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Optimization and Evaluation of Process Variables for Synthesis of PAMAM dendrimer

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Abstract

Dendrimers are synthetic, highly branched, nearly spherical and symmetrical macromolecules with well-defined sizes and compositions. Dendrimers can be synthesized by appropriately selecting the cores, connecting units, branching sites, and terminal groups. PAMAM (Polyamido amine) dendrimers are multifunctional nano platforms for various clinical and diagnostic applications. There are two basic approaches for synthesis of dendrimers viz. divergent and convergent method. The divergent method is still one of the preferred one for synthesis but problems like low yield after purification and uncompleted reactions are disadvantages of this method. In present research work different process variables like solvent system, reaction temperature and reaction time were optimized to increase % yield and to obtain complete product after reaction of each generation. The three different solvents viz. Methanol, Chloroform and DMSO were taken at room and cool temperature for reaction time of 24, 48, 72 and 96 hours. The results of study revealed that highest purity and % yield was obtain at cool temperature by taking methanol as solvent and different reaction times found to be suitable for different generation reaction.

Key words: PAMAM Dendrimer, methanol, process variables, divergent method.

Introduction

Dendrimer is ideal carrier for drug delivery due to advantages like very low size (1-5 nm), feasibility to develop with defined molecular weight, very low polydispersity index (ratio of weight average molecular weight (Mw) to number average molecular weight (Mn) of polymer), good entrapment efficiency and offering surface for fictionalization. Dendrimer contain three different regions: core, branches, and surface groups. The macromolecule constituents radiate in branching form from the central core, creating an internal cavity as well as a sphere of end groups[1].

Synthesis of a dendrimer according to the divergent method[2] (Figure 1) proceeds stepwise, starting from a multi-functionalized core building block, to whose reactive coupling sites new branching units in the form of dendritic branches are attached *via* a reactive terminal functionality. During reaction, other functional groups of the branching unit are protected and after the first reaction step, the protected functional groups are deprotected (activated) and then serve as new reactive coupling sites for further branching units. A new dendrimer generation arises with each branching unit.

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The synthesis of an EDA core PAMAM dendrimers consist of consecutive steps: Michael addition of primary amine (EDA in very first step) to methyl acrylate/ Methyl metha acrylate followed by amidation of formed multiester (tetra ester at very beginning) of EDA[3-4].

Michael addition reaction uses 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 -0.5 PAMAM 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"[5-7].

In present work different solvent medium were taken for reactions at different temperature for time period of 24, 48, 72 and 96 hours. The resultant dendrimer were checked for completion of reaction and % yield after purification.

Material and Methods

Materials

Ethylenediamine, Methylacrylate, Methyl Metha acrylate and DMSO are obtained from Loba Chem Pvt.Ltd., Mumbai. Other chemicals used were of analytical grade and all solvents used were of HPLC grade.

Synthesis of dendrimer

The syntheses of dendrimer were performed by divergent method using EDA as core. It consists of consecutive steps: Michael addition of primary amine (EDA in very first step) to methyl acrylate/ Methyl metha acrylate followed by amidation of formed tetraester of EDA. The reaction was carried out using different solvents (methanol/$CHCl_3$/DMSO) as medium. The reactions was be 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 Suitable (room temperature/ cold temperature), using amber colored round bottom flask, which will be corked tightly as the reaction condition need to be carried out in dark conditions because the moisture may affect the synthesis reaction. The four reaction times were taken to complete both reactions i.e. addition and amidation reactions allowed to complete in 24, 48, 72 and 96 hrs.

Optimization of process variables

The syntheses of dendrimer is chemical reaction that can occur at specific molar ratio, so unlike other delivery systems, quantity cannot be changed but the process variables can be optimized to complete the reaction and obtain better yield. So in present work we synthesized dendrimer using three process variables i.e. solvent system, Reaction time and reaction temperature.

The solvents used for reaction are Methanol, Chloroform and DMSO, all solvents are organic solvents and both reacting substances are soluble in all three solvents.

Dendrimer are not synthesized in light so amber colored glassware were used at different temperatures i.e. at room temperature (25 ± 2^0C) and cool temperature (8 ± 1^0C). The reaction time was also changed to optimize the completion time of both reactions separately after completion of each batch.

Depending up on above variables different batches were synthesized (Table No. 1). The optimization performed very carefully and completion of reaction was checked after each step and then further proceeds for next step.

Characterization:

Copper sulphate Test:

This is basic step for checking and confirmation of the completion of reaction. 10 drops of 10% Copper sulphate [$CuSO_4$] reagent was taken in a small test tube and 2 drops of resultant mixture of dendrimer was added to it. The Deep blue color indicates presence of terminal Carboxylic acid group and violet color indicates presence of primary amine groups. If any batch not showed completion of reaction than that batch was not used for further generations.

UV- Vis spectrometry:

The completion of reaction was further confirmed by using UV-VIS spectrophotometer (Shimadzu UV-1800). The samples were dissolved in distilled water and scanned in the range 200-400 nm for change of λmax after each reaction.

IR interpretation:

The IR spectra of different samples after each reaction were taken using FTIR-2000A, ABB Spectrophotometer ($ZrCl_2$). The change in characteristic peaks of surface group confirms the completion of reaction.

NMR spectroscopy:

^{1}H NMR spectra of the various generations of PAMAM were recorded on Bruker DRX-300 (300 MHz) spectrometer. The samples were dissolved in methanol and chemical shifts are recorded as δ (ppm) relative to Tetramethylsilane [TMS] as a standard. Various shifts in the peaks were interpreted for different groups in all generations.

% Yield of dendrimer

The percentage yields of 4.0 generation of different sets of dendrimers were calculated after purification. The % yield was calculated by following formula for each set of dendrimer:

$$\% \text{ Yield} = \frac{\text{Practical Yield}}{\text{Therotical Yield}} \times 100$$

Results and Discussion

PAMAM dendrimer from -0.5 to 4.0 generations were synthesized by using divergent growth method using different process variables. Completions of the reactions were primarily confirmed by the copper sulphate reaction. The formations of different generations of PAMAM dendrimer were further confirmed by UV, NMR and IR spectral data.

Copper Sulphate Test

Appearance of a violet coloration with full generation dendrimer indicates presence of an amine functional group on the surface of dendrimer. While dark blue color was observed for half generations dendrimer. The data for different generations were given in (table no. 2-5). The data revealed that reaction was not completed in DMSO and at room temperature. In lower generations reaction time 24 and 48 hrs shows completion of reaction but at higher generations 72 and 96 hrs were required to complete reaction. Chloroform was not able to dissolve higher generation of dendrimer so reaction was not completed.

UV Analysis

The Synthesized dendrimer sample was taken in distilled water and estimated in UV spectrophotometer in the range of 200 – 400 nm and λ_{max} was found to be 275 – 290 nm. The Change in λ_{max} values confirmed the completion of reaction. λ_{max} values are reported in table 6

IR Spectroscopy

IR spectra of different generations of PAMAM dendrimer and were analyzed. The IR peaks confirmed the formation of different generations of dendrimer. The diminishing of peaks of ester in the range [1750-1720 cm^{-1}] for full generation PAMAM dendrimer and disappearance of peaks of amine, in the range [3350-3250 cm^{-1}] for half generation PAMAM dendrimer confirmed the completion of reaction. (Figure no 2-3)

NMR Spectroscopy

NMR spectra further confirm the formation of different generations. Significant change in integral values and shifts of Primary -NH2 group was noticed after each step. The important shifts in NMR spectra of 3.0 G and 4.0G PAMAM dendrimers are shown in Fig 4-5

% yield

The percentage yields of different 4.0 PAMAM generations were calculated (table no. 7). The data shows that when dendrimers were synthesized with methanol as solvent at room temperature and 72 hrs reaction time for one generation ,gives highest yield value.

Conclusion

The present work was an attempt to optimize the process variables for synthesis of PAMAM dendrimer. The Divergent method was used to synthesize 4.0 generation dendrimer by Michael addition followed by amidation reaction. The three process variables: solvent system, reaction temperature and reaction time were changed to synthesize 24 batches. The completion of reaction was checked by copper sulphate test after each generation and then it was further confirmed by UV, IR and NMR spectra.

The results obtained from various reactions depicts that when reaction was done at cool temperature using methanol solvent for 72 hrs reaction time showed completion of reaction and gave highest Yield value. The reaction time of 24/48 hrs can be used to synthesize lower generations (0.0 and 1.0) but for higher generations (2.0 onwards) reaction time to complete reaction was 72 hrs.

Hence it can be concluded that these variables are optimized for synthesis of PAMAM dendrimers by divergent method.

Table 1: Formulation codes for different process variables

Formulation code	Reaction Temperature	Solvents	Reaction time
PD 1	room temperature (25±2^{0}C)	Methanol	24 hrs
PD 2	room temperature (25±2^{0}C)	Chloroform	24 hrs
PD 3	room temperature (25±2^{0}C)	DMSO	24 hrs
PD 4	cool temperature (8±1^{0}C)	Methanol	24 hrs
PD 5	cool temperature (8±1^{0}C)	Chloroform	24 hrs
PD 6	cool temperature (8±1^{0}C)	DMSO	24 hrs
PD 7	room temperature (25±2^{0}C)	Methanol	48 hrs
PD 8	room temperature (25±2^{0}C)	Chloroform	48 hrs
PD 9	room temperature (25±2^{0}C)	DMSO	48 hrs
PD 10	cool temperature (8±1^{0}C)	Methanol	48 hrs
PD 11	cool temperature (8±1^{0}C)	Chloroform	48 hrs
PD 12	cool temperature (8±1^{0}C)	DMSO	48 hrs
PD 13	room temperature (25±2^{0}C)	Methanol	72 hrs
PD 14	room temperature (25±2^{0}C)	Chloroform	72 hrs
PD 15	room temperature (25±2^{0}C)	DMSO	72 hrs
PD 16	cool temperature (8±1^{0}C)	Methanol	72 hrs
PD 17	cool temperature (8±1^{0}C)	Chloroform	72 hrs
PD 18	cool temperature (8±1^{0}C)	DMSO	72 hrs
PD 19	room temperature (25±2^{0}C)	Methanol	96 hrs
PD 20	room temperature (25±2^{0}C)	Chloroform	96 hrs
PD 21	room temperature (25±2^{0}C)	DMSO	96 hrs

PD 22	cool temperature (8±1^{0}C)	Methanol	96 hrs
PD 23	cool temperature (8±1^{0}C)	Chloroform	96 hrs
PD 24	cool temperature (8±1^{0}C)	DMSO	96 hrs

Table 2: Completion of reaction of 1.0 generation

Formulation code	Completion of reaction	Solvents	Reaction time	Status of reaction
PD 1	room temperature (25±2^{0}C)	Methanol	24 hrs	Complete
PD 2	room temperature (25±2^{0}C)	Chloroform	24 hrs	Complete
PD 3	room temperature (25±2^{0}C)	DMSO	24 hrs	Incomplete
PD 4	cool temperature (8±1^{0}C)	Methanol	24 hrs	Complete
PD 5	cool temperature (8±1^{0}C)	Chloroform	24 hrs	Complete
PD 6	cool temperature (8±1^{0}C)	DMSO	24 hrs	Incomplete
PD 7	room temperature (25±2^{0}C)	Methanol	48 hrs	Complete
PD 8	room temperature (25±2^{0}C)	Chloroform	48 hrs	Complete
PD 9	room temperature (25±2^{0}C)	DMSO	48 hrs	Incomplete
PD 10	cool temperature (8±1^{0}C)	Methanol	48 hrs	Complete
PD 11	cool temperature (8±1^{0}C)	Chloroform	48 hrs	Complete
PD 12	cool temperature (8±1^{0}C)	DMSO	48 hrs	Incomplete
PD 13	room temperature (25±2^{0}C)	Methanol	72 hrs	Complete
PD 14	room temperature (25±2^{0}C)	Chloroform	72 hrs	Complete
PD 15	room temperature (25±2^{0}C)	DMSO	72 hrs	Incomplete
PD 16	cool temperature (8±1^{0}C)	Methanol	72 hrs	Complete
PD 17	cool temperature (8±1^{0}C)	Chloroform	72 hrs	Complete
PD 18	cool temperature (8±1^{0}C)	DMSO	72 hrs	Complete
PD 19	room temperature (25±2^{0}C)	Methanol	96 hrs	Complete
PD 20	room temperature (25±2^{0}C)	Chloroform	96 hrs	Complete
PD 21	room temperature (25±2^{0}C)	DMSO	96 hrs	Incomplete
PD 22	cool temperature (8±1^{0}C)	Methanol	96 hrs	Complete
PD 23	cool temperature (8±1^{0}C)	Chloroform	96 hrs	Complete
PD 24	cool temperature (8±1^{0}C)	DMSO	96 hrs	Complete

Table 3: Completion of reaction of 2.0 generation

Formulation code	Completion of reaction	Solvents	Reaction time	Completion of reaction
PD 1	room temperature (25±2^{0}C)	Methanol	24 hrs	Incomplete
PD 2	room temperature (25±2^{0}C)	Chloroform	24 hrs	Incomplete
PD 4	cool temperature (8±1^{0}C)	Methanol	24 hrs	Incomplete
PD 5	cool temperature (8±1^{0}C)	Chloroform	24 hrs	Incomplete
PD 7	room temperature (25±2^{0}C)	Methanol	48 hrs	Complete
PD 8	room temperature (25±2^{0}C)	Chloroform	48 hrs	Incomplete
PD 10	cool temperature (8±1^{0}C)	Methanol	48 hrs	Complete
PD 11	cool temperature (8±1^{0}C)	Chloroform	48 hrs	Complete
PD 13	room temperature (25±2^{0}C)	Methanol	72 hrs	Complete
PD 14	room temperature (25±2^{0}C)	Chloroform	72 hrs	Incomplete
PD 16	cool temperature (8±1^{0}C)	Methanol	72 hrs	Complete
PD 17	cool temperature (8±1^{0}C)	Chloroform	72 hrs	Complete
PD 18	cool temperature (8±1^{0}C)	DMSO	72 hrs	Incomplete
PD 19	room temperature (25±2^{0}C)	Methanol	96 hrs	Complete
PD 20	room temperature (25±2^{0}C)	Chloroform	96 hrs	Incomplete

PD 22	cool temperature (8±1^{0}C)	Methanol	96 hrs	Complete
PD 23	cool temperature (8±1^{0}C)	Chloroform	96 hrs	Complete
PD 24	cool temperature (8±1^{0}C)	DMSO	96 hrs	Incomplete

Table 4: Completion of reaction of 3.0 generation

Formulation code	Completion of reaction	Solvents	Reaction time	Completion of reaction
PD 7	room temperature (25±2^{0}C)	Methanol	48 hrs	Incomplete
PD 10	cool temperature (8±1^{0}C)	Methanol	48 hrs	Incomplete
PD 11	cool temperature (8±1^{0}C)	Chloroform	48 hrs	Incomplete
PD 13	room temperature (25±2^{0}C)	Methanol	72 hrs	Completed
PD 16	cool temperature (8±1^{0}C)	Methanol	72 hrs	Completed
PD 17	cool temperature (8±1^{0}C)	Chloroform	72 hrs	Incomplete
PD 19	room temperature (25±2^{0}C)	Methanol	96 hrs	Completed
PD 22	cool temperature (8±1^{0}C)	Methanol	96 hrs	Completed
PD 23	cool temperature (8±1^{0}C)	Chloroform	96 hrs	Incomplete

Table 5: Completion of reaction of 4.0 generation

Formulation code	Completion of reaction	Solvents	Reaction time	Completion of reaction
PD 13	room temperature (25±2^{0}C)	Methanol	72 hrs	Incomplete
PD 16	cool temperature (8±1^{0}C)	Methanol	72 hrs	Completed
PD 19	room temperature (25±2^{0}C)	Methanol	96 hrs	Incomplete
PD 22	cool temperature (8±1^{0}C)	Methanol	96 hrs	Completed

Table 6: λ max of different generations

S.No.	Dendrimers generation	λ_{max}[in nm]
1	0.0 PAMAM	282.5
2	1.0 PAMAM	280.0
3	2.0 PAMAM	284.0
4	3.0 PAMAM	285.5
5	4.0 PAMAM	281.0

Table 7: % yield of dendrimers

S.No.	Formulation code	% Yield
1.	PD 13	45.34
2.	PD 16	72.54
3.	PD 19	56.34
4.	PD 22	71.68

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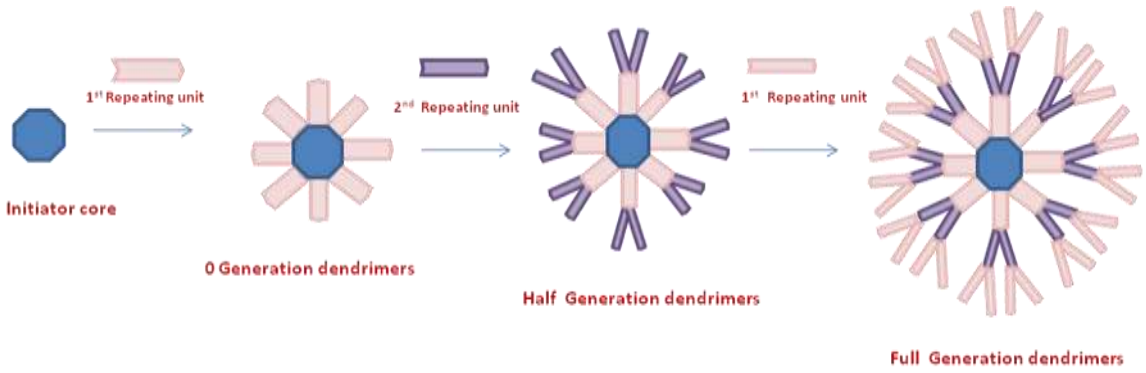

Fig. 1: Divergent method for synthesis of dendrimer

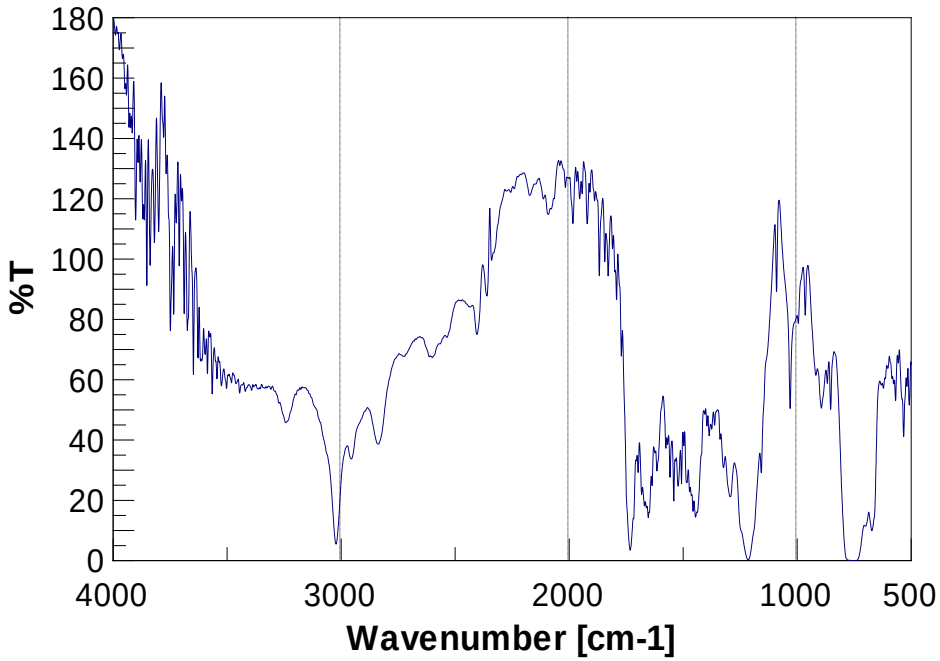

Fig. 2: IR spectra of 3.5 G PAMAM generation dendrimer

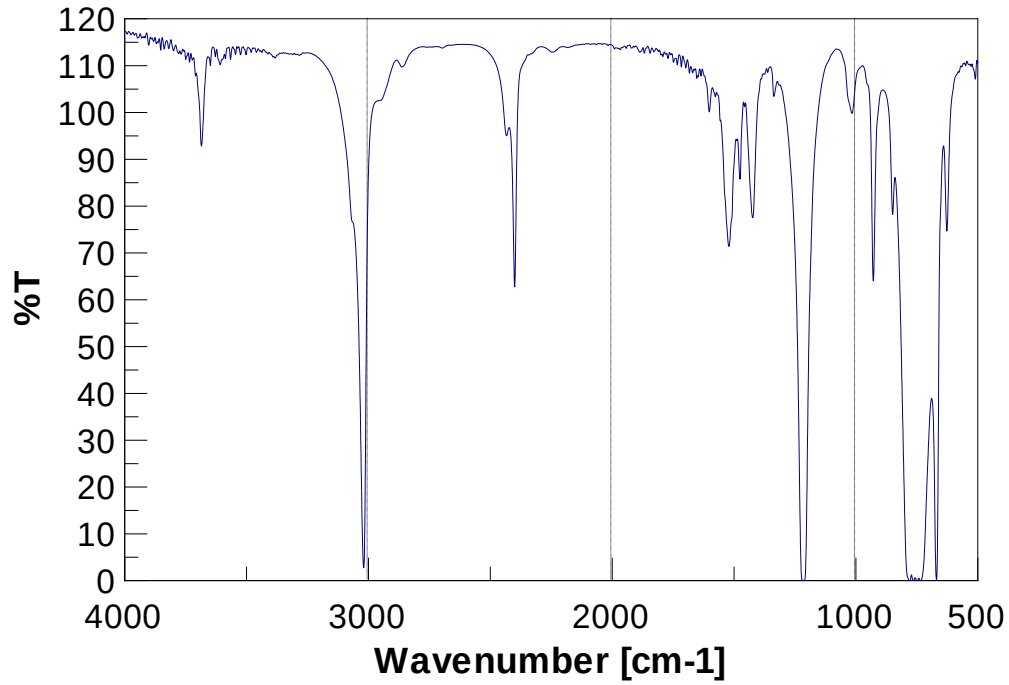

Fig. 3: IR spectra of 4.0 G PAMAM generation dendrimer

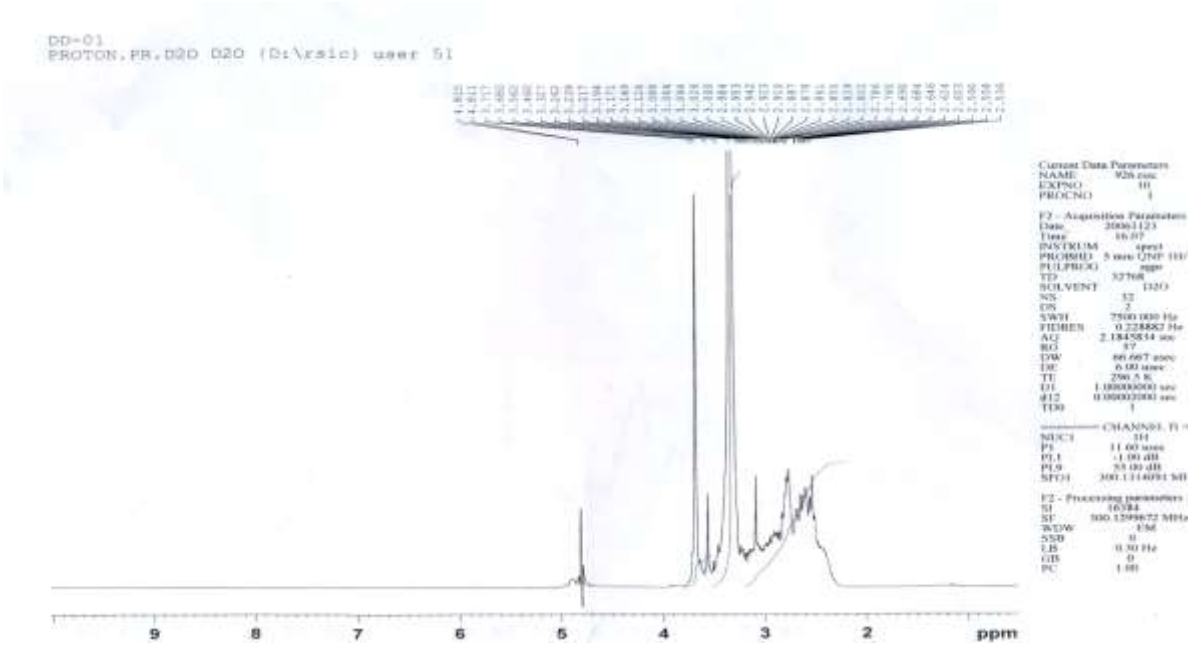

Fig. 4: NMR spectra of 3.5 G dendrimer

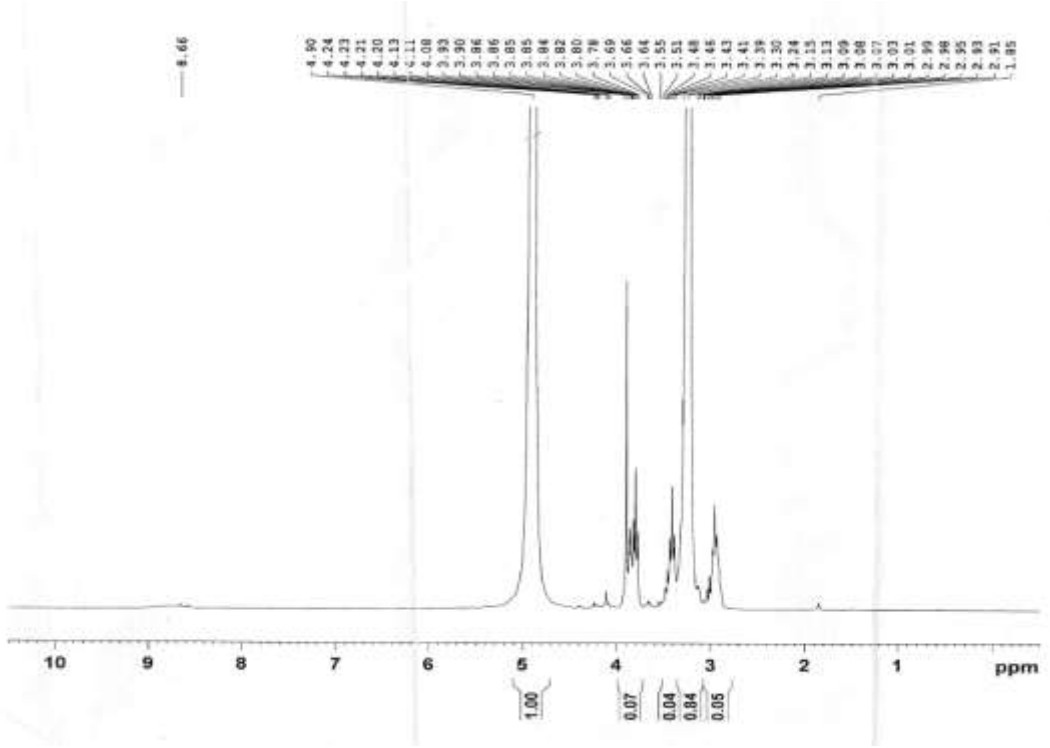

Fig. 5: NMR Spectra of 4.0 G PAMAM Dendrimer

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