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Optimization and computational studies evaluating molecular dynamics of EDA cored polymeric dendrimer

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In this work we report the results acquired from molecular dynamics simulations as well as the optimization of different generations of polyamidoamine dendrimer. The analysis data revealed synthesized dendrimer as a suitable nanostructured candidate suitable for neutral as well as charged molecule delivery due to the presence of both electrostatic potential and van der Waals forces. The methyl ester terminating groups of half-generation dendrimers with characteristic IR peaks for carbonyl at 1670.41 cm^{-1} tends to shift to 1514.17 cm^{-1} on conversion to amide group of full-generation dendrimer. The study includes the usage of detailed analysis, demonstrating how molecular dynamics affect the dendrimer complexation. The present investigations provide an unprecedented insight into the computational and experimental system that may be of general significance for the clinical application of dendrimers.

The modern age revolutionized the foundation of a new era of molecular polymers i.e. polymeric dendrimers. The nanostructured polymers are repetitively bifurcated morphological spherical molecules with distinguishing chemical elegance, pertaining to flexible, versatile and promising molecular representatives[1,2]. The polymers are constructed via a repetitive successive process based primarily on two different approaches: the divergent[3,4] and the convergent[5–7]. Former one deals with the addition of monomers to the core forming layers and the later one starts from the periphery to the core. Since the initial reports of dendrimers various systems have been reported and received widespread attention. In addition, due to structural and chemical diversity, they have produced several opportunities for modification. They have come up as an excellent platform for terminal attachment of multiple close-packed functionalities, as well as cavities around the focal core permit various opportunities[8,9].

The multifunctional hyperbranched analogues with tunable physicochemical attributes of the Tomalia's polyamidoamine[10] make them promising candidates for poorly soluble drugs[11–32] such as fluorine[33], complexes with metal ions[34], catalysis[35,36], gene delivery[37,38], drug delivery[39], sensors, and many other. The influence on chemical and physical properties of dendritic polymers by peripheric moieties have been confirmed by an assortment of reports. The peripheral functionalities are also responsible for impacting the stability, solubility, viscosity, flexibility, aggregation and chemical reactivity as well as spatial and surface shape of the dendrimer[40]. The terminal impact on molecular properties increases with the exponential growth of dendrimer generation. Moreover, a molecule periphery functionalization is the most promising and straightforward option in terms of creating novel dendrimer properties.

In the direction towards the contemplation of molecular dynamics inclusive of molecular interaction at the molecular level, the molecular modeling emerged as a potential means. The algorithmic methodology provides better and broader molecular perception, making molecule designing effective and simpler. The hyperbranched dendritic polymer encompassing repetitious structure can be competently taken advantage of in designing novel porters for both therapeutic and diagnostic agents. The studies discussing the optimization of its properties in various solvents, interaction with drugs, nucleic acids, proteins and lipid membranes have been studied. The pondered researches have brought characteristic features such as dendrimer size and surface to light, that can be altered to augment the performance of anti-TB[41], anti-cancer[42–44] drugs and many other. There are reports of dendritic geometry optimization using forcefield such as AMBER[45,46], CHARMM[47–49], GROMOS[50], MARTINI[51–53] CVFF[54,55], OPLS[56,57] and DREIDING Force[58].

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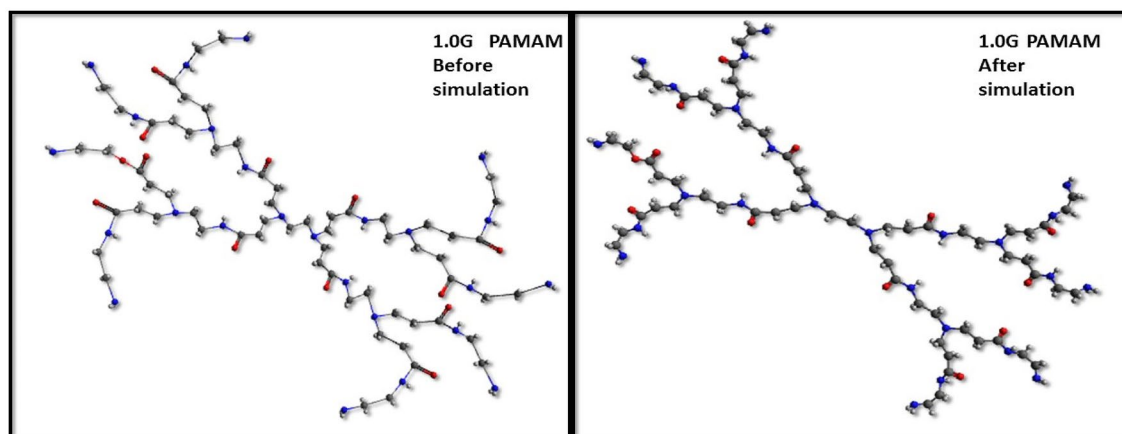
Figure 1. Illustrations showing parameterized simulations on 1.0G PAMAM (Avogadro 1.1.1, http://avogadro. cc).

The knowledge of molecular dynamics by computational studies have advanced experimental work. For enhanced envision and insight of dendrimers and their interactions, computational designs provide sustenance to ameliorate efficiency. This investigational approach is converged on the optimization and application of molecular modeling to analyze the layout of dendrimers. The emphasis has been made to improve the efficacy of this class of molecules. In this paper, dendrimer molecular models were parameterized using the UFF forcefield and molecular dynamic atomistic simulation approaches have been employed. Simultaneously, the dendritic polymers were optimized at laboratory scale. To meet the augmenting requirement of dendrimers for multiple applications, the co-application of computational and experimental approach can significantly enhance the clinical application of dendrimers.

Results

Evaluation of computational studies. Essentially like all molecules, the PAMAM (Polyamidoamine) dendrimer structure shows correspondence with properties and its utilization. The synthesis of highly branched structures with potential practice is directly or indirectly dependent on intrinsic features such as molecular weight, van der wall forces. The UFF elucidates the forces workable for simulation of atoms within dendrimer structure, exhibiting the sum of molecular properties as well as electrostatic and van der Waals interactions, Fig. 1.

In this study we also contemplate the effect of size of dendrimer generation on the conformational characteristics. The deportment of the simulations conducted divulge the augment in average radius corresponding to the increased dendrimer generation. Consequently, the increment in radius of central atom magnifies the distance among atoms. Hence, these alterations toil synergistically with the entrapment or encapsulation of moiety by dendrimer. The simulation studies of PAMAMs overseen in this communication (Fig. 2) manifest the use of high generation dendrimer molecules as a competent approach for drug delivery.

Taking into cognizance, both experimental and simulation studies, the moderate size of the dendrimer concede them to acquit as soft deformable particles rather than compact spheres, reproducing them as an attributable candidate for drug delivery. This is conclusively confirmed in the present study. Comparison between the computational and theoretically calculated molecular weight are shown in Table 1 and Fig. 3. The inferences distinctly substantiate the close agreement between computational and theoretical data.

The detailed effect of the different degree of protonation (i.e., different pH values), was studied by executing simulations of molecules at four different pH levels. The effect of pH was scrutinized using molecular editor, Avogadro, that concede the pH disposition of the molecular environment. All MD simulations were carried out in aqueous solutions under realistic requisites of different pH levels. The assessment of van der Waals spheres was found to be significant in the characteristic determination of dendrimers. These spheres stipulate information in correlation with internal cavities[59], which can be further utilized for the determination of number of molecules dendrimers will be competent to sustain (Fig. 4). The pretense of both electrostatic potential and van der Waals region conjointly validates the dendrimer efficient for binding neutral as well as charged molecule.

Evaluation of dendrimer. Progress of synthesis and differentiation of full and half-generation of dendrimers were confirmed spectrophotometrically. The synthesis of dendrimers reported here employed the usage of ethylenediamine and methyl acrylate, where, former act as initiator core and later as a repeating unit. The yields of 1.0G, 2.0G, 3.0G and 4.0G were reported to be 95, 92, 84 and 81%, respectively: and 0.5G, 1.5G, 2.5G and 3.5G were 90, 84, 81 and 72%, respectively. The copper sulfate color chelation reactions affirmed the completion of each synthetic step. The copper sulfate on reaction with terminal amine group gave deep blue color signifying the presence of half generation, whereas, reaction with terminal carboxyl group gave purple color distinctly affirming the presence of full generation, Fig. 5.

The inquisition detailed here affirm the termination of each synthetic step included in the preparation of the PAMAM dendrimer. A startling change in color was noticed after admixture of carboxyl/amine terminated
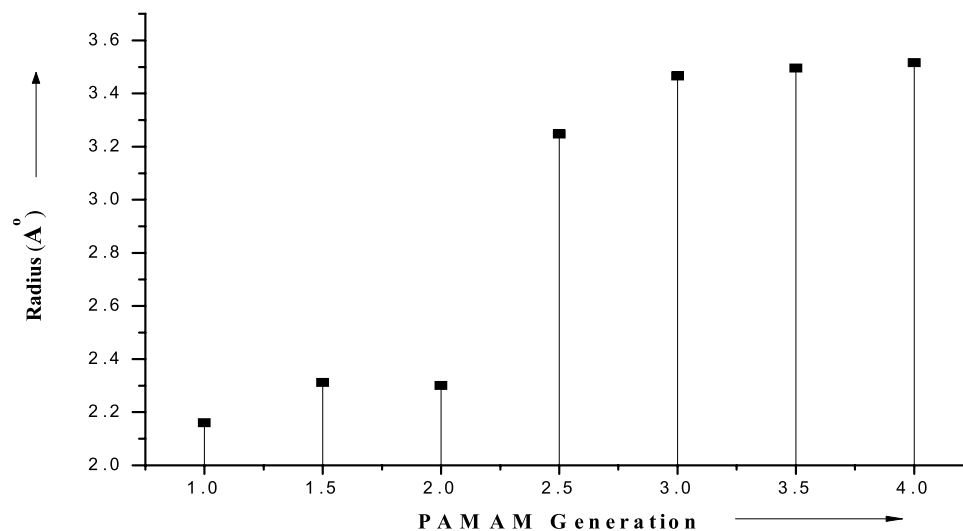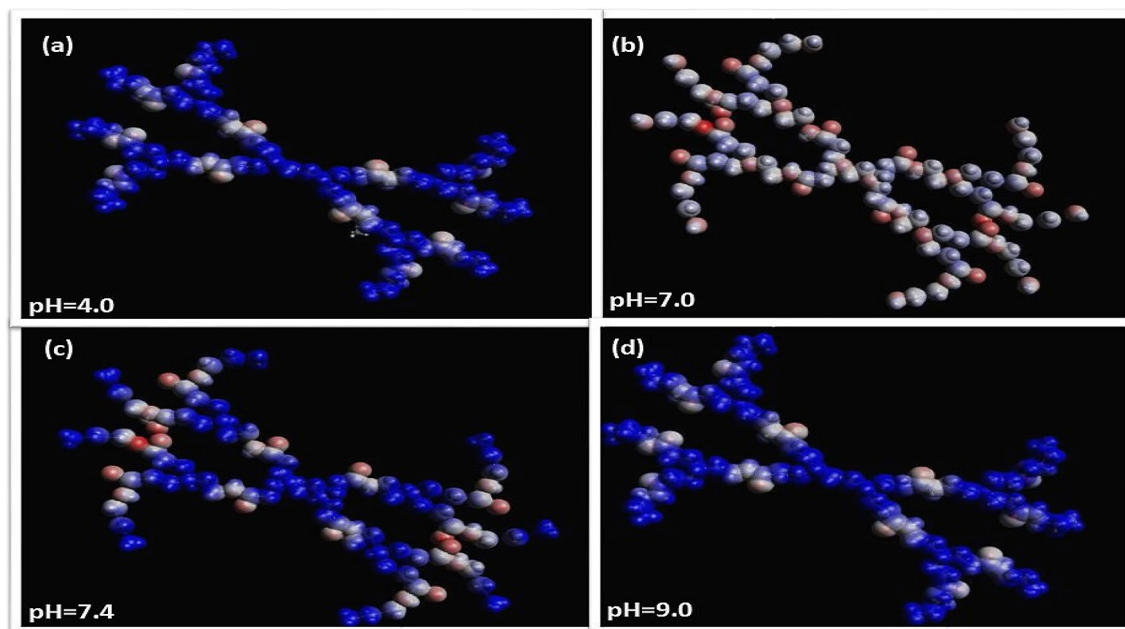
Figure 2. Polyamidoamine generation dependence of the average radius between central atom of dendrimer (OriginPro 8 SR0, v8.0724 (B724), OriginLab Corporation: http://www.OriginLab.com).

| Generation	Number of end groups	Theoretical molecular weight (g/mol)	Calculated Molecular weight post simulation (g/mol)
− 0.5	4	405	404.455
0.0	4	517	516.681
0.5	8	1205	1205.395
1.0	8	1430	1430.831
1.5	16	2807	2836.312
2.0	16	3256	3266.065
2.5	32	6011	5974.471
3.0	32	6909	6846.870
3.5	64	12,424	11,422.589
4.0	64	14,215	13,212.462

Table 1. Molecular dynamics of different generation of polyamidoamine dendrimer.
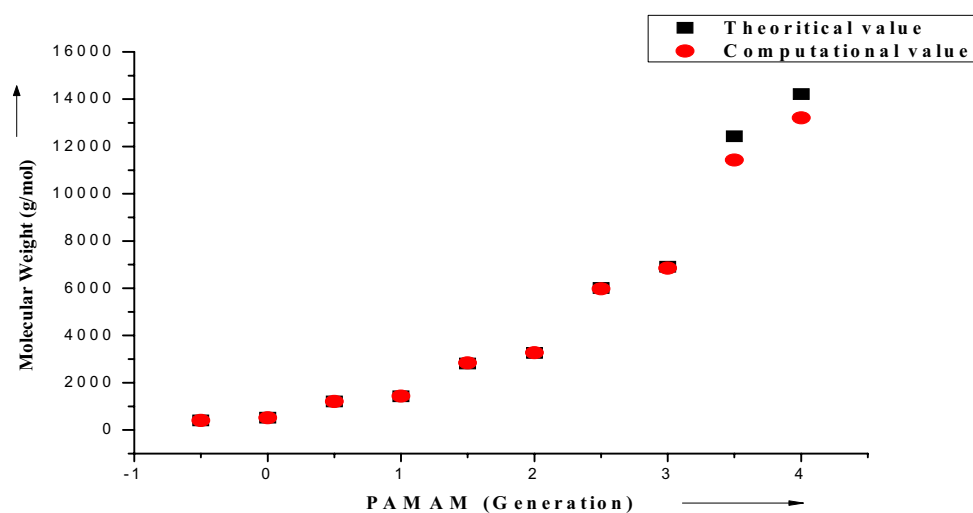
Figure 3. Graphical plot showing alter in molecular weight obtained from theoretical and computational studies of dendrimers (OriginPro 8 SR0, v8.0724 (B724), OriginLab Corporation: http://www.OriginLab.com).

Figure 4. Comparison of Van der Walls effect on 1.0G polyamidoamine generation at (**a**) pH = 4.0, (**b**) pH = 7.0, (**c**) pH = 7.4 and (**d**) pH = 9.0 (Blue region: Van der Waals sphere; Other region: Electrostatic potential) (Avogadro 1.1.1, http://avogadro.cc).
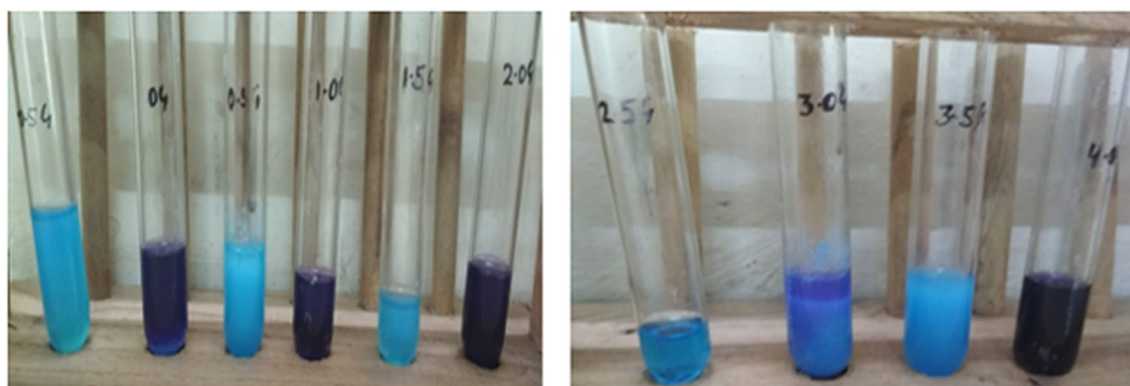

Figure 5. Copper sulphate test results of different generations of polyamidoamine.

dendrimer with copper sulfate solution. The observation led researchers to conclude the formation of complex on combination of copper with terminal groups. Consequently, the blue precipitate is formed due to the reaction of hexaaquacopper(II) complex ion $-[Cu(H_2O)_6]^{2+}$ present in copper sulphate solution with terminal amine group. Similarly, admixture of carboxyl terminated dendrimer and copper sulfate solution produced the purple color precipitates.

The synthesis of dendrimer was overseen by usage of UV–Visible spectroscopy. The variation in λmax values perceived from half-generation to full generation i.e. from 290 to 270 nm impart the structural modification of PAMAM (Polyamidoamine) dendrimers, as shown in Fig. 6. The proportional correlation between absorption and number of chromophoric units explicate the alter in the intensity of the absorption band with the growing generation. This manifests the conformation of synthesis of dendrimers.

The Infrared (IR) spectrum of different generations of dendrimers was acquired. The IR spectrum of the synthesized dendrimer 4.0G showed absorption peaks at 3315.74 cm⁻¹ for N–H stretching of primary amine, the peak at 2827.74 cm⁻¹ for aliphatic C–H stretches, 1514.17 cm⁻¹ and 1448.59 cm⁻¹ peaks for N–H bending and 1149.61 cm⁻¹ for C–C bending. A peak at 1654.5 cm⁻¹ was assigned for amide carbonyl absorption while a peak at 1568.4 cm⁻¹ was due to a core N–C stretching. The IR spectrum of the 3.5G showed absorption due to the presence of characteristic quaternary ammonium ion peak at 3282.95 cm⁻¹ as well as carboxylic carbon at 1670.41 cm⁻¹. Other peaks include 3001.34 cm⁻¹ for N–H stretch, 2700.43 cm⁻¹ for C-H stretch and 1188.19 cm⁻¹ for C–C bending. Half generation carboxyl terminated shows intense peaks in the -C=O region while full generations show intense peaks in the -N–H stretch for primary amine. The appearance-disappearance reappearance of distinctive peaks stipulates the evidence of synthesis, as shown in Table 2 and Fig. 7.
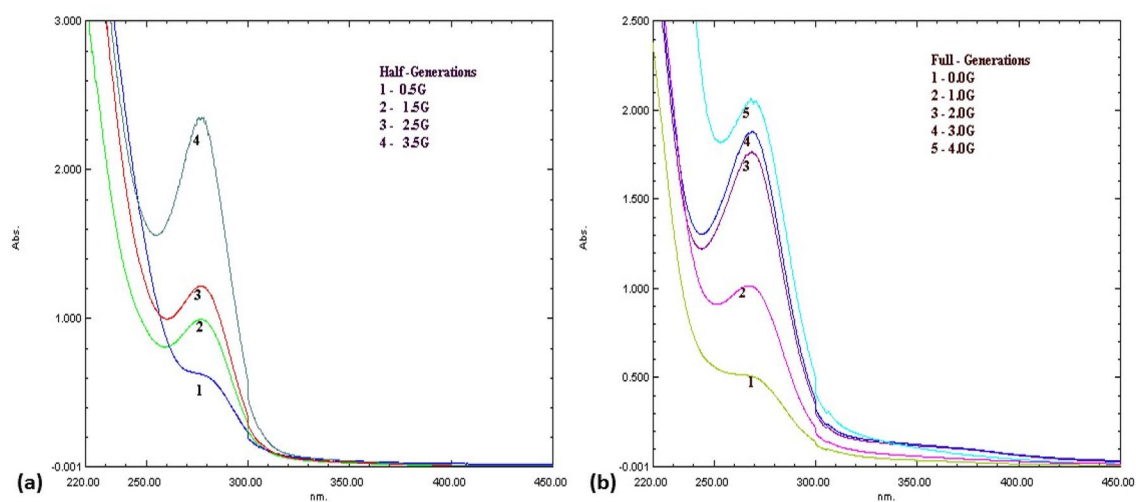
Figure 6. UV–Vis absorption spectra obtained from different generations of PAMAM dendrimer in methanol (**a**) half generation; (**b**) full generation.

| S. No	Functional groups	Frequency (cm^{-1})
4.0G PAMAM dendrimer		
1	N–H stretch of primary amine	3315.74
2	N–H stretch anti-symmetric primary amine	2991.69
3	C-H stretch	2827.74
4	N–H bending of N-substituted amine	1514.17, 1448.59
5	C–C bending	1149.61
3.5G PAMAM dendrimer		
1	Quaternary ammonium ion peak	3282.95
2	N–H stretch anti-symmetric primary amine	3001.34
3	C–H stretch	2700.43
4	C=O stretch of carbonyl	1670.41
5	C–C bending	1188.19

Table 2. IR interpretation of 4.0G and 3.5G PAMAM dendrimers.

Solubility studies. The different generations of PAMAM dendrimers dissolved in solvents with varied solubility parameters were employed for the determination of solubility. The dissolvability of polymers is subjugated by internal energy interactions. The relative solvency of particular solvent, consequential of the solvent's cohesive energy density delineates the solubility parameter. Solubility reflects the degree of change in polymer composition. The studies were evaluated under steady and tumbling conditions. However, it was observed that even after tumbling for 36 h dendrimer solvency have significant effect by solvents with solubility parameter greater than 26.6 (MPa)$^{1/2}$. Whereas, in both conditions negligible effect on solvency was observed on admixture of solvents with solubility parameter between 15.8–24.6 (MPa)$^{1/2}$. The results in Table 3 clearly show the significant solubility with the incremental solubility parameter of solvents.

Conclusion

Through a combined computational and experimental approach, a substantial endeavor has been dedicated for the synthesis of dendrimer. In this work we performed an extensive MD simulation on PAMAM dendrimers, with the purpose of elucidating several structural aspects. The effects associated with changes in pH (Fig. 4) are reflected by the electrostatic, and van der Waal forces. The work was pondered to develop minimal step, defect free, rapid and cost-effective synthesis. This study investigated the optimization and molecular dynamics as an approach to enhance the potency of molecules. The structural composition of dendrimer was successfully achieved via a divergent approach by reacting EDA and methyl acrylate in repeated steps. The synthesized dendrimer was characterized by UV–Vis, IR spectroscopy. For the assessment of morphological information UV–Vis spectroscopy was used. The Infrared spectroscopy was employed for the evaluation of chemical transits under way at the periphery of dendrimers, such as the disappearance of amine groups in the synthesis of half generation of dendrimers. The analysis data revealed synthesized dendrimer as suitable nanostructured candidate suitable for neutral as well as charged molecule delivery. Understanding the molecular dynamics as well
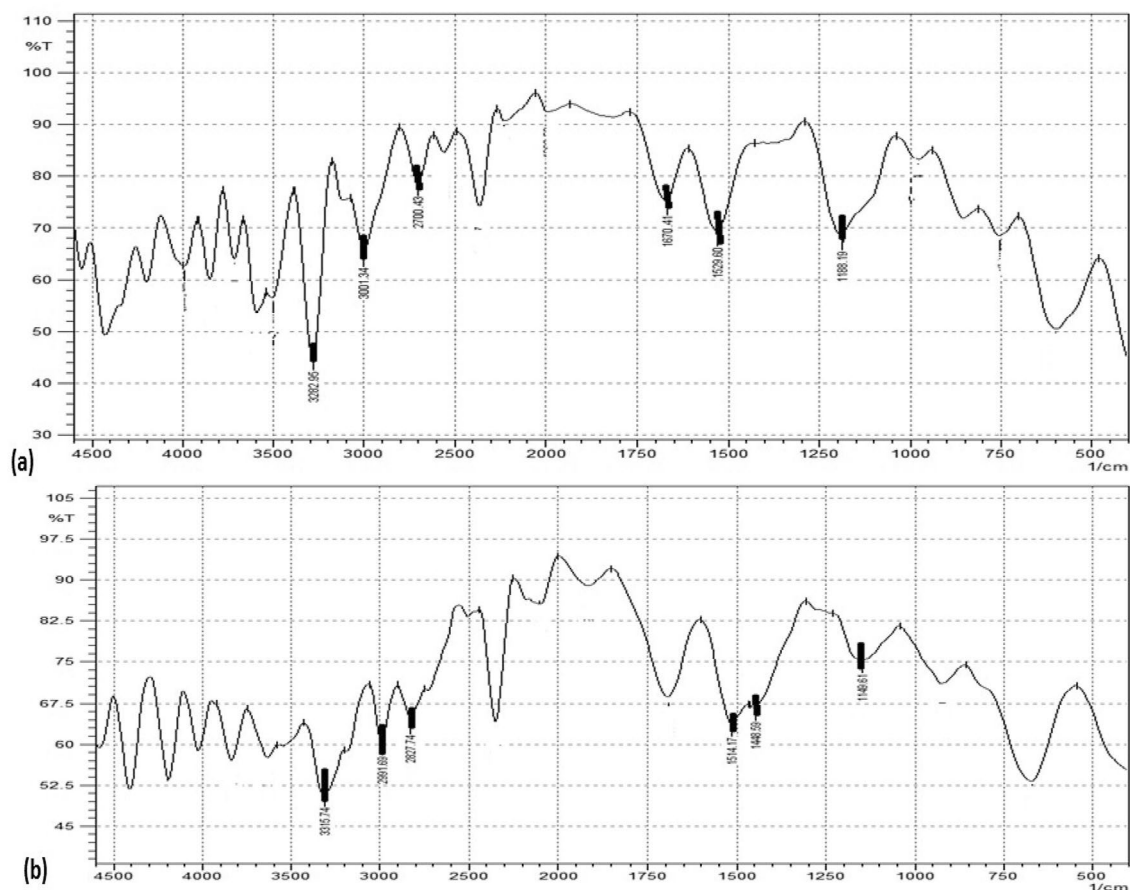
Figure 7. FTIR analysis subjected for 32 scans and scanning ranges from 4000–400 cm^{-1} using Shimadzu FTIR-8400S of (**a**) 3.5GPAMAM; (**b**) 4.0GPAMAM.

Solvents	Solubility parameter (MPa)$^{1/2}$	PAMAM generation												
		1.0G		1.5G		2.0G		2.5G		3.0G		3.5G		4.0G	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2
Diethyl ether	15.8	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Cyclohexane	16.8	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Chloroform	17.8	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Toulene	18.2	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Acetone	20.1	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Acetic acid	21.3	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Acetonitrile	24.6	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Ethanol	26.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Methanol	29.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Ethylene glycol	32.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Water	47.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 3. Solubility of different generations of polyamidoamine dendrimer in some common solvents. *1-Solubility 15 min after addition of solvent; 2-Solubility after 36 h of tumbling; *I* Insoluble *S* Soluble.

as experimental studies governing the synthesis of dendrimer alludes that an interplay of essential parameters needs to be considered in order to achieve enhanced clinical application of dendrimers.
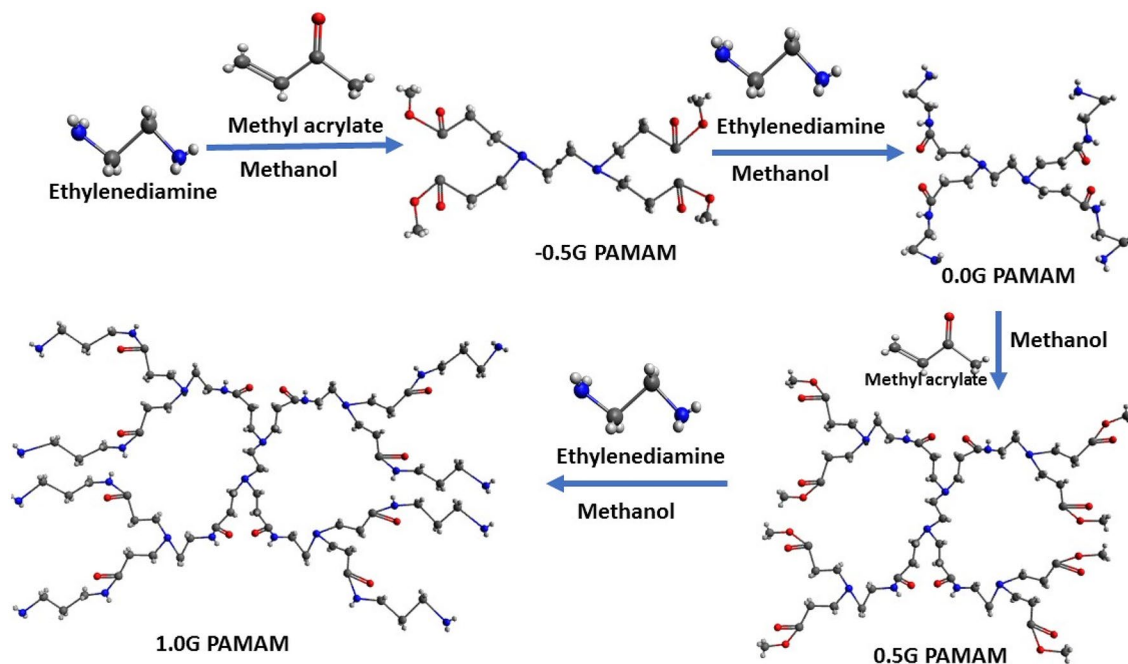
Figure 8. 3D Molecular model illustrating the synthesis of PAMAM dendrimer (Blue: Nitrogen; Grey: Carbon; Red: Oxygen) (Avogadro 1.1.1, http://avogadro.cc).

Methods

Material. Ethylenediamine (Merck Life Science Private Limited, Mumbai), Methyl acrylate (Central Drug House (P) Ltd., New Delhi), methanol (Merck Life Science Private Limited, Mumbai), Copper sulfate (Central Drug House (P) Ltd., New Delhi) were used. All the chemicals were used without further purification. The double-distilled water was used during all related studies.

Determination of molecular dynamics. In an effort to retrieve knowledge about the molecular properties of dendrimer, the 3D simulations were carried out. The different generations of dendrimers were simulated using Avogadro software[60] and further studied for the estimation of various molecular dimensions. In the exploration of molecular simulations, the force field act as an essential key element. The force field proportionally studies the estimation of potential energy and computation of forces acting on the atom, of the system under assessment. In this work, the polymeric dendritic molecules were parameterized employing the UFF i.e. Universal Forcefield. This forcefield is a broad spectra and non-reactive approach towards introduction of weighted atom types and weighted bonds, used to update topologies and atom parameterizations at every time step of a simulation. Each molecular model was then subjected to an amalgamation of steepest descent and conjugate gradient energy minimization steps of 25,000 cycles, in order to relax close atomic distances. The minimization methods steepest descent and conjugate gradient were employed in combination for effective results, where, former is faster and latter productive. Moreover, the system was equilibrated by performing 8–12 ns MD simulations in the isobaric-isothermal (NPT) ensemble. In the MD simulation, the atoms included in molecules move according to the Newtonian equation of motions. Subsequently, the 1 fs time step algorithm was employed to achieve the process of integrating the equation of motion, as integration method.

The resulting simulated molecules were subsequently energy minimized to yield van der Waals sphere at variable pH from 4.0 to 9.0 depicting electrostatic potential[59]. Equations used for the electrostatic interactions is Coulomb's law, and the equations used for the van der Waal's component is the 6–12 Lennard–Jones potential. Both type of interactions contributes to the adhesion of particles. For the ease of clarity 1.0G PAMAM generation was chosen here, as simulations of all generations are beyond the scope of this paper.

Preparation of dendrimers. The composition of dendrimers principally encompasses two steps; Michael addition and amidation. The reported method here involved ethylene diamine (EDA) as core, which was attached by acrylate resulting in −0.5G tetra ester. The next step followed the amidation of terminal carbomethoxy group by EDA. This tetra ester with an excess amount of EDA gave 0.0G tetra amine, Fig. 8. The process was repeated until the desired generation was achieved i.e. 4.0G. To circumvent the obstruction due to incomplete reaction, excess of EDA was used[61]. Excess of reagents was removed employing the usage of rotary vacuum evaporator maintained at 50–60 °C, in every step. All the reactions were advanced using tightly corked amber colored round bottom flasks obscured at room temperature for minimal step, defect-free, rapid and cost effective synthesis. The addition reaction took 2 days, whereas the amidation reaction took 4 days for the accomplishment of the array of reactions.

Evaluation of dendrimers. The validation of synthesis of half and full generation dendrimers was determined by UV–Vis as well as FTIR spectroscopy. For the differentiation among half and full generation of dendrimer, 0.1% w/v dendrimers solution was stirred with freshly prepared 1% w/v aqueous solution of copper sulphate. To investigate the structural changes in dendrimer, FTIR spectroscopy was used. The samples were prepared in the form of KBr pellet method. It was subjected to 32 scans and scanning ranges from 4000–400 cm^{-1} using FTIR-8400S, Shimadzu, Kyoto, Japan.

Solubility studies. Solubility parameters were determined according to the standardized American Society for Testing Materials (ASTM) D 3132–84 procedure with slight modifications[62]. In the experiment, 1 ml of 0.1 mg/ml each PAMAM dendrimers generations 1.0G, 1.5G, 2.0G, 2.5G, 3.0G, 3.5G and 4.0G along with solvents with varied solubility parameters were kept in test tubes. The test tubes were shaken at room temperature for 36 h in a shaker and allowed to stand to attain equilibrium. Visual inspections were conducted for the evaluation of solubility.

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Authors contributions

M.C. worked out on the methodology, investigation, preparation of original draft preparation as well as handled the software. J.S. conceptualized, handled data curation, method validation and final review. R.D.K. supervised the entire work and reviewed for final submission. M. directed the interpretation done on part of software used. All authors reviewed the manuscript.

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