synthesis of 0.5G PPI was confirmed by IR peaks, mainly of nitrile at 2248 cm^{-1}. All the nitrile terminal 0.5G PPI got converted to (NH2)4, which was confirmed by IR of PPI 1.0G that exhibited major peak at 3284.78 cm^{-1} for amine (N-H stretch). Likewise, IR peaks also confirmed the synthesis of PPI 5.0G dendrimers. The main peaks are of C-C bend (1115.21 cm^{-1}); C-N stretch (1243.44 cm^{-1}, 1374.50 cm^{-1}); C-H bend (1477 cm^{-1}); N-H deflection of amine (1665.40cm^{-1}) and primary amine at 3410 cm^{-1}(N-H stretch), confirming that nitrile terminal groups of dendrimer were converted to amine terminals. The results matched with the reported synthesis of PPI dendrimers. The synthesized dendrimers were PEGylated using DCC and PEG 4000. IR and NMR data proved the synthesis of PEGylated dendrimers. The IR spectrum of PEGylated PPI 5.0G dendrimer exhibited major peak of N-H stretch of amide at 3324.70 cm^{-1}. An important IR peak at 1242.75 cm^{-1} of ether linkage (C-O) appears in the spectrum of PEGylated dendrimers.C-O stretch of amide group has been found near 1624.29cm^{-1}. The important peak of C-N stretch of amide also appears at 2925.43 cm^{-1} (Figure 1-4).

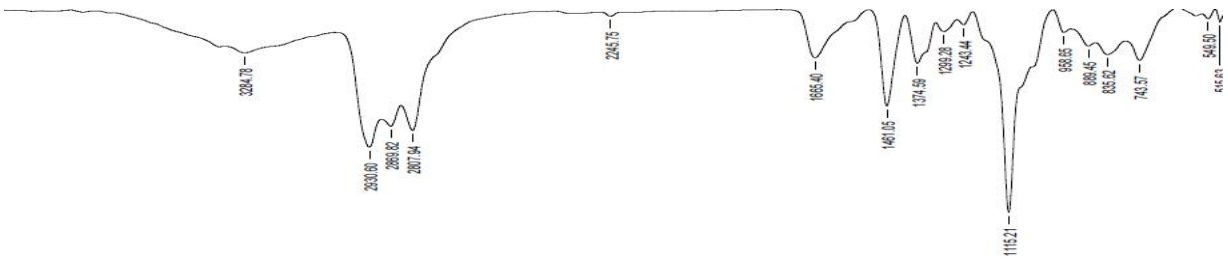

Figure 1: FTIR Spectrum of Plain 5.0GPPI dendrimer

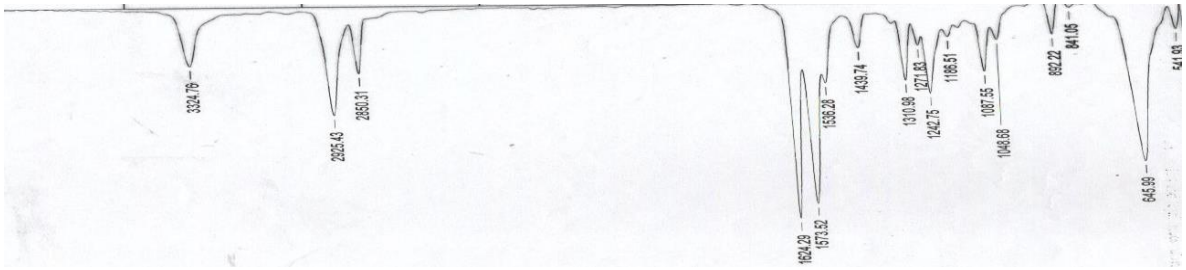

Figure 2: FTIR Spectrum of PEGylated 5.0GPPI dendrimer

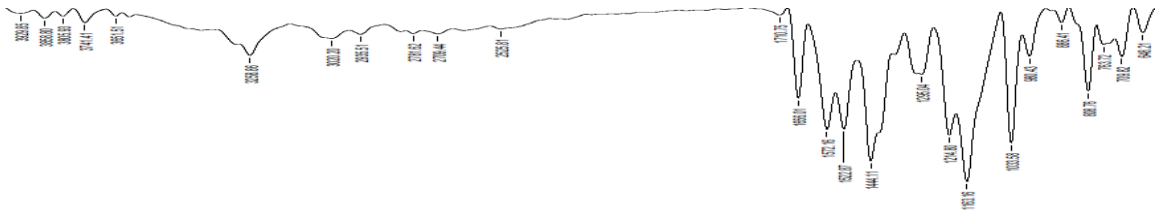

Figure 3: FTIR Spectrum of plain Imatinib

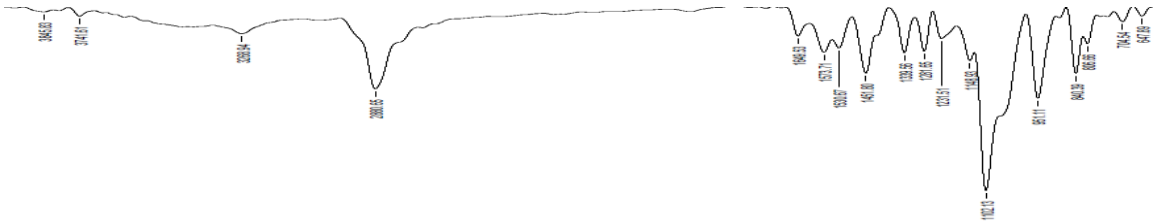

Figure 4: FTIR Spectrum of Imatinib loaded PEGylated 5.0GPPI dendrimer

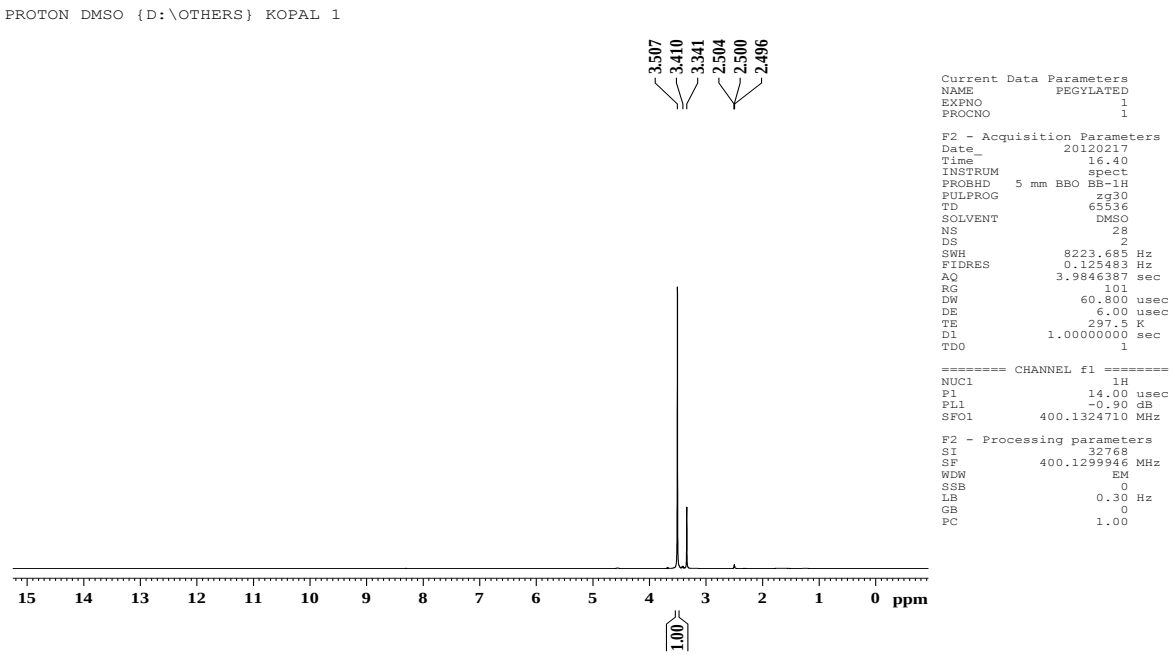

Figure 5: NMR Spectrum of PEGylated 5.0GPPI dendrimers

NMR spectrum and shifts of PEGylated dendrimers as compared to that of simple dendrimers proved PEGylation. There was increase in integral value for the shift of secondary -CH2 groups on PEGylation. This is due to the increase in number of secondary -CH2 groups in PEG that are linked on PEGylation. Similarly, strong peak of ether linkage appears at 3.507 ppm due to the presence of ether linkages in PEG in high

amount, remaining free amines $–CH_2-NH_2$ appears at3.341-3.410 ppm. The characteristic peak of amide linkage appeared near 2.504 ppm and 2.496ppm for carbonyl $-CH_2C=O$ in NMR spectrum of PEGylated dendrimers as shown in **Fig. 5**.

Drug loading in to the PEGylated dendrimers:

The known molar concentrations (1:0.5, 1:1, 1:2) of PEGylated-PPI dendrimers and Imatinib was used to load the drug in to PEGylated dendrimer system for getting optimized formulation. Non-covalent interactions between Imatinib and PEGylated PPI 5.0G dendrimers, such as hydrophobic interaction and hydrogen bonding, contributed to the physical binding of drug molecules inside dendritic micelles and surface PEG layers. The percentage loading of both the drugs in PEGylated PPI 5.0G dendrimers was significantly increased in 1:1 ratio of dendrimer: drug for the formulation (p value 0.0001, extremely significant) compared to 1:0.5 and 1:2 molar concentration of both the drugs respectively. PEGylation increases the Imatinib loading capacity of the PPI 5.0G dendrimers due to more interaction of drug and PEG at the peripheral portions of dendrimers. Imatinib entrapment in PEGylated dendrimers increased significantly due to more sealing of dendrimeric structure by PEG at the peripheral portions of dendrimers as coat, which prevented drug release by enhancing complexation probably by increasing steric hindrance over dendrimer periphery [11].

Drug entrapment efficiency:

Number of moles of both the drugs entrapped in 1 mol of PEGylated dendritic architecture was found to be in 1:1 ratio of dendrimers and drug is suitable as 92.08 ± 1.2 mol for Imatinib as compared to 7.28 ± 1.9, 12.42 ± 0.8 mol in 1:0.5 molar concentration and 48.4 ± 1.2 and 51.1 ± 1.0 molar concentrations in 1:2 ratio. If the drug entrapment is more than the required quantity leads to toxic to the host, increase in size leads to internal pressure were by leakage of drug from the system may happen. So the study considered to take up only the 1:1 ratio molar concentration followed in the preparation. The entrapment efficiency of PEGylated formulation of both drugs shown in Table 1**.**

Table 1: Drug entrapment efficiency of Imatinib loaded PEGylated dendrimer

S.No	Formulation code	Ratio of (dendrimer: drug) In mol. Con	% of drug entrapped
1.	DLDI	1:0.5	12.42±0.8
2.	DLDI	1:1	92.08±1.2*
3	DLDI	1:2	51.01±1.0

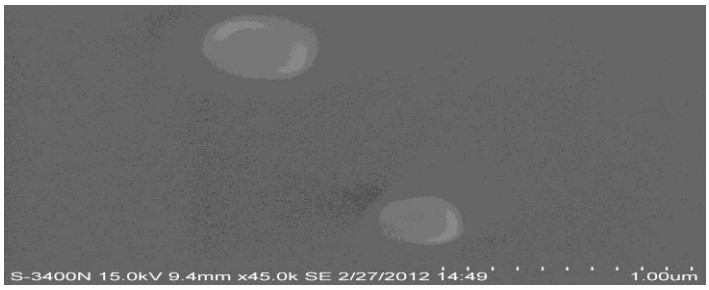

Figure 6: SEM photograph of Imatinib loaded PPI dendrimer

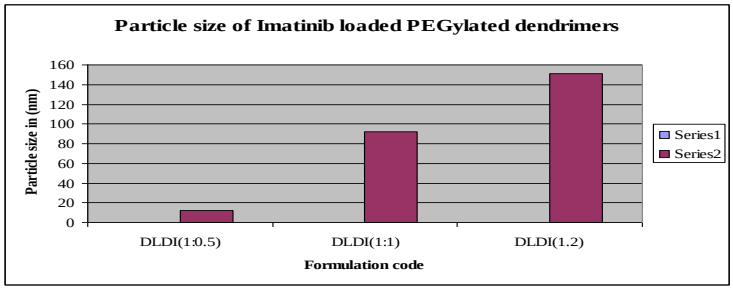

Figure 7: Particle size of various formulation of Imatinib loaded dendrimers

Morphology of the dendrimers:

The scanning electron micrographs of PEGylated dendrimers and Imatinib dendrimers were shown in Figure 6, which revealed the formation of spherical shape with irregular surface. SEM micrographs of drug loaded PEGylated dendrimers of respective drugs showed that the drug loaded dendrimers were more or less spherical in shape (PEGylated 5.0G EDA-PPI dendrimers) and that the dendrimers were agglomerated (Figure. 6).

Particle size and polydispersity index:

The particle size of synthesized plain PPI dendrimers, PEGylated dendrimers and Imatinib loaded PEGylated dendrimers were analyzed by Malvern particle size analyzer. The formulations are intended to know the size, the sizes varied with the molar concentration of PEGylated dendrimer and drug substances. It was observed that when the drug ratio is less the size altered slightly but the drug ratio is higher the size is increasing considerably due to the non -covalent bond of drug and PEGylated dendrimer proves the agglomeration were by the size is large(Vijayarajkumar,2006). Even though overall size distribution of all the formulations size were seen between 78 ± 0.8 to 110.6 ± 2.2 nm. This will allow the bioadictive nature of the formulation. The Polydispersity index value of both the optimized formulation is indicated as 1.000. The particle size of dendrimers was the main factor for diffusion through lipid layers in the system. Particle size of 20-200nm were easily transported in the cell wall of the cancerous cells by passive diffusion [12].The particle size of both formulation were shown in Figure 7.

In vitro drug release:

The release of Imatinib was 54% while drug loaded dendrimer of Imatinib is only 9.14%in 91.4% in 8h and 80h, respectively. The cumulative % release of both prednisolone and Imatinib loaded PEGylated dendrimers was decreased with increase of dendrimer generation. This may be due to greater hydrophobic interaction between the drug and the core of higher generation dendrimer (5.0G). The difference in number of terminal PEG groups also contributes to the slower drug release profile whereby both dissolution as well as diffusion of drug occurs through small channels in the PEGylated dendrimers. The release pattern of the optimized formulation was shown in Figure 8.

Stability study of drug loaded PEGylated PPI dendrimers:

The stability study was performed for optimized formulations of Imatinib dendrimers at 40±2°C for 4 weeks neither change in its appearance and redispersing ability nor significance difference in potency. The drug content and the release also not changed. The appearance and drug content was tabulated in **Table 2.**

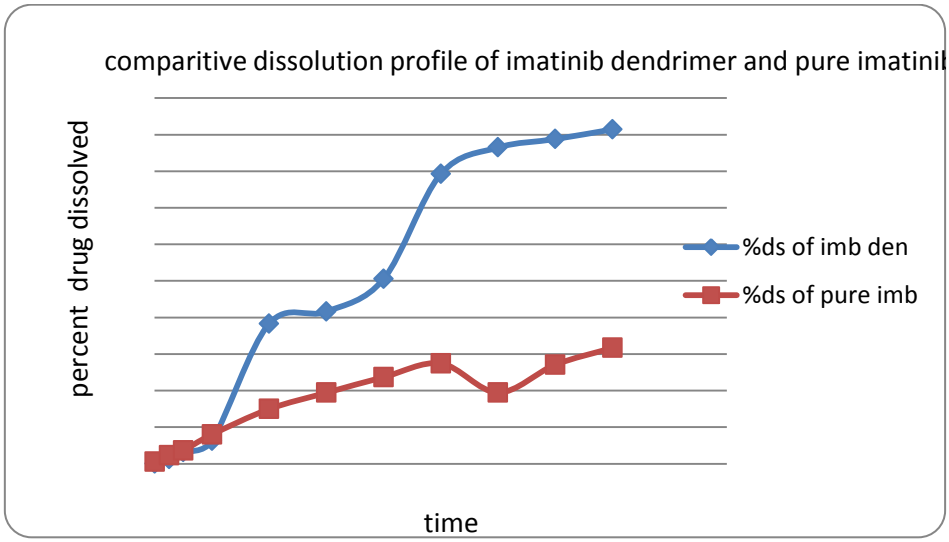

Figure 8: Comparative dissolution profile for Imatinib dendrimers and pure imatinib

Table 2. Stability studies of optimized formulation

S.No	Formulation code	Storage time(4 weeks)
1.	Imatinib loaded PEGylated dendrimers Appearance Drug content Drug leakage	 Pale yellow colour powder 92.08±1.2* Not observed

EX VIVO STUDY

Hemolytic toxicity:

The hemolytic toxicity of the dendrimers was enough to impose a constraint in its use as a drug delivery system. The toxicity is due to the poly cationic nature of the PPI dendrimers. However, PEGylation of dendrimers was found to decrease the hemolysis of the RBC considerably at all concentrations due to the shielding or coating of the charged quaternary ammonium ion that is generally formed on the amine-terminated whole generations of PPI dendrimers, responsible for hemolysis. The whole generation of amine-terminated charged PPI 5.0G dendrimers. Showed hemolytic toxicities of 20±0.82 in 0.2 w/v concentration.. But PEGylation of the dendrimers was found to have decreased the hemolysis of the RBCs significantly to 2.72±1.10 at 0.2 w/v concentration. (Table 3.) This was due to the inhibition of interaction of RBCs with the charged quaternary ammonium ion as determined by interaction with RBCs using the method suggested [13].

Table 3. Nomenclature, % hemolysis of plain and PEgylated PPI 5.0G dendrimers

Formulation code	Concentration	Actual number of terminal amine groups	%hemolytic toxicity
5G-PPI dendrimer	0.2 µg/ml	64	20.39±0.82
PEGylated-PPI dendrimer	0.2 µg/ml	-	2.72 ± 1.10
DLDI(1:1)	0.2 µg/ml	-	2.62± 0.2

% hemolysis produced by 5 mg/ml formulations on 5% hematocrit RBCs on incubation for 1h. mean ±SD. (n = 3).

Table 4: Brine Shrimp Lethality (Cytotoxic) Assay for Drug Loaded Dendrimers

S. No.	Test	Solubility	Control			Live Shrimps				Mean	ED_{50} (µg/ml)	DOF
			Tube 1	Tube 2	Tube 3	Dose µg/ml	Tube 1	Tube 2	Tube 3			
1	DLDI	DMSO	8	7	7	25	5	4	4	9	37.16	0.1874
						50	3	4	3	12		
						100	1	1	3	17		
3	Std	DMSO	8	7	6	2.5	4	3	2	12	2.24	0.1267
						5	1	1	1	18		
						10	0	0	0	21		

DMSO – Dimethyl Sulphoxide; ED_{50} – Effective Dose Concentration at 50%;
DOF – Degrees Of Freedom; DLDI - Imatinib Loaded PEGylated PPI Dendrimers;
Mean [Control –Live Shrimps] = Dead Shrimps;
Standard (Podophyllotoxin)

Brine shrimp lethality assay:

Brine shrimp lethality assay was used according to method of (Meyer et al.1982). Percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. The ED_{50} values were obtained using Fenny probed analysis software. The result for test compound was for imatinib loaded PEGylated dendrimers showing the ED_{50} of 37.16 and the degree of freedom is 0.1874 in 24 h of incubation period. The ED_{50} values are matching the expected level of cytotoxicity in 24 h. The ED 50 values of both the optimized formulations were calculated and tabulated in Table 4.

Antileukemic activity:

The antileukemic activity was assessed by use of the criterion T/C % [14]. The results obtained from this study on the effect of Imatinib and its Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer on BDF_1 hybrid mice-bearing *AML-193* leukemia are shown on the Table 5.

According to these results, the free Imatinib exhibited a pronounced and dose-related

antileukemic activity on mice-bearing *AML-193* leukemia. An increase of the free Imatinib dose over 0.25 mg/kg x 3, i. p., caused an increase in its acute toxicity. This fact was registered by the progressive decrease in the ratio T/C (treated/control). The dose of the free Imatinib of 2 mg/kg x 3, i. p., was toxic (T/C% < 125%). The Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer exhibited an antileukemic activity against ascitic myelogenous leukemia *AML-193* in BDF_1 mice, in four of the used doses – from 0.5mg/kg x 3 to 8.0 mg/kg x 3, i. p., with T/C% varying between 197.2% and 278.7%. The experimental results on activity of the Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer showed that an increase in dose levels of equivalent to the free drug led to an increase in the ratio T/C, indicating lower toxicity. The dose of 8.0 mg/kg x 3, i. p., was not toxic (T/ C% = 278.7%). The results obtained from the effect of Prednisolone and its Prednisolone loaded PEGylated Polypropyleneimine (PPI) dendrimer on BDF_1 hybrid mice-bearing K563 leukemia are shown on the Table 5.

Table 5: Antileukemic activity of free Imatinib and Imatinib loaded PEGylated olypropyleneimine (PPI) dendrimer on BDF1 hybrid mice-bearing *AML-193* leukemia

Drug and formulation	Dose (mg/kg) x 3, i.p	MST (in days)	T/C (%)
Imatinib	0.25	27.7	256.4
	0.5	27.4	253..7
	1.0	22.5	208.3
	1.5	13.8	127.7
	2.0*	8.3*	76.8*
Imatinib loaded PEGylated (PPI) dendrimers	0.5	21.3	197.2
	1.0	23.7	219.4
	2.0	25.9	239.8
	4.0	27.3	252.7
	8.0	30.1	278.7
Untreated control	0	10.8	-

MST – mean survival time (days); T – survival time of treated mice (days);C – survival time of control mice (days); Significant antileukemic effect at T/C% > 125% was accepted.

* Toxic dose at T/C% < 125%.

According to these results, the free Imatinib exhibited a pronounced and dose-related antileukemic activity on mice-bearing *AML-193* leukemia. An increase of the free Imatinib dose over 0.25 mg/kg x 3, i. p., caused an increase in its acute toxicity. This fact was registered by the progressive decrease in the ratio T/C (treated/control). The dose of the free Imatinib of 2 mg/kg x 3, i. p., was toxic (T/C% < 125%). The Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer exhibited an antileukemic activity against ascitic myelogenous leukemia *AML-193* in BDF_1 mice, in four of the used doses – from 0.5mg/kg x 3 to 8.0 mg/kg x 3, i. p., with T/C% varying between 197.2% and 278.7%. The experimental results on activity of the Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer showed that an increase in dose levels of equivalent to the free drug led to an increase in the ratio T/C, indicating lower toxicity. The dose of 8.0 mg/kg x 3, i. p., was not toxic (T/ C% = 278.7%).

CONCLUSION

The dendrimers are linear macromolecules only occasionally contain some smaller or longer branches. In the recent past it has been found that the properties of highly branched macromolecules can be very different from conventional polymers. The PPI was prepared by using double Michael addiction reaction procedure to get the various generations of dendrimers. The 5.0 generations of PPI dendrimers was loaded with Imatinib as anticancer drug. The prepared PPI dendrimers were characterized by FTIR and NMR spectroscopy to assess the formation and drug loaded efficiency. The drug loaded PPI dendrimers were evaluated by various characterization procedures like morphology, size and shape, Polydispersity index, Invitro release studies. The optimized formulation was subjected to various stability studies. The stable prednisolone loaded PPI dendrimers were subjected to Hemolytic toxicity studies, ED 50 calculation and antileukemic activity. The antileukemic activity of

the Prednisolone loaded PEGylated dendrimer was shown more significant activity than the activity of free Prednisolone that was favorable by clinical point of view to treat the cancer.

REFERENCES

[1] Abhinav Agarwal, Umesh Gupta, Abhay Asthana, Narendra K Jain. Dextran conjugated dendritic nanoconstructs as potential vectors for anti-cancer agent, *Biomaterials*, 30, 3588–3596 (2009).

[2] D Prajakta, J Ratnesh , K Chandan, S Suresh , S Grace, V Meera, P Vandana. Curcumin loaded pH-sensitive nanoparticles for the treatment of colon cancer. *J. Biomed.Nanotechnol, 5*(5), 445-455 (2009).

[3] Prashant Kesharwani, K Rakesh Tekade, Virendra Gajbhiye, Keerti Jain, K Narendra Jain. Cancer targeting potential of some ligand-anchored poly(propylene imine) dendrimers: a comparison, *Nanomedicine: Nanotechnology, Biology, and Medicine*, 7 , 295–304(2011)

[4] Puneet Sharma, Sanjay Garg. Pure drug and polymer based nanotechnologies for the improved solubility, stability, bioavailability and targeting of anti-HIV drugs, *Advanced Drug Delivery Reviews*, 62, 491–502 (2010).

[5] Quan Zheng, Cai-yuan Pan, Preparation and characterization of dendrimer-starPNIPAAM using dithiobenzoate-terminated PPI dendrimer via RAFT polymerization, *European Polymer Journal*, 42, 807–814 (2006).

[6] Bruno Sarmento , Domingos Ferreira , Francisco Veiga (2006). Characterization of Insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies, *Carbohydrate Polymers*, 66, 1–7 (2006).

[7] Carey Munsick, Robert Murray, Tom Dziubla, Anthony.Quantitation of Humalog Insulin by UV spectrophotometric methods, *Journal of Diabetes Science and Technology*,3, 603-607 (2007).

[8] D Bhadra, , A K Yadav, S Bhadra, NK Jain. *Int.J. Pharm*, 295, 221 (2005).

[9] Gopinath, RAS Naidu. Pharmaceutical Preformulation Studies – Current Review, *International Journal of Pharmaceutical & Biological Archives*, 2, 1391-1400 (2011).

[10] Maltesen, S Bjerregaard, L Hovgaard, S Havelund. Analysis of Insulin allostery in solution and solid state with FTIR, *Journal of pharmaceutical science*, 98, 3265-3277 (2009).

[11] Mansour Mansouri, Hamid Reza Pouretedal, Vida Vosoughi. Preparation and Characterization of Ibuprofen Nanoparticles by using Solvent/ Antisolvent Precipitation, *The Open Conference Proceedings Journal*, 2, 88-94 (2011).

[12] Masotti, F Marino, G Ortaggi, Cleofe Palocci. Fluorescence and Scanning Electron Microscopy of Chitosan/DNA Nanoparticles for Biological Applications, *Modern Research and Educational Topics in Microscopy*, 43, 690-700 (2007).

[13] P V Kumar, A Asthana, T Datta, N K Jain, Intracellular macrophage uptake of rifampicin loaded mannosylated dendrimers. *J. Drug Targ*. 14: 546-556 (2006).

[14] BN Meyer, NR Ferrigni, JE Putnam, LB Jacobsen, DE Nichls, JL Mc Laughlin. A Convenient general bio assay for active plant constituents. *Planta medica*, 1982, 45:31-34(1982).