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Keywords : *poly (amido) amine; PAMAM dendrimers; divergent approach; polydispersity.*

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Synthesis and Analytical Characterization of Ester and Amine Terminated PAMAM Dendrimers

Surya Prakash Gautam [α], Arun Kumar Gupta Anupama Sharma [σ] & Tapsya Gautam, Madhu [ρ]

Abstract - PAMAM dendrimers containing ethylene diamine core and methyal acrylate as repeating unit were synthesized by divergent approach. Analytical characterization of PAMAM dendrimers amine terminated full generation 4.0G and ester terminated half generation 3.5G were performed using UV-Vis spectroscopy, FT-IR spectroscopy, differential scanning calorimetry, NMR spectroscopy and MASS spectroscopy. The half generation dendrimers have the methyl ester terminating groups, which have the characteristic IR peaks for carbonyl at 1730-1750 cm^{-1}. For the full generation dendrimers, when the methyl ester groups were converted to amide groups, the corresponding carbonyl shifted to 1660 cm^{-1}.The characteristic methyl ester peak, which appeared in all the ^{1}H-NMR spectra of the ester terminating dendrimers, whereas it is absent in all the amine terminating dendrimers. The molecular weight was determined by ESI mass spectroscopy which further confirms the preparation of PAMAM dendrimers and provides information about the structural defects, polydispersity and purity.

Keywords : poly (amido) amine; PAMAM dendrimers; divergent approach; polydispersity.

I. Introduction

Dendrimers are spherical, well defined, highly branched macromolecules with dense surface functional groups (**Fig. 1**) [1–3]. Ethylenediamine (EDA) core based Poly (amidoamine) (PAMAM) dendrimers synthesis needs repetitive Michael addition and amidation steps in which each iteration yields the next higher generation of the dendrimer. Multifunctional platform of dendrimers provides endless applications in drug delivery [4-8]. The synthesized PAMAM dendrimers are characterized for UV, FT-IR, NMR, DSC and MASS analysis. In the biomedical field dendrimers had been used for drug delivery, gene therapy, antigen conjugates, NMR contrast agents and synthetic vaccines [9-15]. UV-Vis spectrometry provides the proof of synthesis as well as the conjugation (surface modification) on dendrimers due to characteristic absorption maximum or shift in Lambda Max value due to conjugation [16-20]. Appearance disappearance and reappearance of characteristic peaks in FTIR spectroscopy provides the proof of synthetic. Disappearance of nitrile groups in the synthesis of PPI dendrimers, disappearance reappearance of amine groups in PAMAM dendrimers generation, Pegylation of PAMAM dendrimers, disappearance of the aldehydes during the synthesis of PMMH dendrimers reflects the synthesis and surface modifications [21-23]. Nuclear magnetic resonance (NMR) spectroscopy permits determination of the structure and dynamics of molecules in solution. PAMAM dendrimers and complexed PAMAM are characterized by Rotational-Echo Double Resonance (REDOR) solid-state NMR spectroscopy [24]. Multidimensional NMR spectroscopy ((2D)-NMR, (3D)-NMR) is also acquiring increasing importance in the characterization of dendrimers [25]. NOESY experiments permit quantitative determinations of internuclear distances for nuclei in different parts of the dendrimer molecule [26]. The dynamics of dendritic branches can be investigated by measurement of ^{1}H- and ^{13}C-spin-lattice relaxation times (T_1). Since the mobility of a dendrimer segment is proportional to its T_1 value, the change of mobility of the various dendrimer segments [27]. The DSC technique is generally used to detect the Glass Transition Temperature (Tg). The Tg is affected by the end group substitutions, and the molecular mass. DSC and Temperature Modulated Calorimetry (TMC) were also used to detect physical aging of PMMH dendrimers. Generation has practically no influence on the Tg values of liquid crystal dendrimers based on poly (phenyl acetylene) [28-30].MALDI-TOF-MS and ESI-MS are among the few analytical methods suitable for detailed studies of structural defects in dendrimers on the basis of characteristic fragmentation patterns. The polydispersity and the purity of dendrimers explain the percentage of defect-free dendritic material [31-32]. The principal objective of the work presented here is to analytically characterize and investigate their structural characteristics by UV-Vis spectrometry, FT-IR spectroscopy, Differential Scanning Calorimetry (DSC), NMR spectroscopy and ESI Mass spectroscopy. The presented studies provide new insights into the understanding of the structure and properties of PAMAM

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dendrimer nanocomposites for future drug delivery and cancer treatments.

II. Materials and Methods

a) Materials

Ethylenediamine (Merck Specialities (P) Ltd. Mumbai) and Methylacrylate (Loba Chem (P) Ltd., Mumbai) were used after distillation. Rest of the chemicals was purchased from Loba Chem (P) Ltd., Mumbai. For synthesis HPLC grade solvents were used.

b) Preparation of PAMAM Dendrimers

Dendrimers were prepared by a divergent synthesis scheme using the reagent excess method starting from Ethylenediamine (EDA) by consecutive Michael addition and ester amidation reaction. Dendrimers were prepared according to following step: Michael addition of primary amine (EDA in very first step) to methyl acrylate followed by amidation of formed multiester (tetra ester at very beginning) of EDA.

Michael addition reaction used ethylene diamine (EDA) as an initiator core for starting the synthesis of dendrimers by attaching four acrylate moieties on each amino group of EDA. The resulting compound referred to as "generation -0.5PAMAM tetra ester". This caused the branching in the structure of the dendrimer. The second step used is amidation of terminal carbomethoxy group ($COCH_3$) of methyl acrylate with EDA. This tetra ester with excess EDA gave "generation 0.0 PAMAM tetra amine". EDA was used in excess to about twenty to hundred times to avoid incomplete reactions and hence improved yield. The reaction was carried out using methanol as medium. The reactions were followed by removal of excess reagents by rotary vacuum evaporation at 55°C-60°C, in every step. The whole reaction was carried out in dark at room temperature, using amber colored round bottom flask, which was corked tightly. Addition reaction was allowed to complete in two days, whereas amidation reaction complete in four days.

c) Evaluation of PAMAM Dendrimers

Synthesis of half and full generation dendrimers were confirmed through UV spectroscopy, FT-IR spectroscopy, DSC, ^{1}H-NMR and ESI-Mass spectroscopy.

III. Results and Discussion

a) Identification of Dendrimers by UV-Spectroscopy

The change in λ_{max} values was obtained from half generation to full generation. The change in λ_{max} values from 284.0 (3.5G) to 277.5 (4.0G) nm revealed the change in structure of PAMAM dendrimers **(Fig. 2 & 3)**. The λ_{max} of 4.0 G PAMAM dendrimers was 277.5 nm.

b) FT-IR Spectroscopy

The important peaks in FT-IR spectra of 3.5 G dendrimers were of Quaternary ammonium ion peak 3218.61 cm^{-1}, N-H stretch anti- symmetric sub. Primary amine 3021.91 cm^{-1}, C-H stretch 2402.87 cm^{-1}, 2834.22 cm^{-1}, C=O stretch of carbonyl group1731.61 cm^{-1}, 1650.81 cm^{-1}, C-C bending 1215.91 cm^{-1}.The important peaks in FT-IR spectra of 4.0G dendrimers were of N-H stretch of primary amine at 3310.21 cm^{-1}, N-H stretch of anti-symmetric substituted primary amine at 3021.87cm^{-1}, C-H stretch at 2947.66cm^{-1}, C=O stretch of carbonyl group at 1668.12 cm^{-1}, N-H bending of N-substituted amide at 1511.92,1417.42 cm^{-1}, C-C bending at 1215.90 cm^{-1}. The results obtained are given in **Table 1**. The FT-IR spectra of 3.5G and 4.0G PAMAM dendrimers are shown in **Fig. 4 & 5** respectively

c) Differential Scanning Calorimetry (DSC)

The changes in endothermic peak were analyzed. The changes in endothermic peak from 120.03 to 120.56 ^{0}C were observed which shows the change in structure of PAMAM dendrimers. The results obtained are given in **Table 2**. The changes in endothermic peak are shown in **Fig. 6 & 7** respectively.

d) NMR Spectroscopy

The PAMAM dendrimers was solubilized in deuterated methanol and analyzed at 300 MH_z. Important shifts in NMR spectra of 3.5G dendrimers were 2.68 and 2.93ppm for carbonyl methylene proton -(CH_2C=O), 3.40ppm amide methylene proton proton –CONHC$\underline{H_2}CH_2$N-. Important shifts in NMR spectra of 4.0G dendrimers were 3.03 ppm for carbonyl methylene proton -(CH_2C=O), 3.40 ppm amide methylene proton proton –CONHC$\underline{H_2}CH_2$N-, 3.84 (var) ppm for –NH_2 terminal group (**Fig. 8 & 9 and Table 3**).

e) ESI Mass Spectroscopy

The ESI Mass spectra further confirm the preparation of PAMAM dendrimers. The molecular weight peak of 3.5G PAMAM dendrimers was 11944 Da and the molecular weight peak of 4.0G PAMAM dendrimers was 14483 Da. The ESI Mass spectra of 3.5G and 4.0G PAMAM dendrimers are given in **Fig. 10 & 11** respectively, while data are shown in **Table 4.**

IV. Conclusions

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 cycle. Increasing amount of reactant in every progressive step was added to avoid incomplete reaction and hence to improve the yield. Completion of the reaction was confirmed by the copper sulphate solution reaction. The whole generation gave purple color, whereas half generation gave 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

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differentiation of 3.5G and 4.0G was confirmed by UV, IR, NMR and MASS spectroscopy.

The λ_{max} values were found out to be in range of 277-286 nm. The λ_{max} of 4.0G PAMAM dendrimers was found to be 277.5 nm. In IR spectrum peaks of N-H stretch for primary amine were obtained at 3310.21 cm^{-1}, which was due to NH_2 periphery of 4.0G PAMAM dendrimers. Half genenration carboxyal terminated shows intense peaks in the-C=O region while full generations shows intense peaks in the-N-H stretch for primary amine. Appearance-disappearance reappearance of characteristic peaks provides the proof of synthesis. The changes in Endothermic peak from 120.03 to 120.56^{0C} were observed which shows the change in structure of PAMAM dendrimers. In NMR spectra terminal amino group proton peaks (-CH_2NH_2) were obtained at 3.84 ppm and 2.68, 2.93, 3.03 ppm for carbonyl methylene proton (-CH_2C=O). Characteristic shifts in NMR spectra of 3.5G PAMAM dendrimers was due to terminal groups of –$COOCH_3$ at 3.73 ppm and 4.0G PAMAM dendrimers was for terminal groups of –$NHCH_2CH_2NH_2$ at 3.84 ppm. NMR spectral characteristic like shielding effects, deshielding effects, peak intensity, and integral value provides information about synthesis of dendrimers (Characteristic peaks in the spectra); conjugation chemistry (Shielding deshielding effects shifts in peaks); hydrodynamic radii (NMR pulse-field gradient spin echo); number of protons (Intensity of peaks and integral value); conformational changes (unique NMR signals from the core to the periphery); isomer populations observed by ^{1}H NMR reveal the onset of globular structure; NOE complexity emerges with globular structure: variable temperature NOESY studies show that the peripheral groups; Variable temperature coefficients for NH protons suggests that solvent is largely excluded from the interior of the dendrimer. Relaxation studies show that peripheral groups are more dynamic than groups at the core. The NMR data corroborated well with the FT-IR data to confirm the structure of the dendrimers.

The molecular weight peak of 3.5G PAMAM dendrimers was 11944 Da and the molecular weight peak of 4.0G PAMAM dendrimers was 14483 Da, which was approximate to theoretical molecular weight of PAMAM dendrimers. Deviation may be due to incomplete Michael addition causing the appearance of unsymmetrical dendrimeric structures, intramolecular cyclization, and the *retro*-Michael reaction. Synthesis of PAMAM dendrimers always generates "structural errors". Therefore it needs more attention for improving the synthesis and exploring the novel possible applications.

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Table 1 : IR Interpretation of 3.5 G dendrimers

Generation	S. No.	Peaks Value (cm^{-1})	Interpretation
3.5G	1	3218.61	Quaternary ammonium ion peak
	2	3021.91	N-H stretch anti- symmetric sub. Primary amine
	3	2834.22	C-H stretch
	4	1731.61, 1650.81	C=O stretch of carbonyl group
	5	1215.9	C-C bending
4.0G	1	3310.21	N-H stretch of primary amine
	2	3022.87	N-H stretch anti symmetric of sub. primary amine
	3	2947.66	C-H stretch
	4	1668.12	C=O stretch of carbonyl group
	5	1511.92, 1417.42	N-H bending of N- substituted amine
	6	1215.90	C-C bending

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Table 2 : DSC spectra of 3.5 G and 4.0 G PAMAM dendrimers

S. No.	Generation of dendrimers	Endothermic peak (^{0}C)
1.	3.5G	120.03
2.	4.0G	120.56

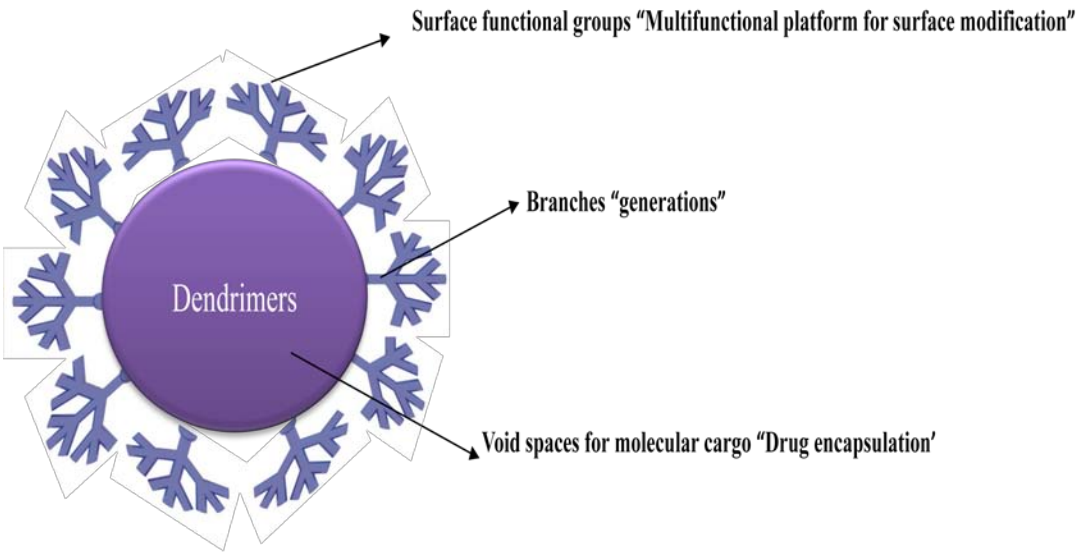
Table 3 : NMR spectra chemical shifts and interpretation of 3.5 G and 4.0G PAMAM dendrimers

S. No.	Generation	δ values (ppm)	Interpretation
1	3.5G	2.44 and 2.46	–NC**H**$_2$C**H**$_2$N-
		2.52	–NHCOCH$_2$C**H**$_2$N-
		2.68	–NHCOC**H**$_2$CH$_2$N-
		3.40	–CONHC**H**$_2$CH$_2$N-
		2.93	–NCH$_2$C**H**$_2$COOCH$_3$
		3.73	–NCH$_2$CH$_2$COOC**H**$_3$
2	4.0G	2.93	–NC**H**$_2$C**H**$_2$N-
		2.98	–NHCOCH$_2$C**H**$_2$N-
		3.03	–NHCOC**H**$_2$CH$_2$N-
		3.40	–CONHC**H**$_2$CH$_2$N-
		2.95,2.99	–CONHCH$_2$C**H**$_2$N-
		3.84	–CONHCH$_2$CH$_2$N**H**$_2$ (var)

Table 4 : ESI Mass spectra interpretation of 3.5 G and 4.0 G PAMAM dendrimers

S. No.	Generation of dendrimers	Theoretical molecular weight (Da)	Practical molecular weight (Da)
1.	3.5 G	12424	11944
2.	4.0 G	14215	14483

Figure 1 : Core structure of dendrimers



Core structure of Dendrimers

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Figure 2 : UV spectra of 3.5 G PAMAM dendrimers

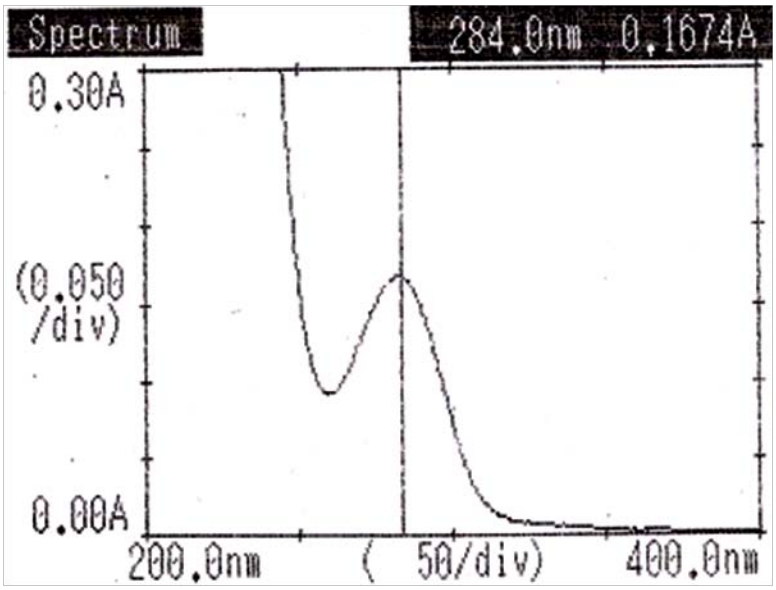


Figure 3 : UV spectra of 4 G PAMAM dendrimers

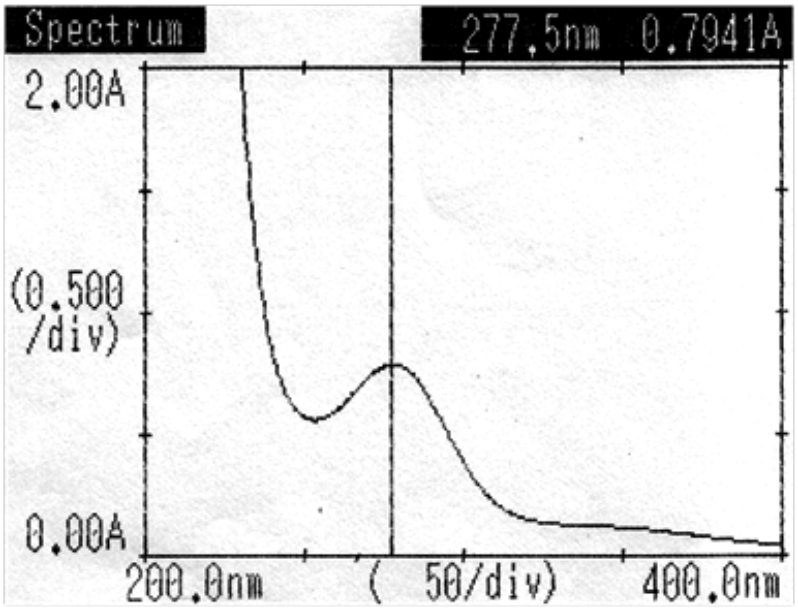
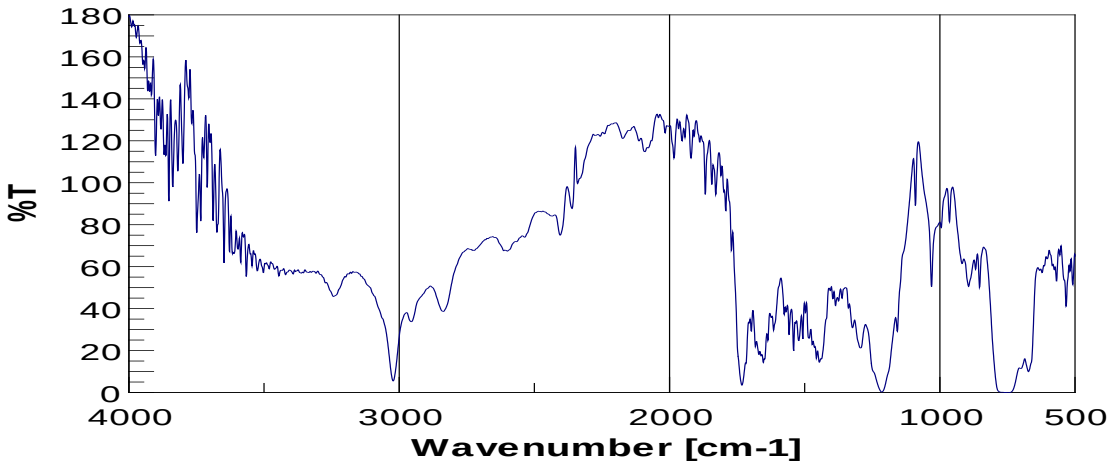


Figure 4 : FT-IR spectra of 3.5 G PAMAM dendrimers



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Figure 5 : IR spectra of 4.0 G PAMAM generation dendrimer

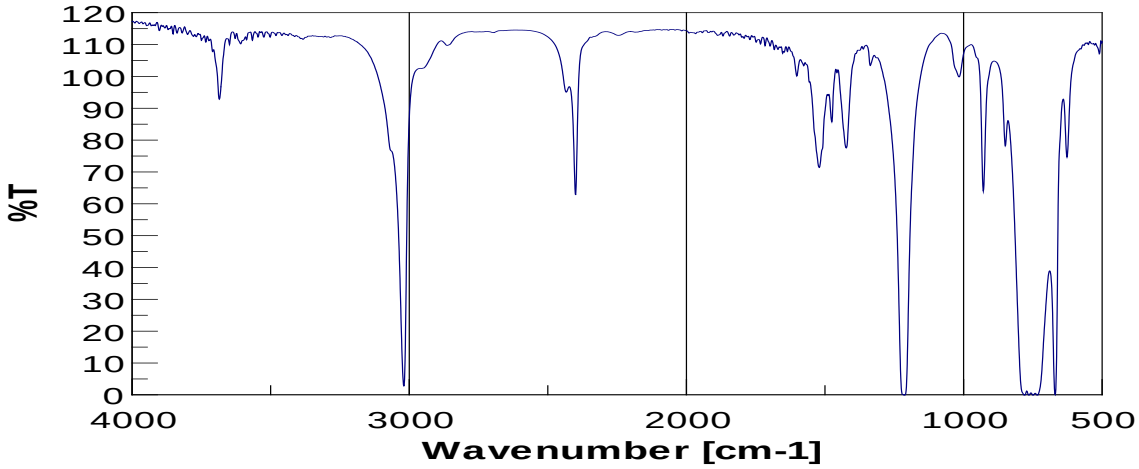


Figure 6 : DSC spectra of 3.5 G PAMAM dendrimers

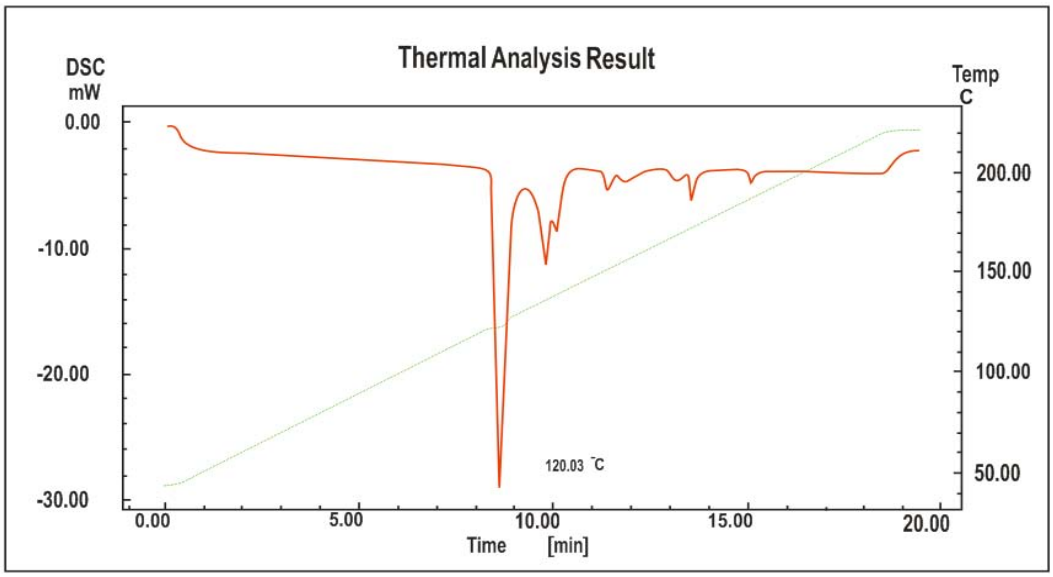
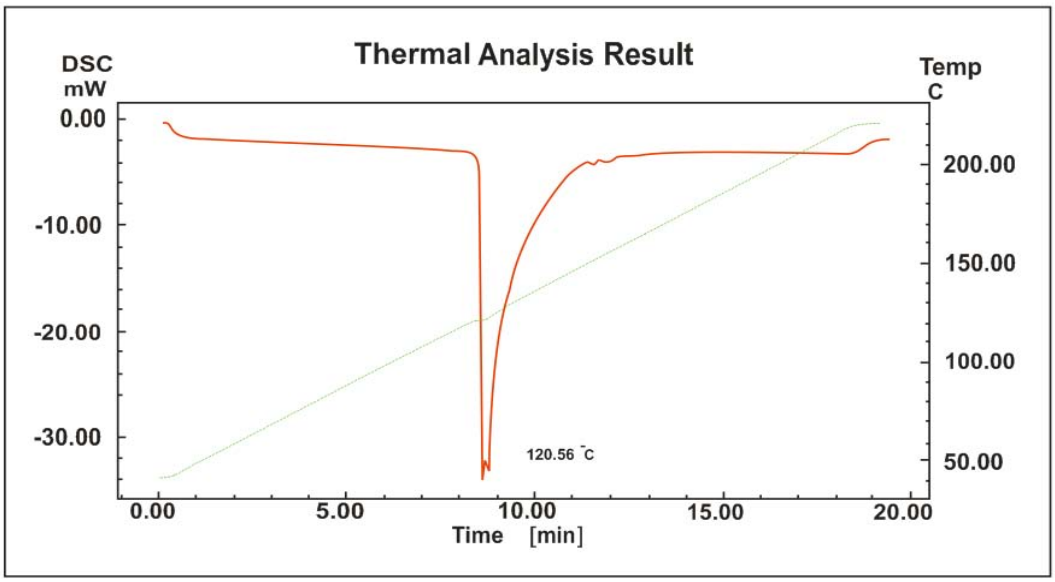


Figure 7 : DSC spectra of 4.0 G PAMAM dendrimers



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Figure 8 : NMR spectra of 3.5 G PAMAM dendrimers

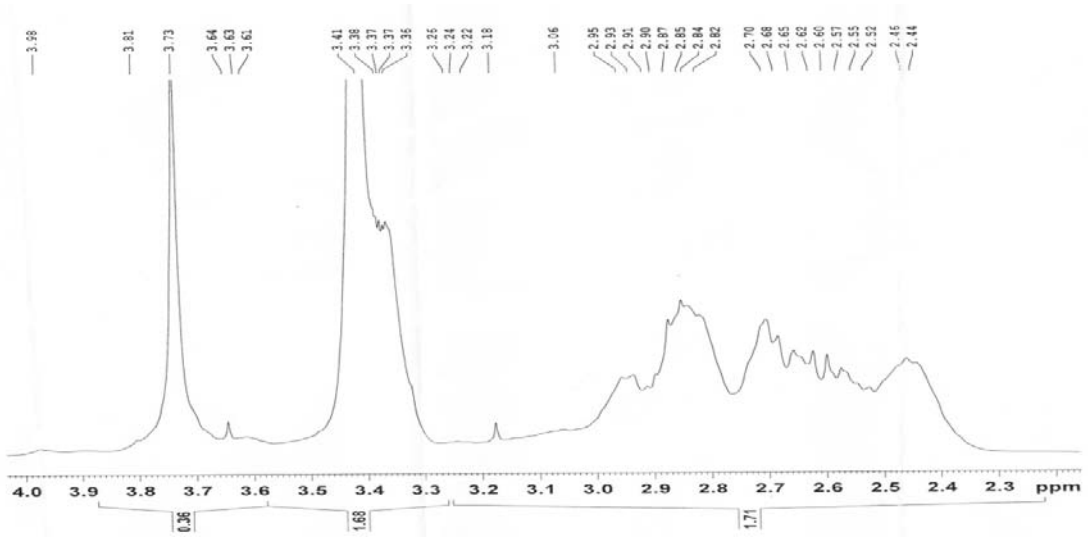


Figure 9 : NMR spectra of 4.0 G PAMAM dendrimers

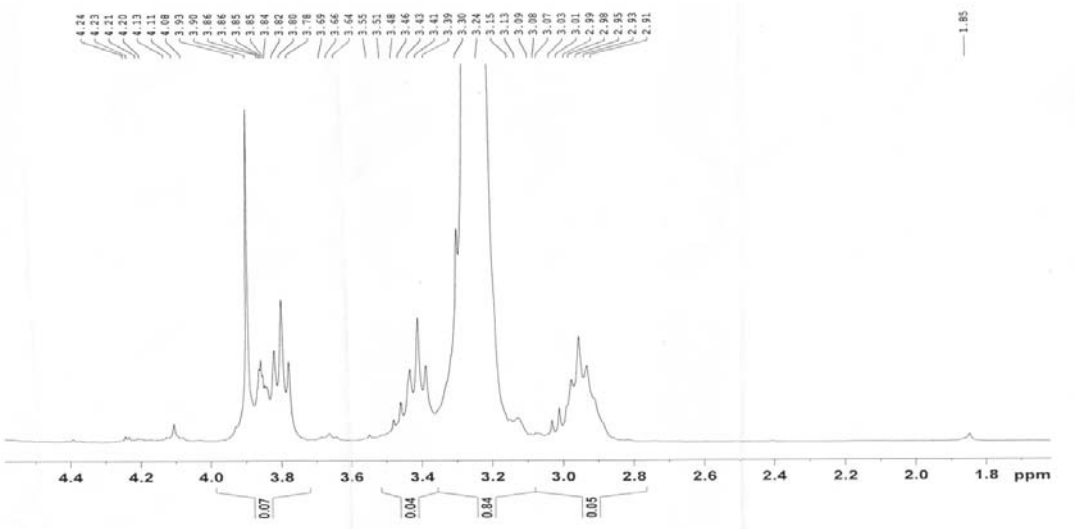
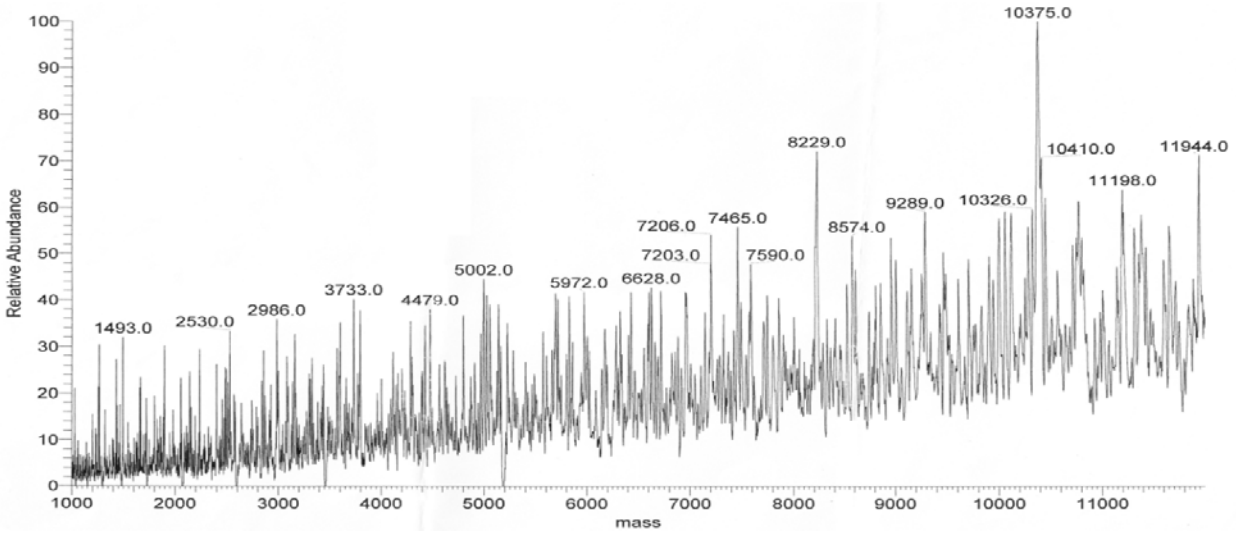
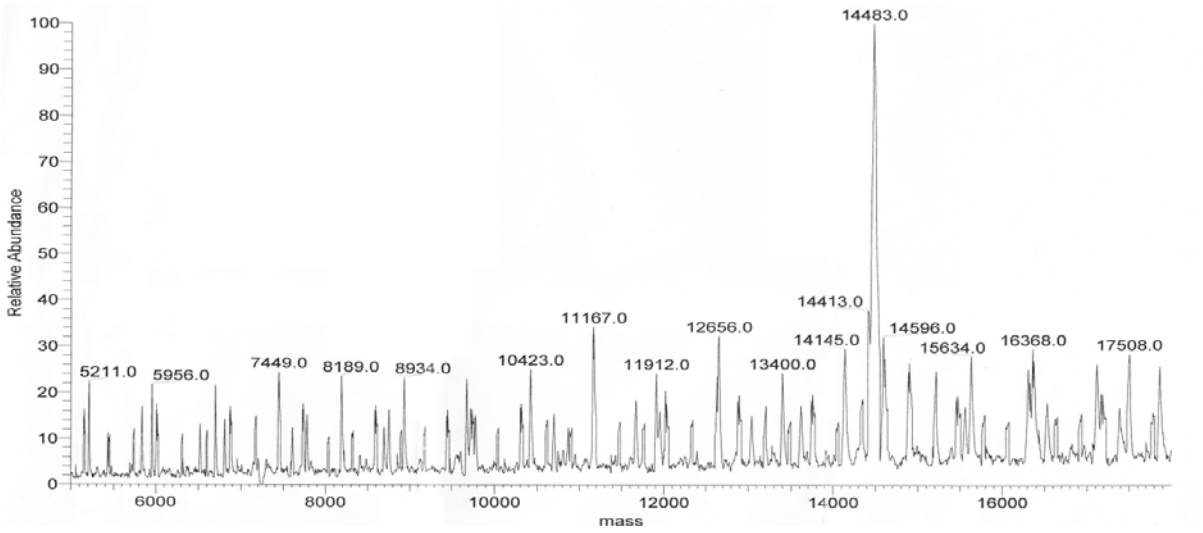


Figure 10 : ESI Mass spectra of 3.5 G PAMAM dendrimers



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Figure 11 : ESI Mass spectra of 4.0 G PAMAM dendrimers



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